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## Genomics: applications to Antarctic ecosystems

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**Abstract** Biological research in Antarctica has made considerable progress in science over recent decades. As little as 50 years ago, there was scant knowledge even of the species inhabiting the region. Since then, understanding has developed rapidly, across diverse disciplines including physiology, biochemistry, ecology and biogeography. Some dramatic global-scale discoveries and advances have been made, including the characterisation of antifreeze proteins from notothenioid fish and the finding that some fish lack a heat shock response, the identification of microbial communities living within the surface layers of rocks and description of the simplest faunal communities known, the identification that possibly the fastest environmental and ecological change on earth is occurring in Antarctic lakes, and that the biodiversity of the Southern Ocean is much greater than previously thought. Findings such as these

have made biology in cold extreme environments one of the most stimulating areas for research in recent decades. Now, the advent and widespread applicability of the novel genomic technologies promise to move us into a period of equally, or possibly even more, rapid advance. At present, genomic information on Antarctic species is limited mainly to a number of fish species and microbes. However, an increasing number of Antarctic genomics projects are being funded and will significantly increase the amount of molecular information available on a much wider range of species in the near future. Hence it is timely to review progress so far in the use of genomic methods in Antarctic research and identify exciting prospects for dramatic future advances.

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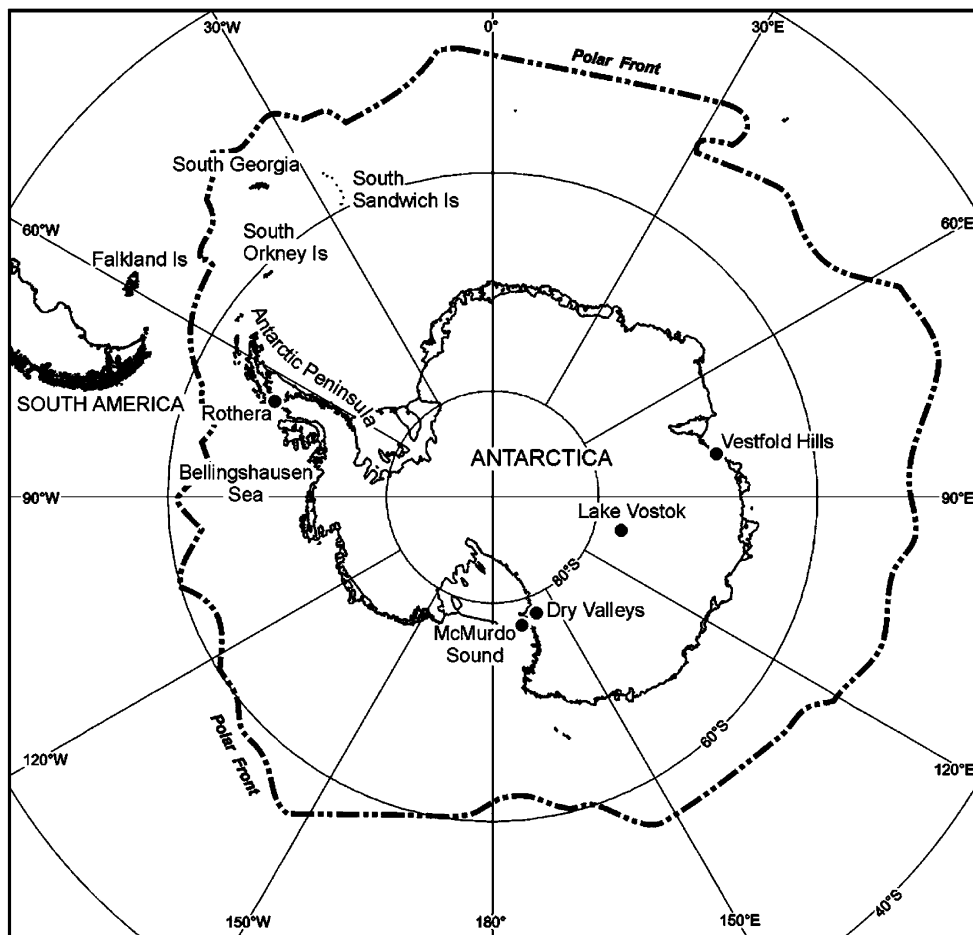
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### Introduction

Possibly the greatest biological breakthrough of the twentieth century was the determination of the structure of DNA and thereby the genetic code. This breakthrough spawned a wide variety of new disciplines, technologies and industries. Recent advances, combined with the advent of genomic approaches, have now reached the point where we stand on the threshold of dramatic changes in our understanding of how living organisms interact with their environment. Genomics is a high-profile science that impacts on all areas of biology, and it is not surprising that it is now playing an increasing role in Antarctic research. The isolation of the Antarctic, and the poor understanding we have of many of its environmental and biological processes, mean it is often viewed as one of Earth's last great frontiers (Fig. 1). The extreme climate, combined with months of complete or near darkness provide inhospitable conditions for life. Despite this, the evolutionary and geographical history of the Antarctic has produced a unique environment, rich in species adapted to extreme conditions. The importance of genomic research is highlighted by the widespread interest in genome mining or bio-

**Fig. 1** Map of Antarctica and the Southern Ocean



prospecting, which is high on industrial and political agendas, despite associated political controversy (c.f. Lake Vostok: George 2004; Bowyer 2003; Watson 2003, Antarctica: Lohan and Johnston 2003). It also provides unparalleled tools for studying natural selection in action and investigating the link between organisms and environment (environmental genomics). With the publication in 2003 of a US National Research Council position paper entitled “Frontiers in Polar Biology in the Genomic Era” (NRC 2003) and a 2003 workshop meeting on polar genomics at the British Antarctic Survey, a review of progress prospects for the future is now timely.

### Evolution of the Antarctic environment

The biota of Antarctica has evolved under the influence of a range of geological and climatic factors, including geographic isolation of the landmass and continental shelf seas, extreme low temperatures and intense seasonality. Antarctica (Fig. 1) emerged from the disintegration of the Gondwana supercontinent that started around 120 Ma BP during the Cretaceous period. Isolation progressively increased through time until the continent finally separated from South America about

31 Ma BP (Lawver et al. 1992; Lawver and Gahagan 2003), at the Eocene/Oligocene boundary. This period also marked the onset of major cooling in the Antarctic with the first appearance of sea ice (Lear et al. 2000; Zachos et al. 2001). Since then Antarctic temperatures have generally decreased, but the trend has been interrupted by either global or regional warming episodes. The first formation of permanent icecaps on the Antarctic continent occurred within the first few million years after separation from South America. Nevertheless, during warmer periods, the continent hosted considerable development of cool temperate vegetation and fauna, probably comparable to that seen today in southern South America and New Zealand. In particular the early Miocene period (23–17 Ma BP) appears to have been marked by significant warming, and brief warming periods also occurred in the late Miocene and early Pliocene (4.8–3.6 Ma BP). Sharp climatic cooling after this period caused further expansion of the ice sheets and culminated in the bipolar glaciation of the Pleistocene (reviewed in Clarke and Crame 1992).

