ANNUAL REVIEWS

Annual Review of Animal Biosciences

Adaptations and Diversity of Antarctic Fishes: A Genomic Perspective

Jacob M. Daane and H. William Detrich III

Department of Marine and Environmental Sciences, Northeastern University Marine Science Center, Nahant, Massachusetts, USA; email: w.detrich@northeastern.edu

Annu. Rev. Anim. Biosci. 2022. 10:39–62

First published as a Review in Advance on November 8, 2021

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

https://doi.org/10.1146/annurev-animal-081221-064325

Keywords

Antarctic notothenioid fishes, adaptive radiation, Southern Ocean, genome evolution, comparative genomics, extreme environments

Abstract

Antarctic notothenioid fishes are the classic example of vertebrate adaptive radiation in a marine environment. Notothenioids diversified from a single common ancestor ~22 Mya to between 120 and 140 species today, and they represent ~90% of fish biomass on the continental shelf of Antarctica. As they diversified in the cold Southern Ocean, notothenioids evolved numerous traits, including osteopenia, anemia, cardiomegaly, dyslipidemia, and aglomerular kidneys, that are beneficial or tolerated in their environment but are pathological in humans. Thus, notothenioids are models for understanding adaptive radiations, physiological and biochemical adaptations to extreme environments, and genetic mechanisms of human disease. Since 2014, 16 notothenioid genomes have been published, which enable a first-pass holistic analysis of the notothenioid radiation and the genetic underpinnings of novel notothenioid traits. Here, we review the notothenioid radiation from a genomic perspective and integrate our insights with recent observations from other fish radiations.

INTRODUCTION

Life in a Freezing Ocean

Adaptive radiation: rapid diversification from a single species to multiple descendant lineages

Benthic: environment at or near the seafloor

Key innovation: trait that facilitates novel interactions between a species and its environment

Pelagic: environment within the water column (between the seafloor and surface)

Swim bladder: gas-filled organ that allows fish to regulate buoyancy

The adaptive radiation of Antarctic notothenioid fishes (order Perciformes; suborder Notothenioidei), which like most teleost fishes are ectotherms, began after the Southern Ocean (SO) had cooled to near-freezing temperatures. Circumscribed by the Antarctic Circumpolar Current, the SO is thermally isolated from waters to the north, and temperatures south of the Antarctic Polar Front range between −2 and +2°C year-round. The low temperatures, strong currents, geographical isolation, and abyssal depths that characterize the SO have led to high degrees of species endemism that are similar to fish radiations in lakes (1).

The SO ecosystem is highly dynamic, with consequences for understanding the evolutionary history of its inhabitants. The SO is split into multiple ecologically distinct habitats, including the ice-free zone, seasonal pack-ice zone, and High-Antarctic zone (2). Antarctic marine ecosystems are reshaped by cycles of glacial expansion and retreat. Icebergs and glacial flow continually scour and rework the seafloor of the continental shelf, such that benthic communities are frequently in states of recolonization and recovery (3). At glacial maxima, many species retreat to sub-Antarctic island refugia and then recolonize high-latitude waters following glacial retreat (4). Furthermore, the currents in the SO form multiple gyres, filaments, jets, eddies, and subfronts that appear, disappear, and rearrange over time (5). These ephemeral physical barriers result in complex genetic histories and speciation patterns.

Adaptive Radiation—the Southern Ocean Notothenioids

Adaptive radiation, defined as the evolution of a large number of related species within a geographical area over a short time period (6), is thought to be an important mechanism underlying the generation of biodiversity (7). Central to adaptive radiation is ecological opportunity, whereby founding populations exploit a new adaptive zone, diverge genetically into separate populations, and ultimately speciate (8). The major triggers of adaptive radiation involve a combination of expansion into new habitats, loss of antagonists, and/or evolution of a key innovation that unlocks access to novel niches (8).

Approximately 35 Mya, the SO was temperate and hosted a cosmopolitan fish fauna (9), but most fish species became locally extinct over time as the SO cooled. Marine teleosts have higher freezing points (approximately −0.7°C) than natural seawater (−1.86°C); contact with environmental ice nucleates ice crystals within their cells, which rapidly propagate throughout the body and cause catastrophic tissue destruction. A key innovation that enabled the notothenioid radiation was the evolution of genes that encode antifreeze glycoproteins (AFGPs). AFGPs bind to nascent ice crystals and prevent their further growth (10). This innovation provided access to the SO, but the majority of extant notothenioid biodiversity originated well after the origin of AFGPs, indicating other drivers of adaptive radiation in this clade (11).

Adaptive radiations typically follow a characteristic progression (12). Species first diverge in habitat utilization, followed by trophic adaptation, and finally by behavioral differences in communication and reproduction. The two main morphological axes of notothenioid habitat partitioning are variation in body size [ranging from <10 cm to >200 cm in length (13)] and in body density [ranging from 0% to 7% relative weight in seawater (%*B*) (14)]. Notothenioids are found at depths ranging from tide pools to >2,900 m and include pelagic, semipelagic, demersal, and benthic biotopes (14, 15). Although the most recent common ancestor of notothenioids lacked a swim bladder, notothenioids diversified throughout the water column via buoyancy adaptations based on reductions in skeletal density and increases in corporeal lipid content (14). Though an early burst in trophic-associated morphological change was detected in notothenioids (16), an

important distinction between the notothenioid radiation and the radiations of other fishes is the absence in notothenioids of large disparities in dentition and diet that relate to fine-scale partitioning of trophic resources (17). However, the notothenioids demonstrate extensive variation in behavioral, mating, and reproductive biology, which suggests that the clade is at an advanced stage of adaptive radiation (18).

The 140 recognized species of Notothenioidei are classified into 8 families (19). Three sub-Antarctic families are distributed along coastal South America, Australia, New Zealand, and sub-Antarctic islands, including the thornfishes (Bovichtidae; 9 species) and 2 monotypic families: Pseudaphritidae (*Pseudaphritis urvillii*) and Eleginopsidae (*Eleginops maclovinus*). The adaptive radiation of Antarctic notothenioids (Cryonotothenioidea) is classified into 5 families: notothens (Nototheniidae; 49 species), spiny plunderfishes (Harpagiferidae; 11 species), barbeled plunderfishes (Artedidraconidae; 36 species), Antarctic dragonfishes (Bathydraconidae; 16 species), and icefishes (Channichthyidae; 16 species). Notably, although cryonotothenioids are defined by the adaptive radiation within the Antarctic SO, several cryonotothenioid lineages have returned and readapted to sub-Antarctic, cool-temperate waters.

Polar ichthyologists are now devoting considerable effort to understanding the genomic features that fueled the adaptive radiation of SO notothenioids. We describe recent work in notothenioid genetics and genomics that addresses this problem. Furthermore, we compare and contrast trends in notothenioid evolution with the adaptive radiations of cichlids (order Cichliformes), for which there is an extensive genomic literature.