Isolation and an extreme environmental history have led to a unique Antarctic biota, both on land and in the sea. Many groups of organisms became extinct in the Antarctic as a result of the increasingly extreme climatic conditions, although this probably took place episodically.

cally over the last 30 million years rather than as a single event. Examples include many decapod crustaceans and most marine fish groups, whilst higher plants are now absent from the land (with the exception of two species only found on the Antarctic Peninsula and associated island archipelagoes). Environmental and geographic isolation and strong selection driven by the extreme conditions have produced a highly adapted and predominantly endemic biota. In the marine environment, for example, over 90% of Southern Ocean pycnogonid (sea spider) species are endemic to the Antarctic (Clarke and Johnston 2003), while all terrestrial nematodes currently known are endemic to the continent (Andrássy 1998). Some of the groups of animals that have adapted to extreme polar conditions have also undergone marked radiation events. A single family, the notothenioid fish, contributes over 50% of species diversity and 90% of biomass of the Antarctic continental shelf fish fauna, and is one of the few examples of a species flock in the marine environment (Eastman and McCune 2000).

The marine fauna of the Antarctic is relatively diverse and in some cases ancient (Clarke and Crame 1989). There is evidence that some groups of animals invaded the Antarctic during warm periods and subsequently speciated in situ, as they were isolated by steepening environmental gradients from north to south in the Southern Ocean (e.g. Page and Linse 2002). The terrestrial fauna has an extremely low diversity, with some soil communities being the simplest on Earth (Freckman and Virginia 1997; Convey 2001a). In some areas, communities are limited to a few species of algae, fungi and bacteria living endolithically a few millimetres below rock surfaces (Friedmann 1982; Hughes and Lawley 2003). Much of the coastal flora and fauna may have arisen from the relatively recent immigration of species, as almost all of this habitat was destroyed by recent glaciations (O’Cofaigh et al. 2002; Convey 2003). There is evidence, however, that a significant part of the continental Antarctic terrestrial biota may have survived glaciations in refugia and some elements may even represent ancient Gondwanan relicts (e.g. Marshall and Pugh 1996; Stevens and Hogg 2003).

The contrast in environmental variability in terrestrial versus marine systems has led to markedly different evolutionary pressures on organisms in these two ecosystems. In the most southerly marine areas, temperature variations may be <0.5 K annually, although recent data suggest that McMurdo Sound and, by inference, other high-Antarctic locations are not quite as thermally stable as originally thought, with fluctuations of up to 1.5 K annually (Hunt et al. 2003). In contrast, terrestrial organisms can be exposed to daily temperature fluctuations of 20–40 K and annual temperature fluctuations as high as 40–80 K (Convey 1996). Seasonality in the marine system is extreme, with phytoplankton blooms lasting as little as 8–10 weeks in some locations, while winter periods have productivity levels lower than any other large marine region and last for as

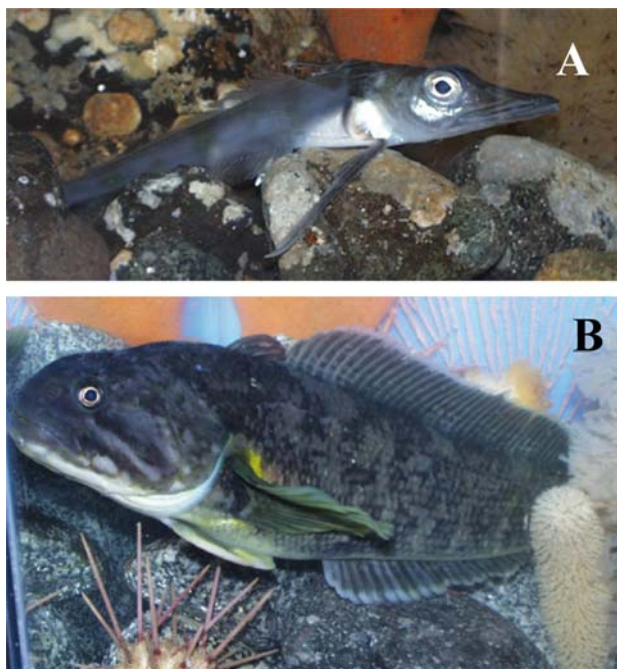
much as 7 or 8 months (Clarke 1988; Clarke and Leakey 1996). Shallow marine species face the additional problems of intense and frequent physical disturbance from impacts of floating ice (Peck et al. 1999; Brown et al. 2004). Terrestrial species also experience long periods of restricted or zero resource availability in winter because the environment is frozen and, even in summer, may be further limited by chronic shortage of liquid water. These conditions have prevailed in Antarctica for at least 10 million years, a long evolutionary period and considerably longer than the glaciation of high northern latitudes. Given this unique evolutionary history and current environmental setting, the Antarctic biota provides many opportunities to address fundamental biological problems, in particular the links from genome to survival of organisms and the functioning of ecosystems.

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### The development of Antarctic fish as model systems

Among polar organisms, the phylogenetic history of the Antarctic fishes of the suborder Notothenioidei (Teleostei) is undoubtedly the best understood (Clarke and Johnston 1996; Chen et al. 1998; Eastman 2000; Eastman and McCune 2000; Near et al. 2003; Ritchie et al. 1996). Living in a stable, extremely cold, and well-oxygenated marine environment, these fishes have evolved numerous adaptations in their biochemical and physiological functions, several of which are unique. Cheng has referred to the Antarctic notothenioid fishes as “swimming libraries” of cold-adapted genes and proteins. These compensatory adaptations have involved both the alteration of existing genes to produce enzymes that function well at cold temperatures, and the restructuring of the genome to gain new physiological capabilities. In recognition of this potential, NHGRI funding in the United States has been given for the creation of two BAC libraries [large insert (120 kb) genomic clone libraries in Bacterial Artificial Chromosome vectors] for *Chaenocephalus aceratus* and *Notothenia coriiceps* (Fig. 2) (<http://www.genome.gov/10001852>). This will make these Antarctic genomes far more accessible to the wider scientific community, enabling any researcher to identify and clone their “favourite” gene and functionally compare psychrophilic and mesophilic adaptations, in addition to gaining information on biological variability.

One major example of these adaptations is the acquisition of genes for antifreeze glycoproteins from the modification of the control region of a pancreatic trypsinogen-like gene (Chen et al. 1997, 1998; Cheng and Chen 1999). By contrast, adaptation to a stable, cold environment has also led to the loss of capabilities usually assumed to be essential to vertebrate life. Hence these fish present many natural examples of gene knock-out experiments (an experimental procedure most commonly used in mice, where genes are targeted for inactivation and the effects of non-expression studied in relation to development and disease). One striking



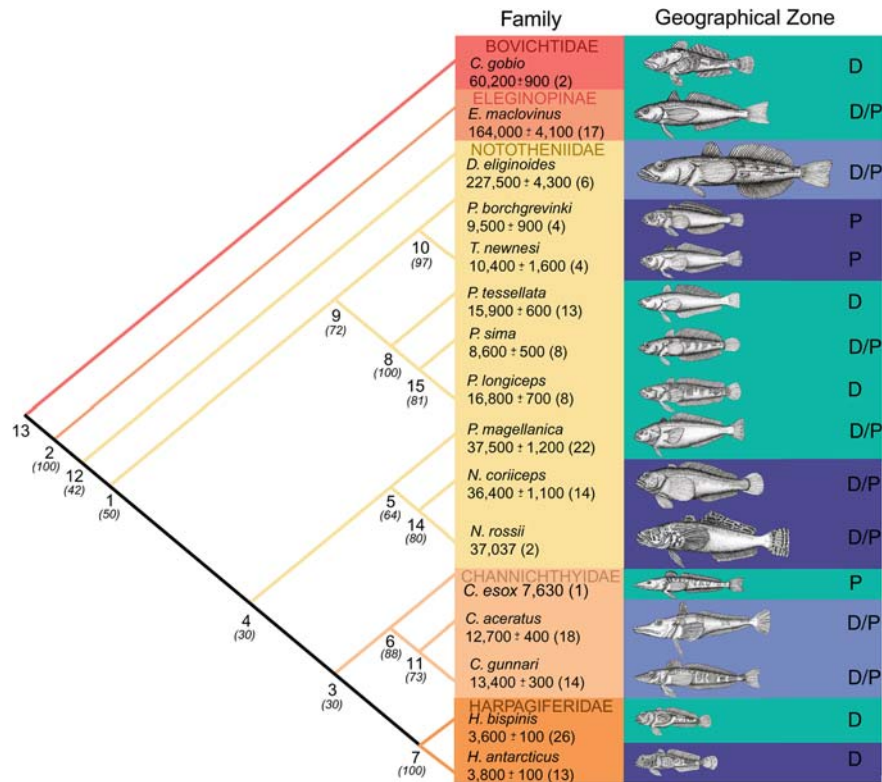
**Fig. 2** BAC libraries will be produced from: **a** The blackfin icefish *Chaenocephalus aceratus*. Specimen total length approximately 25 cm. **b** The yellowbelly rockcod (also known as bullhead notothen) *Notothenia coriiceps*. Specimen total length approximately 35 cm. Both photographs courtesy of H. William Detrich III. For details contact either H. William Detrich III (iceman@neu.edu) or Chris Amemiya (camemiya@benaroyaresearch.org)

example of such is the heat-shock response that appears absent in at least one Antarctic fish. Heat shock proteins (HSPs) are highly conserved and were thought to be universally present in all animals, acting to protect cells from damage associated with stresses such as those induced by significant variations in temperature, or desiccation in some terrestrial species. The Antarctic notothenioid fish *Trematomus bernacchii* has lost the ability to inducibly express HSPs when exposed to elevated water temperatures (Carpenter and Hofmann 2000; Hofmann et al. 2000; Place et al. 2004), probably as a result of dysfunctional heat shock elements (HSE) within the promoter region of the HSP70 gene (Carratù et al. 1998). Possibly the most unusual of these phenotypes is the loss of the respiratory oxygen transporter haemoglobin (Cocca et al. 1995; di Prisco et al. 2002; Ruud 1954) and the ability to produce functional erythrocytes (Barber et al. 1981; Hureau et al. 1977) by icefish (Channichthyidae), the most derived family of the notothenioid suborder. In these fish, oxygen is transported around the body solely in solution, not bound to a pigment, and the blood is colourless. Scanning the genomes and transcriptomes of red-blooded notothenioids for genes that are no longer expressed by channichthyids, may identify novel erythroid genes. These can then be functionally characterised by cloning and analysing their orthologues in better-described vertebrate genetic models, such as the zebrafish (so-called “model-

hopping”). Knock-out experiments of these genes in zebrafish (or other model species such as mice) will indicate if the genes have a role in erythropoiesis and will help to elucidate the genetic programme of erythropoiesis in all vertebrates, including humans. They could then be exploited to develop new treatments for blood-related diseases and syndromes, such as anaemias associated with kidney dialysis treatment and cancer chemotherapy.

In addition to the loss of haemoglobin, 6 of the 15 members of the Channichthyidae lack myoglobin in their cardiac muscle (Moylan and Sidell 2000). Comparative studies indicate a diversity of evolutionary mechanisms leading to the loss of the myoglobin genes (Small et al. 2003). The relatively high solubility of oxygen at low temperatures may have obviated the need for myoglobin-facilitated diffusion. Cardiac and skeletal red muscles in notothenioids contain amongst the highest densities of mitochondria found in vertebrates, reflecting a temperature-induced reduction in respiratory capacity at the level of the individual mitochondrion (Johnston et al. 1998). However, other physiological processes, such as the cell cycle time of myogenic cells, show elevated rates at 0°C relative to temperate species (Brodeur et al. 2003). Some species derived from the core radiation of Antarctic notothenioids have subsequently invaded more temperate waters. These include *Paranotothenia* species that inhabit the Southern Ocean including southern New Zealand and associated islands (*Paranotothenia magellanica*) and the South West Pacific, New Zealand and Macquarie Island (*P. microlipidota*). In a similar way, representatives of the Antarctic plunderfish genus *Harpagifer* also occur in the Falkland Islands and South America. Comparative genomic studies of these closely related taxa therefore provide opportunities for investigating critical genetic adaptations associated with cold adaptation and separating these from constraints of phylogenetic history.