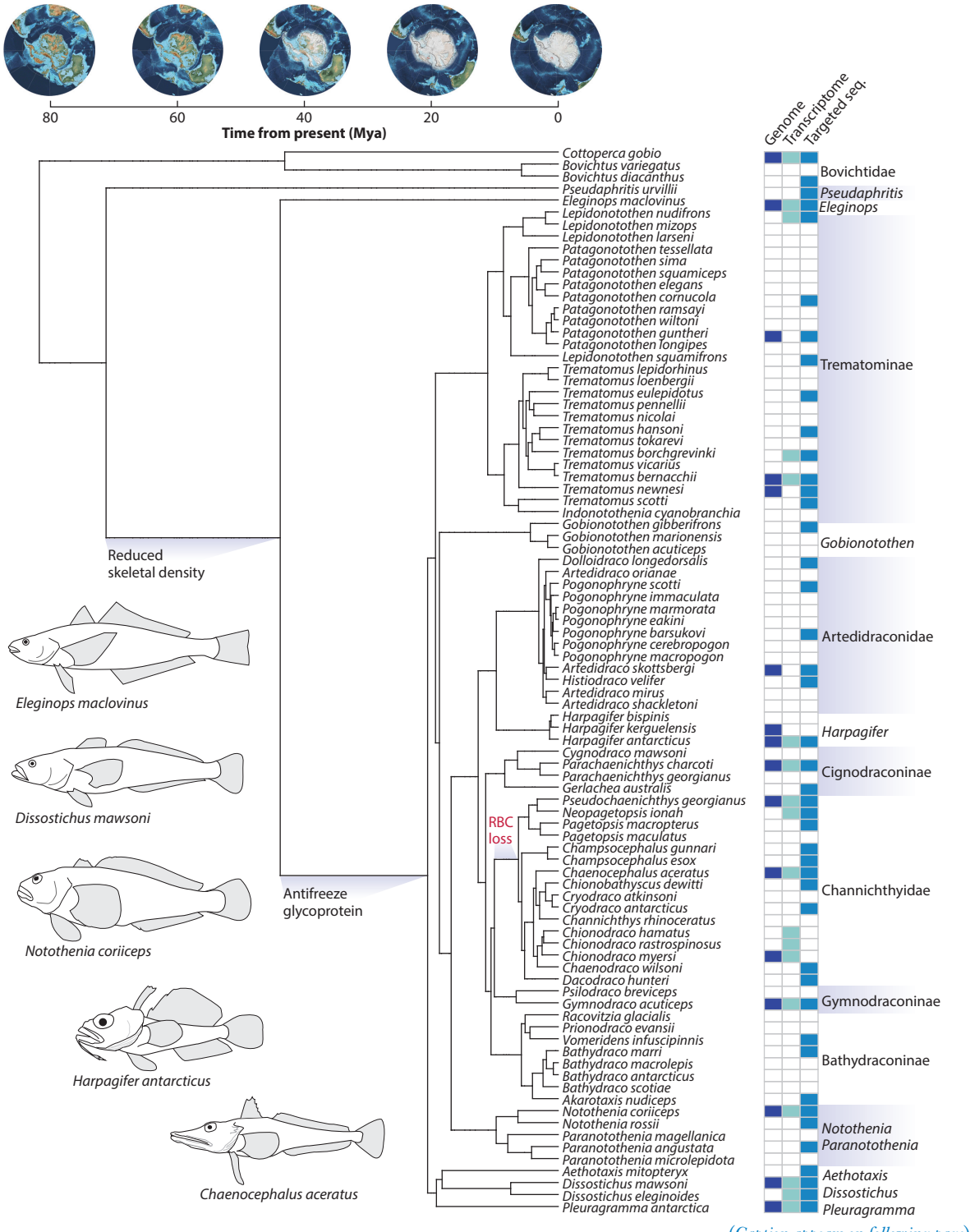
Genome, Exome, Transcriptome, and Regulatory Element Sequencing of Notothenioids

Since 2014, sequencing of notothenioid genomes has expanded rapidly (**Figure 1**). Whole-genome sequences have now been reported for the Antarctic bullhead notothen (*Notothenia coriiceps*) (20); two icefish species [Myer's icefish (*Chionodraco myersi*) (21) and the blackfin icefish (*Chaenocephalus aceratus*) (22)]; Charcot's dragonfish (*Parachaenichthys charcoti*) (23); the commercially important Antarctic toothfish (*Dissostichus mawsoni*) (24, 25); a keystone Antarctic silverfish (*Pleuragramma antarctica*) (26); the dusky rockcod (*Trematomus newnesi*); the yellowfin notothen (*Patagonotothen guntheri*); and some ancestral, non-Antarctic clades [the Patagonian blenny (robalo; *E. maclovinus*) (24) and the channel bull blenny (*Cottoperca gobio*) (27)]. Furthermore, targeted sequencing of highly conserved noncoding elements (CNEs) and protein-coding exons from 44 notothenioids and from 2 outgroup perciforms, including the sister taxon to the notothenioids, *Percophis brasiliensis* (28), has enabled phylogenomic analyses of functionally annotated genetic regions across the radiation (29). Finally, transcriptomic resources are available for several species, tissues, and experimental treatments (**Figure 1** and references therein). Considering that sequencing of many more species is underway, notothenioid genomics is poised to make numerous ecological, evolutionary, and biomedical discoveries.

GENOMIC EVOLUTION IN ANTARCTIC NOTOTHENIOIDS—CONTRIBUTIONS TO THE ADAPTIVE RADIATION AND THE ORIGIN OF KEY TRAITS

Chromosomal Variation and Karyotype Evolution

Structural variation in chromosomes, including inversions, translocations, and large deletions or insertions (indels), can promote adaptive radiation. Chromosomal variants are more likely than other genomic lesions to produce large-effect phenotypes, which could generate ecologically



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

The adaptive radiation of Antarctic notothenioid fishes. Time-calibrated phylogeny adapted from Daane et al. (29) and Dornburg et al. (4). Shown are 89 out of ∼140 extant species. Available genomic resources (whole-genome sequencing, transcriptome sequencing, targeted sequence enrichment) are indicated. Specific branches are highlighted to note the timing of the evolution of reduced skeletal density, the origin of antifreeze glycoprotein, and the loss of red blood cells (RBCs). Global paleogeography models created using PALEOMAP for Gplates (152). Targeted sequencing resources from Daane et al. (29). Citations for genome and transcriptome resources: *Eleginops maclovinus* (24, 71, 153), *Dissostichus mawsoni* (24, 25, 59, 154), *Dissostichus eleginoides* (155), *Pleuragramma antarctica* (26, 156), *Patagonotothen guntheri* (26), *Lepidonotothen nudifrons* (157), *Trematomus bernacchii* (78, 158), *Trematomus borchgrevinki* (71), *Trematomus newnesi* (26), *Notothenia coriiceps* (20, 156, 159), *Harpagifer kerguelensis* (26), *Harpagifer antarcticus* (160), *Artedidraco skottsbergi* (26), *Gymnodraco acuticeps* (78, 154), *Parachaenichthys charcoti* (23, 160), *Neopagetopsis ionah* (160), *Pseudochaenichthys georgianus* (160), *Chaenocephalus aceratus* (22, 156), *Chionodraco myersi* (21), *Chionodraco hamatus* (78, 161, 162), and *Chionodraco rastrospinosus* (71).

relevant variation for natural and sexual selection (30). Furthermore, chromosomal inversions are known to suppress recombination, thereby protecting adaptive loci from the homogenizing effects of gene flow and maintaining regions of high genetic divergence between populations (30). Finally, chromosomal variation causes errors in segregation during meiosis and disrupts epistatic genetic interactions, ultimately establishing postzygotic mating barriers (30).

Substantial alterations to chromosome number and structure have occurred in the notothenioid adaptive radiation (**Figure 2*a***). The plesiomorphic notothenioid karyotype consists of 48 (2*n*) acrocentric chromosomes (31, 32). Ancestral, sub-Antarctic notothenioids, such as *C. gobio*, *Bovichtus variegatus*, *P. urvillii*, and *E. maclovinus*, have haploid chromosome numbers ($1n = 24$) similar to those of other perciform fishes. Within the genus *Trematomus*, multiple chromosomal fusions and fissions have generated haploid chromosome numbers between 12 and 29 (33) (**Figure 2*a***). Pairwise Robertsonian chromosomal fusions in the genus *Notothenia* reduced haploid chromosome numbers from 24 to 11–13 (34) (**Figure 2*a***). Despite extensive chromosome rearrangements, total genome sizes were conserved in the genera *Trematomus* and *Notothenia* (**Figure 2*b***) compared to in other cryonotothenioids (35).

Notothenioids are also noteworthy with respect to sex chromosomes: Approximately 26% of high-latitude notothenioid species possess heteromorphic sex chromosome pairs, compared to ∼4% of teleost species (36) (**Figure 2*a***). Sex-related chromosomes appear to be absent in sub-Antarctic notothenioids but have evolved independently on several occasions in the high-latitude Antarctic lineages (36). The role of sex chromosome changes in the adaptive radiation of Antarctic notothenioids has yet to be determined.