The elevated oxygen solubility in water at low temperatures has allowed several important unusual characters to arise, including gigantism in marine invertebrates (Chapelle and Peck 1999) and the evolution of giant muscle fibres in some notothenioids (Johnston et al. 2003) and isopod and amphipod crustaceans (Young 2004; J. Young, personal communication). The increase in muscle-fibre size is possible because constraints of diffusion are relaxed, both by the increased ambient oxygen levels and reduced metabolic rates at low temperature (Clarke and Johnston 1999; Peck and Conway 2000) and, in fish, is associated with a dramatic reduction in muscle-fibre number for a given body size. Phylogenetic analysis revealed this trend has been progressive during the radiation of the group (Johnston et al. 2003) (Fig. 3). In most teleosts (comprising the major commercial fish species), new muscle fibres are produced from discrete germinal zones in the larval stage (stratified hyperplasia) and then from scattered myogenic progenitor cells during the subsequent juvenile and adult stages (mosaic hyperplasia). In



**Fig. 3** Maximum likelihood phylogenetic tree estimated from 12S mitochondrial rRNA sequences and the trait values for the final number of fast muscle fibres (FN<sub>max</sub>: presented as mean ± SE, number of individuals) for notothenioid fishes prepared using PhyIip. The bootstrap support values obtained from the PhyIip analysis are shown in *parentheses* by the nodes. The size of the fish gives some indication of their relative sizes, but they are not drawn to scale. The locomotory habit of each species is also shown: *D* demersal; *DP* demerso-pelagic and *P* pelagic. The colours on the *right-hand side* show the geographical zone of capture for each species: Beagle Channel (*green*), Shag Rocks, South Georgia (*light blue*) and Antarctic Peninsula (*dark blue*). The colours on the *left-hand side* illustrate the family relationships of the species studied. Reprinted from Comparative Biochemistry and Physiology, Part B, volume 136, Johnston I.A, Muscle metabolism and growth in Antarctic fishes (suborder Notothenioidei): evolution in a cold environment, 701–713., Copyright 2004 with permission from Elsevier

contrast, the icefish *C. aceratus* appears to grow entirely by stratified hyperplasia, with impairment of mosaic hyperplasia (Johnston et al. 2003). These clear differences in muscle development present ideal opportunities for using subtractive genomic techniques for the discovery of novel genes associated with mosaic hyperplasia, and for understanding the genetic mechanisms controlling fibre number. The study of such processes is of considerable economic importance for farmed species such as salmon, trout and cod, because a high muscle-fibre number is associated with improved flesh quality, particularly firmness, and better processing characteristics (Johnston 2001).

The utilisation of Antarctic fish as natural knock-out models is a situation by no means unique in biology. A considerable number of “natural” animal models

for human disease already exist, such as dogs, cats, sheep, racehorses etc. (a comprehensive list can be found on [http://www.pirweb.org/pir04a\\_animals.htm](http://www.pirweb.org/pir04a_animals.htm)). For example, it was analysis of a combination of Dobermann, labrador and dachshund pedigrees that enabled the first identification for the gene involved in narcolepsy, and Bedlington terriers suffering from copper toxicosis have identified a novel gene involved in copper transport (Lin et al. 1999; van de Sluis et al. 2000). Both of these are medically important conditions and there is no reason to suppose that investigation of Antarctic fish will not offer similar rewards. As regards functional analysis of null proteins, Antarctic fish are more likely to provide candidate genes for more rapid analysis in other model organisms, the zebrafish being the most obvious first stage candidate. This is because Antarctic fish grow very slowly and tissue culture systems have yet to be reproducibly developed for any notothenioid species. However, this is not a drawback, as the second vertebrate model to be sequenced to draft quality was the Japanese pufferfish (Aparicio et al. 2002), another fish species in which experimental manipulation is not currently possible, but is now extensively used for gene identification and comparative genomic analyses (Clark and Roest Crolius 2004).

### Dissection of cold-adaptation mechanisms

Clearly one of the main uses of Antarctic organisms and analysis of their genome data is to examine cold-adaptation mechanisms. There is the fundamental research

aim of dissecting enzymic adaptation and polypeptide folding, but there is also commercial value in exploiting cold-adapted enzymes. Again, research until recently has focused on fish systems, but this is being increasingly supplemented by microbial genomes.

Both tubulin dimers and the microtubule motors of Antarctic fish are cold-adapted, with their properties and function rates at 0°C being similar to organisms living at much warmer temperatures. The protein structure of tubulin dimers and microtubule motors is substantially conserved (Detrich 1998). Cold-adaptation is the result of small changes in primary sequences and post-translational modifications of component subunits. Microtubule cold-adaptation is probably achieved by hydrophobic re-modelling of the interdimer surfaces (Detrich et al. 1989, 1992), increased flexibility of the domains involved in dimer-dimer contact (Fontana 1991), decreased electrostatic repulsion between tubulin dimers, or some combination of these modifications. Similar changes producing increases in molecule flexibility have also been identified in the A<sub>4</sub>-lactate dehydrogenases from a range of different fish species, including notothenioids (Fields and Somero 1998). Dissecting the mechanisms leading to conformation changes in proteins provides an insight not only into the general principles behind the evolutionary adaptation of organisms, but also into polypeptides that adopt multiple conformations such as pathogenic prion proteins in mammals (Cohen and Prusiner 1998).

Such studies, which originated on Antarctic species, are enhanced by examining enzyme orthologues in a range of congener species showing different degrees of temperature adaptation (Fields and Somero 1997, 1998; Fields et al. 2002). Indeed, it is this latter field of study that has proved tremendously fruitful in defining protein adaptation to temperature. Although the amino acid changes described above play a role in temperature adaptation, it is now being shown that these studies should expand to include examination of micromolecules, in particular low molecular mass organic solutes (Somero 2003). It is hypothesised that these molecules stabilise conformational microstates in the cellular milieu and play an important role in stressful conditions (Somero 2003), of which temperature adaptation is just one example. It will be interesting to compare such microstates in Antarctic species and their temperate congeners.