What species-related and/or environmental drivers facilitated the major karyotype variation in high-latitude notothenioids? Transposable elements (TEs) are prime candidates. TE genes often mobilize in response to thermal and/or oxidative stressors in the environment (37). Members of the *DIRS1* retrotransposon family are found in centromeric and pericentromeric regions of the chromosomes in *Trematomus* species and potentially fueled the observed chromosomal fusions (35). Interspecific hybridization, which is frequently associated with adaptive radiations and species flocks, can activate transposons and induce chromosomal rearrangements (38). Furthermore, the acrocentric chromosomes of notothenioids may be more amenable to chromosomal fusions, as an ancestral metacentric chromosome remains unfused in three of the four *Notothenia* and *Paranotothenia* species investigated (34).

Karyotype change in the cichlid adaptive radiation provides an informative counter-comparison to the notothenioids. Cichlid karyotypes contain a mix of acrocentrics and metacentrics (39), but variation in chromosome number among cichlids is low: Haploid chromosome numbers range between 20 and 23 in East African cichlids and average 24 in South American species (39, 40). Intrachromosomal rearrangements in cichlids are rare (41). The stability of cichlid karyotypes may underlie the group's ability to hybridize and introgress over large evolutionary

Gene flow: transfer of genetic material between populations or species

Acrocentric chromosome: chromosome in which the centromere is at or near one end

Robertsonian translocation: fusion between two acrocentric chromosomes

Transposable element (TE): DNA sequence capable of changing position within the genome

Metacentric chromosome: chromosome with a central centromere and arms of roughly equal length

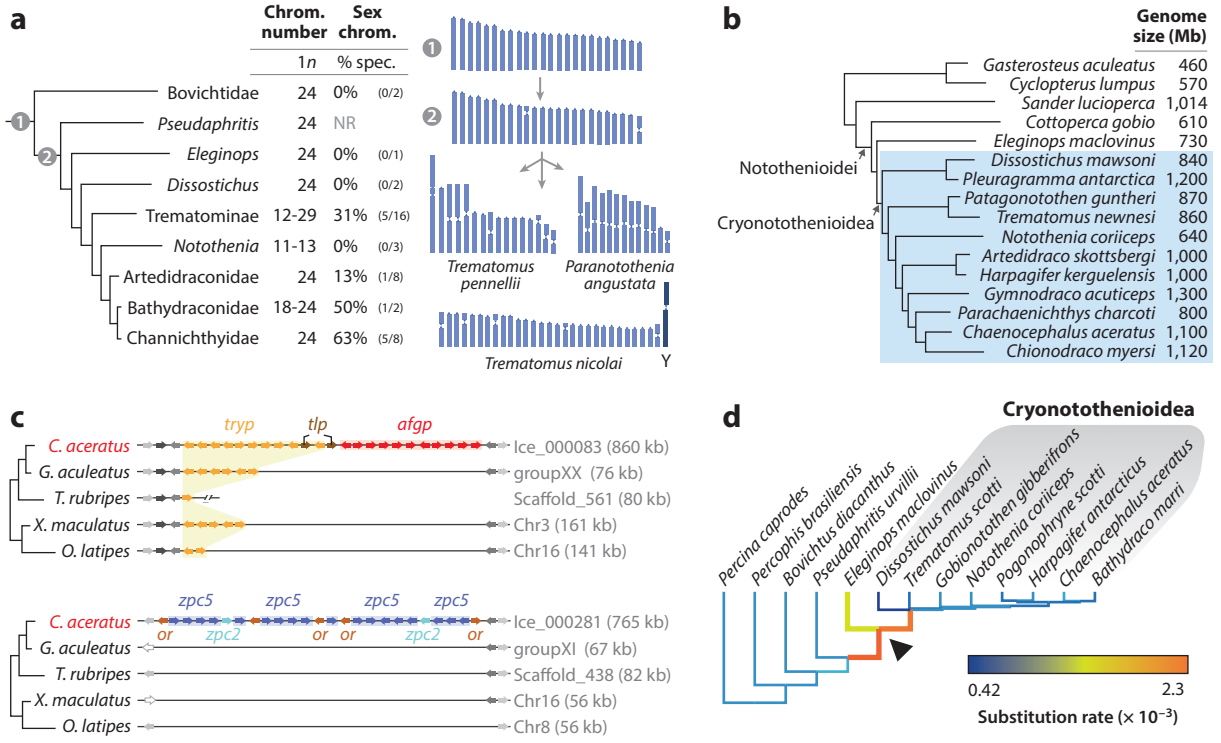


Figure 2

Prominent genomic features of the notothenioid adaptive radiation. (*a*) Karyotype evolution in notothenioids. Independent, large-scale chromosomal fusion and fission events and the emergence of heteromorphic sex chromosomes in Antarctic notothenioids. Karyotype data adapted from Amores et al. (34) and Auvinet et al. (33). Sex chromosome data reviewed in Ghigliotti et al. (36). (*b*) Genome size data from Baalsrud et al. (26), Chen et al. (24), Shin et al. (20), Kim et al. (22), Bista et al. (27), and Bargelloni et al. (21). (*c*) Gene family expansion and neofunctionalization underlying antifreeze protection, including antifreeze glycoprotein (*afgp*) and zona pellucida proteins (*zpc5*, *zpc3*). Diagram modified from Kim et al. (22). (*d*) Transient elevation in substitution rate prior to notothenioid adaptive radiation. Modified from Daane et al. (29).

distances (42), which is thought to be a major factor contributing to cichlid adaptive radiation (43). Although karyotypes are generally conserved, indels >5 bp are positively correlated with species richness across 100 cichlid genomes from multiple lake radiations (44). Whether indels correspond to species richness in notothenioids remains be investigated.

Subfunctionalization: a process in which duplicated genes evolve such that the functions of the ancestral gene are subdivided between them

Neofunctionalization: process in which a duplicated copy of a gene evolves a new function

Whole-Genome and Gene Family Expansions

Whole-genome and gene family duplications provide raw material for selection (45). Provided that one member of each gene pair continues to perform the ancestral function of the single copy gene, the other copy can evolve new functions or expression patterns that enable the emergence of ecologically relevant variation. Furthermore, divergent resolution of paralogs, including subfunctionalization, neofunctionalization, and evolved epistatic interactions, generates genetic incompatibilities in diverging populations that promote reproductive isolation (30).

Genome size has increased markedly in the cryonotothenioids during their adaptive radiation, but there are, as yet, no examples of whole-genome duplication (**Figure 2*b***). Genome sizes

among non-notothenioid perciforms range from 0.58 to 1.30 pg (~560–1,200 Mb), whereas those for notothenioids are 0.98–1.83 pg (~960–1,800 Mb) (46). Notothenioid genome expansion results in part from large increases in TEs and other non-protein-coding DNA. The total number of protein-coding genes is similar across notothenioid species, although disparities in genome completeness and annotation methods render comparisons somewhat fraught. When adjusted for assembly and annotation artifacts, notothenioid gene counts range from 19,585 in *D. mawsoni* to 26,805 in *C. myersi* (21).

Multiple gene families have expanded in notothenioids. Comparison of the genome of the sister taxon to the cryonotothenioids, *E. maclovinus* (Patagonian blenny), to the Antarctic toothfish, *D. mawsoni*, revealed >200 gene families that had expanded in cryonotothenioids (24). The genome of the blackfin icefish, *C. aceratus*, has 373 significantly expanded and 346 significantly contracted gene families compared to a background distribution of gene family copy numbers from 13 teleost species (22). As predicted for gene expansion/subfunctionalization, expression of the duplicated genes of *C. myersi* is upregulated and shows greater tissue specificity with respect to single-copy genes (21).

Many duplicated gene families in notothenioids are related to the unique physiological stresses of life in the SO, including freeze avoidance and protection from reactive oxygen species (ROS). The AFGP gene family, which adsorbs to nascent ice crystals and inhibits further crystal growth, is the key innovation that enabled the Antarctic notothenioids to survive and thrive in the icy SO. AFGPs arose through duplication of an ancestral pancreatic trypsinogen-like gene followed by neofunctionalization and expansion (47) (**Figure 2*c***). The genome of the icefish *C. aceratus* contains 10 tandem copies of the trypsinogen gene, 2 copies of trypsinogen-like protease genes, and 11 copies of the AFGP gene (22). Notothenioid lineages that have secondarily returned to cool-temperate sub-Antarctic environments, such as *P. guntheri*, have lost AFGP entirely (48). Owing to the diversity in AFGP gene copy number, the number of AFGP-encoding units per polyprotein gene, and proteolytic processing of the AFGP polyproteins, the serum AFGPs of notothenioids are a heterogeneous mix of isoforms of differing sizes (2.6–33 KDa). The antifreeze activity of AFGPs is built upon a repetitive tripeptide, Ala(Pro)-Ala-Thr, the threonine in which is subject to glycosylation (10). Isoforms vary in the extent of these tripeptide repeats.