A further example from fish identifies the commercial interest in exploiting Antarctic species. A clear example is the improvement of secretory capacity of cells used in industry. This has been a long-standing goal that has remained largely elusive as a result of limited understanding of the principles governing protein folding and secretion. The protein machinery required for protein translocation, protein folding, and vesicle budding and fusion is highly conserved and well characterised, but its mechanisms of action are still poorly understood (Johnson and van Waes 1999; Alberts et al. 2002). Recently, the genes encoding the core component of the

protein translocation channel in the ER membrane, *SEC61*, have been sequenced from Antarctic and Arctic fishes and compared to their mesophilic counterparts (Römisch et al. 2003). Amino acid changes were identified in a functionally important, ER luminal loop of the Sec61p protein (Römisch et al. 2003). These data suggest that this loop undergoes a conformational change during translocation, which is facilitated at low temperature by the observed changes in amino acids at specific positions. In addition, ER vesicles were isolated from the liver of the Antarctic icefish, *Dissostichus mawsoni*, and protein translocation into the vesicles investigated over a temperature range (Römisch 2003). Compared to mammals and temperate fish, *D. mawsoni* ER imports proteins highly efficiently at 0°C, lending support to the hypothesis that the protein translocation machinery in these organisms is adapted to low temperature (Römisch 2003). It is now possible to introduce the corresponding amino acid changes into the protein translocation channel of a genetically tractable model organism such as *Saccharomyces cerevisiae*, or into tissue culture cells (disabling the endogenous channel by RNA interference), and directly observe their effects on protein translocation across the ER membrane at various temperatures.

As discussed above and in previous sections, fish can provide valuable examples for biological research; however, microbial genomes are increasingly prominent in this field of research. The draft genome sequence of the Antarctic archaeon, *Methanococcoides burtonii*, has enabled a global overview of prokaryote cold-adaptation mechanisms (Goodchild et al. 2004). Gene and protein expression profiles were generated from this archaeon grown at both 4 and 23°C. This enabled identification of key genes involved in thermal regulation, e.g. RNA polymerase subunit E, and also the quantification and comparison of mRNA expression levels, protein abundance and enzyme activities associated with temperature increases (Goodchild et al. 2004). Temperature-dependent gene expression has been examined previously in a limited way in Antarctic fish (e.g. Hardewig et al. 1999), but the ease of manipulation of microbial cultures will significantly progress this area of research in the future.

Also, a further advantage of microbial genomes lies in the power of *in silico* comparative analyses. One recent Antarctic example is provided by the draft genomes of 2 methanogenic archaea, *Methanogenium frigidum* and *Methanococcoides burtonii*, from Ace Lake, in the Vestfold Hills region of Antarctica (Saunders et al. 2003), with 19 other archaeal genomes. These represented organisms with optimal growth rates ranging from 15 to 98 K. Psychrophilic methanogen proteins contained structural features interpreted as conferring molecular flexibility, which increase catalytic efficiency, as well as prevent cold-denaturation. In addition, both genomes contained cold shock domains or folds that have putative roles in RNA stabilisation. Among the most important adaptations to cold conditions are

changes in microbial cell membranes, such as the production of polyunsaturated fatty acids to maintain fluidity at low temperatures and the synthesis of enzymes capable of catalysis at low temperatures (Deming 2003). Cold-adapted enzymes have already been exploited in industries and products as diverse as leather tanning, food processing and laundry detergent developed for cold-water washing of clothes (Feller and Gerday 2003), and certainly this is an area set to expand, particularly in the arena of gene patents (Stix 2004).

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### Genome mining

A significant constraint to research on any Antarctic species is the current lack of sequence data. However, this is not such a problem with Antarctic microorganisms and their small genomes, which are more amenable to exploitation. Genomics is already making an impact here through the analysis of genome sequences from cold-adapted bacteria and archaea (Table 1), and from the ability to compare them to their mesophilic and thermophilic counterparts. The massive increase in worldwide sequencing capacity and improvement in sequence generation and data-handling now means that sequencing of a microbial genome (certainly to draft standard: where there is considerable coverage of the genome, usually a minimum of 6x, but the genome sequence is not contiguous) is now relatively trivial for a number of the major sequencing centres such as TIGR, JGI and the Sanger Institute. The Genomes On Line Database (GOLD: <http://www.genomesonline.org>) now lists 520 prokaryotic genome projects (either on-going or complete), which represent an immensely valuable resource for comparative analyses (Nierman and Fraser 2004).

Most of the microorganism genome data are directly available in searchable databases (Table 1); the number of published analyses is more limited. In addition to the previous examples of *Methanococcoides burtonii*, and *Methanogenium frigidum*, there is also the *Colwellia psychrerythraea* genome. This provides another strong candidate for comparative *in silico* data-mining. It is one of the first whole genome sequences of a strictly psychrophilic bacterium (Methe et al. 2002). As a member of the  $\gamma$ -proteobacteria, the genus *Colwellia* represents a group of obligate marine psychrophiles whose members to date have been obtained exclusively from cold environments, including the Arctic, Antarctic deep oceans, seawater, sea ice and sediment, and in which they play important roles in carbon and nutrient cycling. Recent biochemical investigations have demonstrated that *Colwellia psychrerythraea* is capable of producing extracellular proteases with extraordinarily low temperature activity optima (Huston et al. 2000). Environments in which *Colwellia* have been found include ice formations that are currently under examination as models of past ice ages on Earth (Kirschvink et al. 2000) and also as

potential candidates for life on other planets and moons of our solar system (e.g. Mars) (Deming 2002).

So, although examining cold-adaptation may initially be one of the primary aims behind sequencing these psychrophilic genomes, and certainly analysis of such data can facilitate the discovery of novel proteins and secondary metabolites, environmental biochemistry is now coming to the fore. The elucidation of probable metabolic pathways or metabolic potential of each microorganism is fundamental to understanding its role in biogeochemical cycling. This information in turn is critical to the additional applications of microorganisms as potential agents of wastewater treatment and bioremediation of toxic pollutants in cold environments (including temperate environments that experience a winter season). It is also of fundamental importance to understanding carbon sequestration in the oceans, and hence the prediction of biotic response to, or possible mitigation of, climate change.

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### Ecosystem monitoring and ecological barcodes

Linkages between gene, genome content, gene regulation and expression can provide information about Antarctic terrestrial and marine biodiversity and ecosystem function. Antarctica harbours an incredible variety of habitats dominated by microorganisms, ranging from sea ice, the water column and benthos, including hydrothermal vents and mud volcanoes (Klinkhammer et al. 2001; Vanneste and Larter 2002) in the marine environment, to volcanoes, rocks and soils, and all the different forms of ice and water on the continent. Of those habitats investigated, molecular biological approaches are increasingly being used in combination with classical techniques to describe the diversity of the organisms present. These tools are a great benefit, since as in many other environments on Earth, our abilities are limited for cultivating the majority of, or the ecologically important, microorganisms (Amann et al. 1995). The slow growth rates at low temperatures experienced in the Antarctic, with some endolithic cyanobacteria dividing as few as four times per year (Hughes and Lawley 2003), form an extra complication.