Notothenioid embryos and hatchlings are not protected by AFGPs (49). Rather, Antarctic notothenioids evolved embryonic freeze avoidance through expansion and neofunctionalization of the zona pellucida (ZP) gene family (50) (**Figure 2*c***). Compared to the ZP family genes of many temperate teleosts [e.g., medaka (*Oryzias latipes*) and the temperate notothenioids *B. variegatus* and *E. maclovinus*], which possess single copies of 11 distinct genes, cryonotothenioid ZP gene families possess an average of 70 genes (50). The *C. aceratus* genome contains 131 ZP genes, of which 109 were created by tandem duplications (22). In *D. mawsoni* eggs, transcription of the two most abundantly duplicated ZP genes generates a combined 53% of all expressed mRNA transcripts (50). Some of the ZP genes have neofunctionalized to provide freeze protection through evolved ice melting–promoting activity that lowers both the freezing and the melting points of a solution (50).

ROS, a major cellular threat to notothenioids in their oxygen-rich environment, damage nucleic acids, proteins, and lipids (51). Protection against excessive ROS and maintenance of redox balance is accomplished by antioxidant defenses, which include ROS-degrading enzymes. Genes involved in detoxification of ROS have expanded in cryonotothenioid genomes, as illustrated by the icefish *C. aceratus*, whose genome contains 33 copies of NAD(P)H:quinone acceptor oxidoreductase (*nqo1*), 2 copies of 8-oxoguanine DNA glycosylase (*ogg1*), and 3 tandem copies of superoxide dismutase 3 (*sod3*) compared to 2–10, 1, and 1–2, respectively, in other fish genomes (22).

Genetic incompatibilities: negative interactions between parental alleles that result in decreases in fitness or sterility

Reproductive isolation: inability of two individuals to breed successfully

Reactive oxygen species (ROS): molecules containing oxygen with unpaired electrons, which can cause damage to polynucleotides, proteins, lipids, and other cellular materials

Selenoproteins also protect against ROS (52). The *D. mawsoni* genome contains 84 selenocysteine transfer RNA genes versus one in *E. maclovinus*, and selenoprotein gene expression in *D. mawsoni* is increased relative to that in *E. maclovinus* (24).

Many adaptive radiations have occurred in taxa that have small genome sizes, which indicates that large genome sizes are not required, a priori, for these evolutionary events (8). Cichlid genomes are approximately the same size as those of other teleosts, and no association is apparent between genome size and species richness in cichlids (42, 53). However, gene family expansion is observed frequently among cichlids (54), as with notothenioids. The common ancestor of the East African cichlid radiations had 4.5–6-fold higher rates of gene duplication compared to other teleosts, and many of the duplicated genes (>20%) exhibit expression patterns that differ from the ancestral gene (55). In contrast, gene duplications in the cichlids of Lake Tanganyika are not correlated with species richness (54). With conflicting support for correlations between gene duplication and species diversification, understanding the contribution of gene duplication to adaptive radiation will require the identification and analysis of specific key traits that are facilitated by copy number variation.

Selfish Genes and Notothenioid Genome Expansion: Transposable Elements Run Rampant as the Environment Changes

Why are the genomes of notothenioid lineages larger than those of their non-Antarctic relatives (**Figure 2*b***)? Propagation of selfish DNAs, such as TEs, may explain these genome expansions. TE mobilization spurs genome change and innovation through generation of novel genes, introns, and exons and by alteration of gene expression patterns (56). TE activation also facilitates speciation by causing large-scale genomic variation that creates interspecific genetic incompatibilities (57). Larger TE copy numbers in mammalian genomes are correlated with increased speciation rates (58) (although many TEs may be pseudogenized and therefore inactive). Thus, TEs can create genetic variation that serves as substrate for adaptive radiation (56).

Compared to non-Antarctic perciforms, Antarctic notothenioids have greatly amplified TE copy numbers. Seventeen retrotransposon families, including 13 LINEs (long interspersed nuclear elements), show 8- to 300-fold expansion with respect to sub-Antarctic notothenioids (59). The TE contents of *N. coriiceps* (23.5% of genome) and of *D. mawsoni* (21.4%) are twice that of the cool-temperate notothenioid *E. maclovinus* (10.0%) (24), whereas the genome of the icefish *C. aceratus* has a TE content of 47.4%, fourfold that of cool-temperate perciforms (22). We suggest that TE expansion in notothenioids was a major driver of lineage diversification because it produced larger genomes and may have facilitated novel gene combinations, duplications and losses, and altered gene regulatory networks.

The burst of LINE expansion in notothenioid genomes has been dated to ~6.5 Mya and was probably induced by SO cooling (24). Thus, the Antarctic toothfish, *D. mawsoni*, possesses twice as many LINEs as the cool-temperate *E. maclovinus* (24). Cold-induced mobilization and expansion of the *D. mawsoni* LINE *dmL1* have been demonstrated by transfection experiments in human cells (*HeLa*), zebrafish cells (ZF4), and zebrafish embryos (60). Significantly, cells transfected with *dmL1* had higher survival rates and reduced ROS levels compared to those of control cells (60). Cold-induced mobilization requires p38, a sensor of redox state, which suggests that *dmL1* transposition is activated by elevated ROS (60). These intriguing results argue for further study of mobile genetic elements as drivers of notothenioid trait diversification.

TEs constitute ~35% of cichlid genomes (41), enhance cichlid genetic diversity, and generate trait adaptations when they move. A Lake Malawi cichlid, *Maylandia zebra*, harbors 30% more recent TE insertions (sequence divergence <2%) and has more TE insertions within 15 kb of

genes (1,422 versus 338) than a riverine cichlid, *Oreochromis niloticus* (41). Furthermore, insertion of TEs within 20 kb of 5′ or 3′ untranslated regions (UTRs) correlates with increased gene expression in cichlids. A recent analysis of Lake Tanganyika cichlids concluded that species richness is not correlated with overall TE content (54). However, TE activity is associated with the evolution of specific traits in cichlids, such as enhancement of egg-spot pigmentation patterning and regulation of differential expression of *opsin* genes (61, 62).

Heat shock response: transient increase in chaperone expression in reaction to cellular stress to rescue denatured proteins

Gene Regulation in Notothenioids: *Cis*-Acting Elements and Noncoding RNAs

Changes in gene expression, mediated by *cis*-regulatory variation in proximal promoters and distal enhancers or by the action of noncoding RNAs, are key drivers of adaptation because they fine tune complex genetic networks without impacting protein function directly (63, 64). Mutations in protein-coding sequences, by contrast, often have pleiotropic effects given that the encoded proteins are expressed widely, which makes them blunt, but nonetheless useful, instruments of evolution.

Analysis of *cis*-regulatory evolution in notothenioids has to date been focused on the loss of erythrocytes and the heat shock response. As Antarctic notothenioids diversified in the oxygen-rich SO, they became less reliant on hemoglobin and erythrocytes. At the extreme of this trend, the 16 species of Antarctic icefishes (Channichthyidae) lost both the LA and MN *hemoglobin* (*hb*) clusters of teleost fishes and no longer produce erythrocytes (22, 65). Lau et al. (66) compared the intergenic regions in the LA *α-globin/β-globin hb* gene complex and found that dragonfish intergenic regions are typically shorter (1.5–3.8 kb) than those of notothens (2.9–4.3 kb). Nevertheless, notothen and dragonfish intergenic regions share a conserved, 90-nucleotide element previously shown to be required for *hb* gene transcription (67), and measurements of cellular *α-globin* and *β-globin* messenger RNA (mRNA) levels from one notothen and three dragonfishes showed that their intergenic regulatory regions were equivalent in transcriptional efficacy, and thus other *cis*-regulatory regions are likely involved in reduced globin concentration and hematocrit.

Further analysis of *cis*-regulatory control of erythrocyte production has focused on CNEs, which are defined by high degrees of conservation across teleosts. Many CNEs likely function as *cis*-regulatory enhancer or promoter elements (68). CNE sequences next to genes that are associated with human anemia are enriched for accelerated sequence evolution in the icefish common ancestor. This pattern of accelerated sequence evolution in CNEs is pronounced near genes expressed at high levels late in erythrocyte maturation, but not those CNEs near genes that are expressed earlier in erythrocyte differentiation (69). Remarkably, this pattern of mutation in CNEs is consistent with the unexpected presence of erythroid progenitor cells (normoblasts) within the erythropoietic marrow of icefishes, suggesting relaxed selection on the putative *cis*-regulatory regions governing patterns of gene expression during terminal differentiation (69).

Proteins denature not only at elevated temperatures but also in response to cold stress (70). In their constantly cold environment, notothenioids have lost the ability to express an inducible heat shock response. Rather, they express heat shock proteins constitutively, most likely to rescue proteins denatured by low-temperature stress (71). Hsf1 is a highly conserved master transcription factor that orchestrates the classical heat shock response (72). A recent survey of *cis*-regulatory Hsf1 binding sites in Antarctic notothenioids found that approximately 90% were under accelerated sequence evolution (73), but the functional importance of this relaxed selection remains contentious (74).

Given that notothenioids constitutively express chaperones and lack the classical heat shock response, this suborder provides an intriguing natural laboratory for exploring the interplay of heat shock proteins and adaptive evolution. By stabilizing variant protein forms, chaperones can

modify the penetrance of encoded variation and act as capacitors for evolution (75). Chaperones also suppress transposon activity (76) and thus are able to directly influence de novo mutational processes.

microRNA (miRNA): a small single-stranded RNA (~22 nucleotides) involved in post-transcriptional regulation of gene expression

Among the myriad noncoding RNAs, microRNAs (miRNAs) are perhaps the best understood. miRNAs can regulate patterns of gene expression through translational repression and/or degradation of target mRNAs. Noncoding RNAs are abundant in eukaryotic genomes, but their roles in adaptive radiation are poorly resolved at this time.

The genomes of a cryonotothenioid, the Antarctic toothfish (*D. mawsoni*), and a sub-Antarctic notothenioid, the Patagonian blenny (*E. maclovinus*), contain comparable numbers of annotated noncoding RNA genes: 2,434 and 2,185, respectively (24). Annotated miRNA gene numbers of high- and low-latitude notothenioid species are also similar: Compare *C. aceratus* (290) and *D. mawsoni* (295) to *E. maclovinus* (286) (22, 24). We suggest that expansion of noncoding RNA genes has not been an important driver of the notothenioid adaptive radiation, but further research is clearly warranted. Qualitative changes, such as evolution of novel noncoding RNAs or repurposing of existing ones, are possibilities that we discuss next using miRNAs as an example.

miRNAs have been implicated in notothenioid trait evolution. Desvignes et al. (77) reported that 17 erythropoietic miRNAs are conserved in two white-blooded icefishes (*C. aceratus* and the hooknose icefish, *Chionodraco hamatus*), with one exception of secondary genetic loss in the latter species. Xu et al. (78) found that three miRNA suppressors of erythropoiesis, *mir16b*, *mir152*, and *mir1388*, are expressed at elevated levels in the pronephric kidney of *C. hamatus* compared to those in the red-blooded *Trematomus bernacchii*. Furthermore, overexpression of all three of these miRNAs individually in the zebrafish each results in a partial (50–78%) reduction of erythrocyte production in embryos (78). Therefore, post-transcriptional modulation of erythroid gene expression by miRNAs may contribute to suppression of erythrocyte production in icefishes.

Although cryonotothenioids lack a classical Hsp70 heat shock response, they do exhibit widespread changes to the transcriptome and activation of cellular stress response pathways in reaction to thermal stress (79). Up to 12 miRNAs show differential expression in gills during thermal stress in *T. bernacchii*, and up to 11 of these miRNAs have targets within the FOXO signaling cascade that is central to cellular stress response (80).

Comparison of five cichlids and four non-cichlid teleosts revealed that cichlid mRNAs have significantly longer 3′ UTRs that contain more miRNA binding sites and are evolving at faster rates (81). The number of highly conserved miRNAs in cichlid genomes, approximately 270, is similar to that in notothenioids (55), although lineage-specific miRNAs increase the total to ~400 in some species (81). Polymorphisms within miRNA binding sites show higher allele frequencies and greater genetic differentiation between lineages of Lake Malawi cichlids, suggesting that miRNA-based post-transcriptional regulation is a key facilitator of population diversification (82). Convergent changes in miRNA expression patterns were observed in independent radiations of Midas cichlids from two Nicaraguan crater lakes, which may be involved in the benthic/limnetic diversification of these species (83).

There have been few studies of epigenetics in notothenioids. Intriguingly, polar fishes have strikingly higher levels of DNA methylation compared to temperate and tropical fishes, such that there is a clear negative correlation between body temperature and methylation level (84). Antarctic icefishes possess the highest levels of genome-wide methylation in a comparison of 74 fish species (84).

Additional investigations are needed into gene-regulatory mechanisms in adaptative radiation. The availability of notothenioid and cichlid genomes provides opportunities to study the contributions of *cis*-regulatory elements, epigenetic modifications, miRNAs, and other noncoding RNAs to adaptive radiations, and we anticipate major progress on this subject in the near future.

EVOLUTIONARY MECHANISMS UNDERLYING THE NOTOTHENIOID RADIATION

A Mutation Rate Roller Coaster in Antarctic Notothenioids

Mutation rates are dynamic, depend on the efficiency of genetically encoded DNA repair mechanisms, vary between individuals and species, and are a selectable trait (85). Rates of de novo mutation alone, however, are considered to be too slow to establish the genetic variation and population differentiation required for adaptive radiations (43, 86). Lake Malawi cichlids, for example, have a mutation rate that is among the lowest recorded for any vertebrate (87). Furthermore, many of the loci that underlie key traits in the cichlid and stickleback radiations predate the specific environments in which the adaptive radiation took place (88, 89), indicating that ancestral polymorphism and standing variation, rather than de novo mutations, are more likely to facilitate rapid diversification. Whereas higher mutation rates are correlated with elevated species diversification rates in birds (90), such an association is controversial in mammals (91, 92). Thus, the link between mutation rate and species diversity remains unclear and likely varies on a taxon-specific basis.

Recently, an ancestral, genome-wide mutation rate shift was detected in notothenioids that occurred prior to the onset of polar conditions in the SO and subsequently returned back to baseline during the cryonotothenioid adaptive radiation (29) (**Figure 2*d***). This rate shift occurred prior to the split between cryonotothenioids and the non-Antarctic notothenioid *E. maclovinus*. The biotic and environmental contexts surrounding this mutational spike in ancestral notothenioids remain open questions. Nonetheless, the patterns of mutation in reconstructed ancestral sequences support an enrichment for diversifying selection in genes whose orthologs in humans are associated with skeletal diseases (29) (**Figure 3*c***). This enrichment corresponds with the timing of the evolution of reduced skeletal density in notothenioids (29, 93). Thus, the most recent common ancestor of the cryonotothenioids probably evolved reduced skeletonization concurrent with rapid genome evolution, and both traits may have primed notothenioids to undergo adaptive radiation following Antarctic glaciation.

Genetic Diversity, Heterozygosity, and Standing Variation

Demographic bottlenecks are predicted to deplete nucleotide diversity, shift allele frequencies, create linkage disequilibrium between distant loci, and alter additivity of variance, all of which can greatly influence adaptive radiation (8). Thus, adaptive radiations are thought to require the generation of a large initial pool of genetic variation to combat the loss of genetic variation over time and to provide the raw substrate for selection.