The ability to describe the endemic microbial fauna using molecular techniques greatly facilitates ecosystem monitoring. For example, sequences that are phylogenetically informative in bacteria, such as ribosomal RNA (rRNA), have been used to describe significant temporal shifts in bacterioplankton community composition in the marine ecosystem over the annual cycle. Large changes in bacterioplankton composition have been found towards the end of the austral summer (Murray et al. 1998). Temporal variation was observed also in an abundant group of marine prokaryotes, the group I Crenarchaeota. These archaea have circumpolar distribution in the Antarctic marine environment (Murray et al. 1999) and represent a significant fraction

**Table 1** Status of sequenced psychrophilic microorganisms (NA not available)

Domain	Phylogenetic group	Microorganism	Origin of strain	Status of genome sequence	Web site for blast analysis
Archaea	Euryarchaeotal Methanogen	<i>Methanogenium frigidum</i>	Ace Lake Antarctica	Draft sequence published, Saunders et al. (2003)	<a href="http://psycho.bioinformatics.unsw.edu.au/blast/mf_blast.php">http://psycho.bioinformatics.unsw.edu.au/blast/mf_blast.php</a>
Archaea	Euryarchaeotal Methanogen	<i>Methanococoides burtonii</i> DSM6242	Ace Lake Antarctica	Draft sequence published, Saunders et al. (2003)	<a href="http://www.jgi.doe.gov/JGI_microbial/html/index.html">http://www.jgi.doe.gov/JGI_microbial/html/index.html</a>
Archaea	Group I marine Crenarchaeota	<i>Cenarchaeum symbiosum</i> <sup>a</sup>	Symbiont of marine sponge ( <i>Axinnella mexicana</i> ) off California coast	In annotation	<a href="http://www.jgi.doe.gov/JGI_microbial/html/index.html">http://www.jgi.doe.gov/JGI_microbial/html/index.html</a>
Bacteria	Gamma proteobacteria	<i>Colwellia psychrerythraea</i> 34H	Arctic marine sediments	Manuscript in preparation	<a href="http://www.tigr.org/tdb/mdb/mdbinprogress.html">http://www.tigr.org/tdb/mdb/mdbinprogress.html</a>
Bacteria	Gamma proteobacteria	<i>Vibrio salmonicida</i>	Farmed Atlantic salmon ( <i>Salmo salar</i> ), coast of Norway.	In progress	NA
Bacteria	Gamma proteobacteria	<i>Photobacterium profundum</i> SS9	Amphipod homogenate from 2.5 km deep in the Sulu Sea	In annotation	NA
Bacteria	Gamma proteobacteria	<i>Shewanella violacea</i> DSS12	Deep-sea mud (5.1 km) of Ryukyu trench, Japan	Manuscript in preparation	NA
Bacteria	Gamma proteobacteria	<i>Psychrobacter</i> sp. 273-4	Siberian Permafrost	In annotation	<a href="http://www.jgi.doe.gov/JGI_microbial/html/index.html">http://www.jgi.doe.gov/JGI_microbial/html/index.html</a>
Bacteria	Delta proteobacteria	<i>Desulfotalea psychrophila</i> LSV54	Arctic marine sediments, Svalbard	Manuscript in preparation	<a href="http://www.regx.de/blast/index.html">http://www.regx.de/blast/index.html</a>
Bacteria	Gram positive	<i>Exiguobacterium</i> 255-15	Siberian Permafrost	In annotation	<a href="http://www.jgi.doe.gov/JGI_microbial/html/index.html">http://www.jgi.doe.gov/JGI_microbial/html/index.html</a>
Bacteria	Bacteriodetes	<i>Flavobacterium psychrophilum</i>	Salmonoid pathogen, Montana Fish Hatchery	In progress	NA
Bacteria	Bacteriodetes	<i>Polaribacter filamentous</i>	Surface seawater, 350 km north of Deadhorse, Alaska	In progress	NA

<sup>a</sup>The true psychrophilic nature of *C. symbiosum* remains unknown, since the strain is uncultivated, though it lives in waters at 10–15°C. Adapted from Clark et al. (2004)

(~20% of picoplankton) of the winter surface-water plankton assemblages, in comparison to summer populations, where they account for around 1% of the total picoplankton assemblage (Murray et al. 1998; Church et al. 2003). This ecological pattern is likely to be linked to the ecosystem role that the archaea play, although this role remains unknown. Changes have been detected in freshwater bacterioplankton communities using similar techniques (Pearce 2003).

The relative ease of producing quality short sequence data, such as that described above, from a large number of species has led to the idea of ecological barcoding. The aim is to produce DNA-based identification systems based on sequence diversity of specifically chosen genes [e.g. 18 s rRNA; Floyd et al. 2002 and cytochrome c oxidase subunit 1 (CO1); Hebert et al. 2003]. This is a technology that can be applied to any species; Hebert et al. (2003) studied lepidopterans, but it is a particularly useful technique for mixed microorganism communities, organisms that cannot be cultured, such as viruses (Breitbart et al. 2002) and those in which certain stages of the life-cycle are taxonomically ambiguous or poorly characterised, for example, marine larvae. This also enables taxonomic identification of species by the non-specialist, which is becoming increasingly important with the dwindling pool of taxonomists worldwide. Probes designed from these sequences can also be spotted onto a gene chip, and microarray fluorescent technology can be used to enable estimations of species richness. Such approaches have been used in the identification of pathogenic microorganisms and viruses (e.g. Edman et al. 2000; Wang et al. 2002).

An allied environmental genomics approach is based on the random cloning of large fragments of DNA from the environment. This was exemplified by the environmental genome shotgun sequencing of the Sargasso Sea (Venter et al. 2004). In this study, results based on sequence relatedness identified at least 1,800 genomic species, of which 148 were previously unknown and over 1.2 million previously unknown genes (Venter et al. 2004). Not all environmental investigations are possible on this scale or indeed are necessary to identify novel species or genes. A much more limited study described the genome content and microheterogeneity in the marine planktonic group I Archaea (Beja et al. 2002). A similar approach confirmed the presence of several large genomic clones containing the gene for proteorhodopsin in Antarctic waters, a pigment involved in a recently described novel type of photoheterotrophy in the ocean (De La Torre et al. 2003). For these groups of organisms, and many of those that are thought to be the most abundant in marine ecosystems that have not been cultivated to date, the environmental genomics approach holds promise in helping make key connections between the organisms, their metabolic capabilities, and adaptations to the Antarctic ecosystem. Development of such tools as those described here, although molecular in the first instance, will significantly impact on ecology, enabling access to previously intractable environmental

niches, and dramatically expand the types of questions that ecologists can tackle.