Given the age of the notothen radiation (>10 Mya), extant variation may not reflect the conditions during periods of rapid diversification. Comparison of the *D. mawsoni* and *E. maclovinus* genomes shows comparable densities of heterozygous single-nucleotide polymorphisms (SNPs) per kilobase (2.58 and 2.40, respectively). In contrast, elevated heterozygosity was detected in the genome of the icefish *C. aceratus* (8.79 SNPs/kb) (22). Targeted sequencing of pooled population samples of 44 notothenioids found heterozygosity to vary between 0.4 and 3.9 SNPs/kb, with no clear trend in heterozygosity between the cryonotothenioids, basally branching sub-Antarctic notothenioids, the notothenioid sister group *P. brasiliensis*, or the distant outgroup *Percina caprodes* (29). Although there is a higher genetic diversity reported for pelagic compared to benthic notothenioids (94, 95), nucleotide diversity (π) is generally quite variable between species, subpopulations, and sampling approaches. Notably, as with heterozygosity, populations of *E. maclovinus* have similar nucleotide diversity ($\pi = 0.0039$) compared to that in cryonotothenioids ($\pi = 0.0009$–0.0046) (94, 96–99).

Bottleneck: a reduction in population size that results in a loss of genetic diversity

Nucleotide diversity (π): the average number of nucleotide differences per site between all pairs in a population

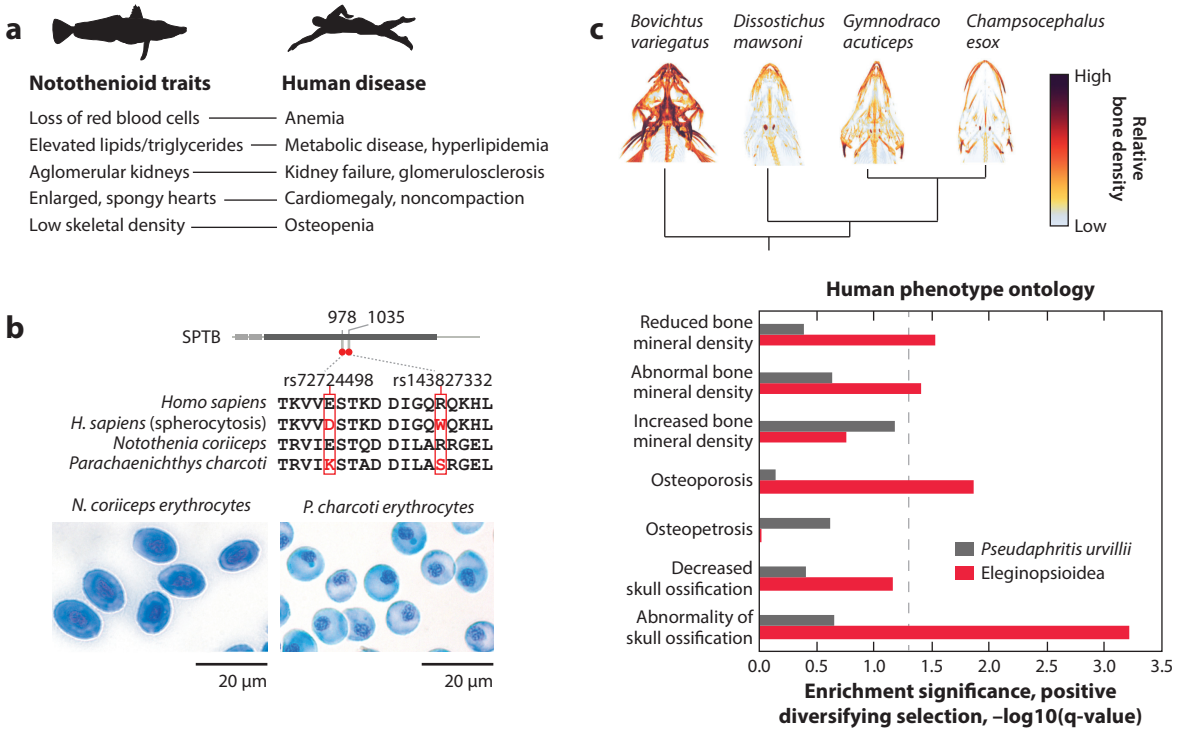


Figure 3

Notothenioids as genetic models for human disease. (*a*) Notothenioid traits that mirror human pathologies. (*b*) Mutations in the *β-spectrin* (*SPTB*) gene in human patients can lead to hereditary spherocytosis. Sites of human patient variation are also mutated in the dragonfish *Parachaenichthys charcoti*, which also has spherocytic erythrocytes. Data adapted from Daane et al. (69). (*c*, *top*) Skeletal density heatmaps showing reduction in skeletal density in notothenioids. (*Bottom*) Enrichment for positive diversifying selection across genes known to cause human skeletal diseases in species with low skeletal density (Eleginopsioidea) compared to a dense sister lineage (*Pseudaphritis urvillii*). Data adapted from Daane et al. (29).

A recent analysis of Lake Tanganyika cichlids found an association between species richness and SNP heterozygosity (54). However, radiating cichlids show low global nucleotide diversity compared to nonradiating vertebrates, and this nucleotide diversity is concentrated in specific regions of the genome (40, 100). The low levels of nucleotide diversity (π) and low nucleotide divergences (d_{xy}) between species and tribes are thought to be due to population bottlenecks, gene flow, low mutation rates, and the youth of cichlid clades (40).

Gene Flow and Hybridization: Universal Features of Adaptive Radiation?

Genetic diversification and reproductive isolation in the presence of the homogenizing effects of gene flow create a paradox that has long complicated our understanding of adaptive radiation. However, admixture of genes, particularly between more distantly related populations, can establish novel allelic combinations [i.e., the combinatorial mechanism (43)] that increase overall heritable genetic diversity in ecologically relevant traits. Adaptive alleles derived through gene flow have a temporal advantage, whereby multiple and ecologically tested epistatic interactions combine through gene flow, an outcome that is unlikely to evolve de novo in multiple lineages during a rapid radiation. Numerous adaptive alleles across multiple fish radiations appear to

predate the environment (e.g., lakes) in which they are found, which indicates that they originated through ancestral standing variation and gene flow (43). Hybridization can also directly influence mutation. In whitefishes, TE expansion is activated in response to hybridization, possibly owing to mismatch between host suppression mechanisms in hybrid genetic backgrounds (101). This can lead to structural variation that can further facilitate adaptation and reproductive isolation (38).

Population demography of Antarctic fishes is shaped by the dynamic oceanographic features of the SO. At glacial maxima, Antarctic fishes are forced into sub-Antarctic refugia, but following ice retreat these species can rapidly expand into newly exposed niches (4, 95). Given the hypothesis that hybridization between distantly related lineages is central to avoiding the homogenizing effects of gene flow (43), these interglacial cycles iteratively isolate and then reestablish contact between populations and species that could provide opportunities for a combinatorial mechanism of adaptive admixture.

Population genetic structure in cryonotothenioids appears to vary on a species-by-species basis (94, 99, 102–106). Notothenioids have long larval stages, up to 6–12 months after hatching (107), during which strong currents can lead to passive dispersal over large circumpolar ranges and spatial genetic homogeneity (108–110). Nonetheless, many species do exhibit spatial genetic structure (111), which likely reflects differences in notothenioid life history strategies.

Gene flow between species has been observed across three species of the icefish genus *Chionodraco*, with 14–36% of sampled individuals from each species having a mixed genetic background and 11–14% of the genomes of these individuals being hybrid in origin (112). These gene flow events are thought to have occurred in two distinct interglacial periods, supporting the hypothesis of secondary contact during range expansion following ice retreat (112). Marino et al. (112) also described two putative F1 hybrids with 50% hybrid genetic background, which indicates ongoing gene flow within this clade. Therefore, postzygotic barriers to gene flow across larger evolutionary divergences (between genera) may be weak or absent, at least for species with compatible chromosome numbers. Desvignes et al. (113) performed an in vitro fertilization experiment using sperm from an ocellated icefish, *Chionodraco rastrospinosus*, and eggs from a blackfin icefish, *C. aceratus*. The resulting embryos developed over 4.5 months, and the hybrid larvae were viable for two weeks posthatching, despite an evolutionary divergence of 4–8 My (4, 113). Unfortunately, termination of the experiment at the close of the field season prevented further observation, and the long generation times of these species precluded analysis of potential F2 hybrid breakdown. Further study of the extent and consequences of gene flow in notothenioids is needed.

Gene flow is a feature in many fish radiations, including clownfish (114), pupfish (115), and whitefish (101). In cichlids, hybridization between two distantly related riverine lineages is thought to have occurred at the base of the Lake Victoria radiation (89), and hybrids can still be formed between distantly related fishes from the Lake Malawi and Lake Victoria radiations (116) or between the different Nicaraguan crater lake radiations (117). Cichlid radiations are characterized by extensive incomplete lineage sorting, widespread hybridization, low genetic diversity, and significant shared genetic variation among divergent morphs. Analysis of 2,543 local phylogenetic trees for Lake Malawi cichlids gave 2,542 different topologies, and 82% of heterozygous SNPs were found in at least one other species (87).

Hybridization is thought to be critical in the generation of cichlid adaptive variation. Among Lake Victoria cichlids, long-wavelength-sensitive opsin haplotypes that are adaptive at different depths and involved in female mate choice were generated through hybridization (89). Intriguingly, there is increased phenotypic novelty with increasing genetic distance in cichlid hybrids, both in the lab (118) and in the wild (119). The effect of hybridization is context dependent. In one example, hybrids of ecologically distinct species performed worse at feeding on parental food types but did better with novel food types (120). Similarly, F2 hybrids from two Lake Malawi

generalist species were able to specialize in feeding on shifting sand, a context for which neither parental species was adapted (121).

Less Is More? Adaptive Evolution Through Loss

Although loss-of-function mutations are often assumed to be deleterious, many such variants are selectively neutral, or even adaptive, in specific contexts (122–124). Adaptive phenotypes isolated in experimental evolution studies are frequently caused by loss-of-function mutations (123). Gene loss may initially result in decreased fitness, but fitness often recovers quickly, and may be enhanced, via compensatory mutations in the genome (125). In the context of adaptive radiation, gene losses often generate large-effect phenotypes, "hopeful monsters" that occupy new niches in which the lack of competition buys time for genetic compensation (123, 126).

Numerous loss-of-function mutations have been found in notothenioids, but determining whether they are adaptive losses or result from relaxed selection that follows trait loss is difficult. The best-studied cases involve erythropoietic genes in icefishes: (*a*) loss of *hemoglobin* genes (*hb*) and erythrocytes in the most recent common ancestor of the Antarctic icefishes and (*b*) independent losses of *myoglobin* (*mb*) expression by several icefish lineages (65). Daane et al. (69) and Bilyk et al. (127) reported that several erythrocyte-associated genes of icefishes are predicted loss-of-function or truncating alleles, including *hemogen* [encodes an erythroid transcription factor (128)], *haptoglobin* [encodes a hemoglobin scavenging protein], *rhd* [encodes the blood-type D antigen], and *alas2* [encodes erythrocyte-specific ALA-synthase]. Other classical erythroid protein-coding sequences have been maintained through pleiotropy (69). Despite coding-sequence conservation, the regulatory regions flanking many erythroid genes show extensive and accelerated sequence evolution (69), consistent with loss of erythroid regulatory function and maintenance of nonerythroid pleiotropy. The adaptive value of erythrocyte loss is unclear, as the energetic benefits from not producing and circulating erythrocytes may be outweighed by compensatory physiological adaptations in the cardiovascular system (129), but one hypothesis is that that iron scarcity in the SO may be a driving force in globin loss (130).

Three-spine sticklebacks provide another example of loss-of-function mutations causing key traits in fish radiations. The repeated loss of pelvic spines in freshwater populations is thought to be an adaptation for protection against invertebrate predators. Loss of pelvic spines in independent stickleback lineages is caused by recurrent deletions within a *pitx1* enhancer (131). This enhancer is enriched for TG dinucleotides, which can induce Z-DNA tertiary structures that are prone to double-stranded breaks with error-prone repair. This inherent DNA fragility ultimately leads to the high frequency of adaptive deletions at this locus (131). Investigations into mechanisms driving deletions, including underappreciated variables such as DNA structure, chromatin packing, and transposon activity, will likely generate insights into mechanisms and adaptability of species.

ADAPTIVE RADIATIONS—IS THERE A COMMON GENOMIC SIGNATURE?

The genomic features that have facilitated the explosive diversification of cichlids have been explored in numerous studies, yet no obvious relationships exist between species richness and transposon numbers, gene family expansions, genome size, chromosome rearrangements, or the number of genes under selection (40, 44, 53, 54). Rather, species richness does correlate with individual heterozygosity (54) and with the frequency of large indels (53). Furthermore, hybridization plays a central, ubiquitous role in the adaptive radiations of species flocks (40). Although the species richness of notothenioids has not yet been examined with respect to these parameters, the rapidly increasing numbers of genomes for this clade promise many opportunities for future discoveries.

From the perspective of selection, there are often many potential genetic routes that lead to the same phenotype, rendering problematic (but not hopeless) searches for universal genomic features of adaptive radiation. For example, horizontal stripe formation in East African cichlids evolved convergently and is associated with the *argp2* locus (132). Among Lake Malawi cichlids, de novo mutations upstream of the 5′ UTR of *argp2* cause stripe formation (132). Among Lake Victoria cichlids, by contrast, the horizontal stripe locus resides in two intronic *cis*-regulatory regions that predate the radiation and are also found in riverine populations (132). Therefore, both new variation (Lake Malawi) and ancient/standing variation (Lake Victoria) underlie this convergent trait (132). We propose that adaptive radiations are more profitably considered by focusing on the interactions between the genome, population genetics, and development rather than on the proximate, and often variable, genetic lesions.

Evolvability: the potential to generate heritable phenotypic variation

Determining the evolutionary mechanisms underlying phenotypes is essential to understand why some lineages undergo adaptive radiation and others do not. Developmental constraint plays a key role in shaping the evolvability and phenotypic potential of a species, which in turn has implications for our understanding of adaptive radiation (133). Phenotypic plasticity is also important, particularly with respect to the flexible stem hypothesis, which suggests that a generalist founding population is more likely to undergo adaptive radiation (134). Cichlid pharyngeal jaw morphology and dentition are highly plastic and most strongly reflect diet (135–137), consistent with a flexible stem common ancestor in cichlids (138). Although there is some evidence for trophic plasticity in notothenioid fishes (139, 140), trait variance and the underlying genetic and environmental causes are not well understood for the group.

The genetic architecture behind adaptive traits is also important to consider as we refine our mechanistic understanding of adaptive radiation in notothenioids. Owing to gene flow between sympatric populations, traits caused by small numbers of large-effect loci may not be important drivers of speciation (141). For example, dark coloration and hypertrophied lips in cichlids are controlled by large-effect loci and are associated with ecological performance and assortative mating, but otherwise little genome-wide differentiation and few postzygotic genetic barriers exist (141). Rather, key polygenic traits in cichlids, such as body shape and pharyngeal jaw morphology, are likely to play a disproportionate role in driving speciation by enabling the stable accumulation of allelic combinations that ultimately increase genomic differentiation (141). Little is known about the genetic structure of key traits in notothenioids, and the large evolutionary distances between species will prove challenging.

As notothenioid genomics advances beyond the initial wave of genome characterization, many important questions remain. What developmental and genetic mechanisms enabled the emergence of key traits in some lineages but not in others? How much genetically encoded phenotypic plasticity remains available to the notothenioids, which have long experienced a cold, thermally stable, and oxygen-rich environment? What are the roles of epigenetics in notothenioid trait evolution? How does species diversity derive from a limited initial gene pool? How do genetics influence the tempo of diversification? What is the balance between selection, contingency, and neutral processes? Concerted study of the connections between notothenioid genomes and the evolutionary and ecological drivers of species diversification in the SO will greatly advance our understanding of this remarkable clade and how it came to be.