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### Dissection of environmental stress responses

The environmental extremes experienced in Antarctica are not unique in the world; for example, low temperatures comparable with those of the maritime Antarctic can be experienced in New York, although the high-Antarctic conditions are unique and the length of evolutionary time that low temperatures have existed is greater than anywhere else on earth. One of the main scientific values of Antarctica lies in the *combination* of a set of environmental stressors and the insights that those stresses may give us about the genomic responses to environmental extremes elsewhere on the planet.

Although 80–90% of the world's freshwater is in Antarctica, most of this is frozen as snow and ice, so despite the abundance of water, many terrestrial organisms experience extreme desiccation. In some habitats, temperatures can drop to below  $-60^{\circ}\text{C}$  and, because of the Earth's obliquity, parts of the Antarctic experience periods of complete darkness during the winter months, when most organisms, except those in ice-covered lakes, are inactive. The winter darkness also means that food availability is highly seasonal for terrestrial organisms, and marine organisms that are dependent on benthic or planktonic primary production (Clarke 1988; Gilbert 1991; Br  thes et al. 1994; Brockington 2001a). Many organisms cease feeding completely, or reduce their food consumption very significantly in winter (Worland and Convey 2001; Brockington 2001b; Fraser et al. 2002; Fraser et al., in press). During the spring, ozone depletion subjects exposed organisms to UV radiation levels elevated up to an order of magnitude higher in terms of potential DNA damage than undepleted conditions (Madronich and Flocke 1997). During freezing at the beginning of winter, small ponds can become salty, resulting in extreme osmotic stress (Schmidt et al. 1991). In summary, on land, extreme low temperatures, desiccation, freezing, osmotic stress and elevated UV radiation are some of the main environmental stressors to be found on the continent; in the sea, extreme seasonality, freezing and physical disturbance from ice are the main stressors (Peck 2002a).

Superficially, these stresses may seem to be unique. Physiological studies of organisms also give the impression that the responses to these different extremes are mediated through different pathways (Davey 1989; Jackson and Seppelt 1997). However, there are some initial results that indicate that the situation may be more complex than previously thought. Studies of the effects of combinations of environmental stresses on the extremophilic cyanobacterium *Chroococcidiopsis* sp. in the McMurdo Dry Valleys showed that increased temperature could synergistically induce an increase in the production of the UV-screening compound, scytonemin, alongside increases in UV radiation (Dillon et al. 2002).

This suggests that there may be links between the pathways for extreme temperature and UV radiation responses. Antarctic bryophytes have also been observed to increase production of screening pigments in response to increased levels of UV radiation (Newsham et al. 2002, 2003).

It is only by examining the expression of the genes themselves to these different stresses that we will be able to determine which genes or suites of genes play common roles in different responses, and unravel how these various cellular processes are linked. This research has global implications:

- First, some of the combinations of stresses experienced in the Antarctic are on a continuum with extreme environments in other regions of the world. Thus, desiccation and extreme UV radiation are experienced by some organisms in hot tropical or subtropical deserts, albeit at high temperatures.
- Second, studies of genomic responses to combined stresses will allow detailed evaluations of resource allocation not previously possible. Depending on the different extremes experienced, organisms must allocate their energy reserves to the different responses required (Convey 2001b). Overlap of genetic responses may allow for a more efficient response to multiple stressors, which should be important to organisms that have limited reserves or nutrient availability, as is often the case in Antarctica and other extreme deserts.
- Finally, by investigating the expression of the specific genes, it will be possible to derive fundamental insights into how similar or variable these responses are in quite different phylogenies, from microorganisms to fish, and to what extent extreme environments require specific adaptations or simply select for more generalist or phenotypically plastic life-styles.

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### Global climate change: Antarctic species as biomonitors

An area strongly linked to the examination of stress responses is that of response to global climate change. Antarctic species act as primary biomonitors for the rest of the world. Global temperatures have increased by  $0.6 \pm 0.2^\circ\text{C}$  during the twentieth century, probably as a result of anthropogenic increases in greenhouse gases (Houghton et al. 2001). On a regional scale, however, the picture is more complex. Some Antarctic areas such as the geographic South Pole are cooling, while some of the most rapid regional warming on Earth is taking place in the Antarctic Peninsula and Bellingshausen Sea (Hansen et al. 1999; Vaughan et al. 2001). Antarctic Peninsula air temperature has increased by more than  $2^\circ\text{C}$  in the last 40–50 years (King and Harangozo 1998). The third climate model produced by the Hadley Centre predicts a  $2^\circ\text{C}$  global increase in seawater temperatures, albeit with large regional variation and confidence intervals associated

with the estimate. At present there are no data indicating that any significant warming of the shallow Southern Oceans has occurred, although there is evidence that mid-depth Antarctic sea temperatures are increasing at a rate twice that seen globally at the same water depth, and that Weddell Sea Deep Waters have warmed by  $0.32^\circ\text{C}$  since 1972 (Gille 2002; Roberston et al. 2002). Some of the fastest environmental and ecological change on record has come from polar freshwater habitats. The mean winter water temperature of lakes on Signy Island (Maritime Antarctic) increased by  $0.9^\circ\text{C}$  between 1980 and 1995, 3–4 times faster than global mean temperature increases (Quayle et al. 2002). As a result, permanent lake-ice cover has reduced by 45% and winter chlorophyll *a* concentrations have doubled (Quayle et al. 2002, 2003).

Regional temperature increases have also been implicated in changes in Antarctic seabird populations, while increased colonisation of previously bare terrestrial habitats has occurred, along with a rapid expansion in extent and numbers of both of the only two higher plant species present on the continent and their associated invertebrates (Fowbert and Smith 1994; Croxall et al. 2002; Walther et al. 2002; Convey 2003). Terrestrial communities already experience large seasonal and annual temperature variations and the biota present is highly eurythermal (Convey et al. 2003). Terrestrial species are, therefore, more likely to be able to tolerate regional increases in air temperature without reaching their critical limits, as predicted global temperature increases are small when compared to the thermal ranges many species already experience (Convey 2003; L.S. Peck, unpublished work). A secondary effect of climate warming in some parts of the Antarctic will be the localised retreat of some glaciers and permanent ice (Smith 1990). In turn, these newly exposed areas will offer opportunities for colonisation of pristine habitats and a novel chance to study genome function and evolution in pioneer species. Because of the vast differences between conditions in newly exposed sites at very high latitude and those in the maritime Antarctic, new colonisation can take from a few months to 5–10 years.

In contrast, Antarctic marine organisms have evolved through several millennia of possibly the most thermally stable conditions on Earth, and are highly stenothermal. In the high Antarctic, marine organisms are exposed to some of the narrowest thermal ranges in the world. At McMurdo Sound, winter seawater temperatures are  $-1.9\text{ K}$  with a small warming in summer (Hunt et al. 2003), while at the more northerly Rothera Research Station on the Antarctic Peninsula, water temperatures only vary by 2–3 K throughout the year (Littlepage 1965; Clarke and Leakey 1996; Grange et al. 2004) (Fig. 1). Such cold conditions have existed around Antarctica for at least 10 million years, and possibly for 15–25 million years (Clarke and Crame 1992). The most stenothermal Southern Ocean species die in experiments at

temperatures above 5°C and have long-term physiological temperature limits of only +2°C (Peck 1989; Pörtner et al. 1999) and are therefore extremely vulnerable to any rapid increase in seawater temperatures (Pörtner et al. 1999; Bailey 2000). Vital functions, such as reburying in the clam *Laternula elliptica*, righting behaviour in the limpet *Nacella concinna* and swimming in the scallop *Adamussium colbecki*, are lost at temperatures between 1 and 4°C, well below the apparent upper lethal temperature limit of these species (Peck et al. 2002, 2004). Evidence suggests that Antarctic marine ectotherms are living close to the limits of their metabolic scopes, and this markedly restricts their ability to cope with elevated temperatures (Peck 2002b; Pörtner 2002). If water temperatures are increased, even by a few degrees, haemolymph oxygen concentrations decrease and anaerobic end products accumulate (Brockington 2001a; Pörtner et al. 1999). When facing environmental change, affected organisms have four options (Clarke 1996; Peck et al. 2004):

- migrate;
- adapt or evolve physiological mechanisms to cope with elevated temperatures;
- cope with the temperature increase within their existing physiological capabilities;
- die.

In the event of predicted water-temperature increases, all of these mechanisms will probably occur to some degree. However, there are limited options for survival:

- Antarctic marine organisms have restricted physiological capacities to cope with change;
- they are predominantly long-lived and exhibit deferred maturity (Peck 2002a, b);
- unlike the other continents, there are no long coastlines spanning a wide range of environmental conditions in Antarctica that allow migration away from deteriorating conditions. These factors appear more severe and restrictive in Antarctic near-shore marine sites than for faunas elsewhere on Earth.

It is in this area of stress analysis (described in the two sections above) that molecular biology can significantly impact on our understanding of physiological processes and responses to stress. So far, there has been no significant work in this area in Antarctic species; however, transcriptome studies will provide a unique ability to detect indications of thermal stress in organisms significantly earlier than would be possible with physiological or other methods. Microarrays are a powerful tool for transcriptome analysis and already have a proven utility in the dissection of stress responses. Gene profiling of hypoxia in the goby fish *Gillichthys mirabilis* (Gracey et al. 2001) and acclimation to constant temperatures and fluctuating daily temperatures in the killifish *Austrofundulus limnaeus* (Podrabsky and Somero 2004) have provided valuable insights into the

gene networks activated in such circumstances. They can also provide an overview of the different tissue-specific responses (Gracey et al. 2001), which provide additional data to explain the physiological observations. Interestingly from the Antarctic point of view, is the finding that different transcriptional responses were observed in response to acclimation to constant temperatures (chronic response) and response to daily temperature fluctuations (acute response) in the killifish (Podrabsky and Somero 2004). These two particular examples also prove that comprehensive gene identification can be successfully accomplished in non-model organisms.

Understanding the molecular mechanisms underlying how a range of Antarctic organisms respond to increased environmental temperature will enable predictions to be made as to how they and other species will adapt to global climate change, in terms of physiological function, distribution patterns and ecosystem balance. This provides a critical link between genomic and population genetic approaches in understanding evolution and adaptation, true eco- or environmental-genomics. Additionally, they will provide an early understanding of these processes that can then be applied to other regions of the world, which may be complex to study and/or are subject to other human influences such as pollution. With the present disparity in climate change in different regions of the Antarctic, an ideal opportunity exists to evaluate genomic responses in related populations of organisms both affected and unaffected by climate warming. Temperate congeners of Antarctic species can then be used to place the responses found in Antarctic species in a global context.

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## Concluding remarks

The application of genomic and functional genomic approaches to the examination of Antarctic organisms is still in its infancy, but there are already tantalising glimpses of the promise for the future, the results of which will not be restricted to Antarctic science (Table 2). The coming years will see an increase in the production of Antarctic genomic resources and sequence data, which will greatly facilitate such studies, providing information across the whole range of biological levels from DNA to ecosystem, linking genomics to allied scientific disciplines such as physiology, microbiology and ecology. It should be realised that genomics, transcriptomics, proteomics, metabolomics etc. will not overpower what are seen as the traditional science disciplines. They are tools to be used to increase our power of understanding our environment, providing added value and extending the range of hypotheses, which can be examined. To be meaningful, sequence data must be placed in a biological context, multidisciplinary approaches are essential, and nowhere is this more strongly emphasised than in the field of Antarctic biology.

Table 2

**Main areas in which genomics will impact on Antarctic biology**

Improved taxonomic classifications via genetic barcodes  
 Improved resolution to evolutionary studies  
 Molecular characterisation of Antarctic species, particularly designated model organisms and description of particular traits/responses across species  
 Identification of genes responding to environmental change  
 Identification of physiological and biochemical transitions with varying environmental conditions  
 Characterisation of suites of genes important in adaptation to extreme environments

**Main areas in which Antarctic genomics will impact on global science**

Psychrophilic bio-mining: identification of commercially important novel compounds  
 Molecular dissection of stress response networks: Antarctic species as biomarkers for global climate change  
 Low environmental complexity will enable efficient linking of genes to ecosystems  
 Provision of model organisms, such as icefish

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