PARALLEL ADAPTIVE RADIATIONS—THE SOUTHERN OCEAN AND BEYOND

We have compared and contrasted genomic and genetic studies of the notothenioid adaptive radiation with those of the cichlids, largely owing to the extensive genetic literature for the latter. One

may ask whether there are more apt taxa for comparison. Zoarcidae (eelpouts, order Perciformes) and Liparidae (snailfishes, order Scorpaeniformes) are abundant in the SO (9), and they diversified under the same environmental transitions the notothenioids experienced. However, whether they constitute adaptive radiations is unclear, in part because they are understudied. Genomic sequencing of Antarctic zoarcids and liparids and suitable outgroups is a logical next step toward improving our understanding of the evolution and adaptation of the SO fish fauna.

The cottid sculpins of Lake Baikal (order Scorpaeniformes) provide a second, intriguing parallel to the notothenioid adaptive radiation (1). Lake Baikal is the world's deepest lake (1,600 m), yet is oxygenated at depth, unlike most other deep-lake environments. As a result, the Baikal sculpins are notable for evolving traits typically seen only in deep-marine lineages. These include retinal photoreceptors composed solely of rods (142); free neuromasts (143); and, in some lineages, specializations for living near abyssal hydrothermal vents and methane cold seeps (144). There are 33 recognized species of Baikal sculpins (145), and, like the notothenioids, they dominate fish biomass (70–80%) and species diversity (57%) in their environment (145). Furthermore, the most recent common ancestor of the group was benthic and lacked a swim bladder, and the main axis of sculpin diversification was into the water column through a reduction in skeletal density and an increase in corporeal lipids (145). Given these parallels to the notothenioid adaptive radiation, genetic and genomic studies of the Lake Baikal sculpins may be particularly informative in revealing universal genetic mechanisms of adaptive radiation and the evolution of key traits.

COMPARATIVE GENOMICS AND TRANSLATION TO MEDICINE

Forward Genomics and Evolutionary Models of Disease—There and Back Again

Elucidation of genotype–phenotype relationships remains a fundamental challenge in genetics. Many genetic association studies in humans identify polymorphisms that have low predicted effect, are found disproportionately in noncoding regions, and/or lack experimental validation, which makes it difficult to link these loci to specific developmental mechanisms (146). One strategy to filter and analyze disease-associated loci is to screen for trait/locus overlaps between patients and evolutionary models.

Notothenioids have evolved a suite of traits that, although adaptive or tolerated in the context of the SO, would be considered pathological in humans (**Figure 3*a***). These disease-like phenotypes include the complete loss of red blood cells (profound anemia) in the icefishes, which is compensated for by enlarged hearts (cardiomegaly) with trabeculated myocardia, increases in total blood volume, and hyperbranching of the vasculature (129). Furthermore, notothenioid lineages that synthesize antifreeze have aglomerular kidneys (a model for kidney disease), which are thought to be an adaptation to prevent loss of AFGPs in the urine (10). Notothenioid buoyancy adaptations are associated with reduced bone mass (osteopenia) and increased corporeal lipids (analogous to obesity) (93) (**Figure 3*c***). Major changes in mitochondrial density and in muscle fiber density are observed in some notothenioid groups (147). Thus, the genomics of adaptive notothenioid traits are poised to facilitate forward genomic interventions in mammalian diseases through identification of shared, conserved genetic regions (148).

Conversely, human population and disease genetics can inform adaptive evolution because granular information about genotype–phenotype variation is often available, in contrast to the paucity of such data for most fish species. Numerous loci in mammals have been statistically associated with a wide spectrum of trait variation; mining these data can reveal candidate genes for natural phenotypes. For example, β-Spectrin is a cytoskeletal protein that is required to maintain the typical ellipsoid shape of red cells, and many human patients with hereditary spherocytic anemia have mutations in the gene (149). Some, but not all, Antarctic dragonfishes have

spherical erythrocytes, and these species share with humans conserved sites of pathological variation in their *β-spectrin* genes (69) (**Figure 3*b***). Thus, patterns of genetic variation in human diseases can be used to predict phenotypes and to guide investigations of natural populations.

As the erythrocytes of dragonfishes illustrate, many interesting traits have been reported in only a handful of Antarctic species, which undermines the power of phylogenomic comparisons. Major efforts will be required to catalog physiological and morphological variation across the spectrum of Antarctic (largely SO) organisms so that expanding genomic resources can be usefully applied. A concerted effort to catalog and digitize phenotypes (including trait measurements already in the literature), as is underway for mammals (150), would promote comparative phylogenomics and contribute to identification of important genotype–phenotype relationships.

ZEBRAFISH AND MODEL ORGANISMS IN ANTARCTIC FISH RESEARCH

Unlike the robust cichlids, which can be maintained easily in laboratory aquaria, many cryonotothenioid species are easily stressed by minor changes in, for example, water temperature, oxygen levels, or salinity. Furthermore, the long generation times of notothenioids make them unsuitable for genetic manipulation, whether via mutagenesis or targeted gene intervention, which clearly hinders efforts to identify genetic mechanisms of trait evolution and adaptive radiation. Fortunately, many of the genes of notothenioids have orthologs in well-established and genetically manipulable laboratory model organisms, such as the zebrafish. Several recent notothenioid studies have used the zebrafish to explore the fundamental biology of notothenioids, from erythrocyte (78, 128, 151) and skeletal (29) development to transposon mobilization (60) and egg freezing protection (50). The combination of comparative phylogenomics and experimental genetics provides powerful and synergistic tools to study trait evolution and adaptive radiation in fishes.

SUMMARY

From a genomic perspective, the notothenioid adaptive radiation is characterized by frequent chromosomal fusions, large-scale TE mobilization, and gene family expansions and contractions that are functionally associated with the physiological stresses of life at subzero temperatures. Unstable karyotypes and dramatic changes to mutation rate in notothenioids distinguish this clade from genetic trends observed in other fish radiations. As observed in other fish species flocks, gene flow may also play an important role in the genetic history of the notothenioids. Future research on the genetic architecture and developmental mechanisms of key traits in notothenioids will help to synthesize the principles and mechanisms of adaptive radiation.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by an American Heart Association Postdoctoral Fellowship (17POST33660801) to J.M.D. and by a US National Science Foundation grant (OPP-1955368) to H.W.D. This is contribution No. 420 from the Marine Science Center at Northeastern University.

LITERATURE CITED

1. Eastman JT, Clarke A. 1998. A comparison of adaptive radiations of Antarctic fish with those of non-Antarctic fish. In *Fishes of Antarctica: A Biological Overview*, ed. G di Prisco, E Pisano, M Clark, pp. 3–26. Milan, Italy: Springer Milan
2. Kock K-H. 1992. *Antarctic Fish and Fisheries*. Cambridge, UK: Cambridge Univ. Press
3. Barnes DKA, Conlan KE. 2007. Disturbance, colonization and development of Antarctic benthic communities. *Philos. Trans. R. Soc. B* 362:11–38
4. Dornburg A, Federman S, Lamb AD, Jones CD, Near TJ. 2017. Cradles and museums of Antarctic teleost biodiversity. *Nat. Ecol. Evol.* 1:1379–84
5. Chapman CC, Lea MA, Meyer A, Sallée JB, Hindell M. 2020. Defining Southern Ocean fronts and their influence on biological and physical processes in a changing climate. *Nat. Clim. Change* 10:209–19
6. Simões M, Breitkreuz L, Alvarado M, Baca S, Cooper JC, et al. 2016. The evolving theory of evolutionary radiations. *Trends Ecol. Evol.* 31:27–34
7. Wiens JJ. 2017. What explains patterns of biodiversity across the Tree of Life? *BioEssays* 39:1600128
8. Naciri Y, Linder HP. 2020. The genetics of evolutionary radiations. *Biol. Rev.* 95:1055–72
9. Eastman JT. 2005. The nature of the diversity of Antarctic fishes. *Polar Biol*. 28:93–107
10. DeVries AL. 2020. Fish antifreeze proteins. In *Antifreeze Proteins*, Vol. 1: *Environment, Systematics and Evolution*, ed. H Ramløv, D Friis, pp. 85–129. Cham, Switz.: Springer
11. Near TJ, Dornburg A, Kuhn KL, Eastman JT, Pennington JN, et al. 2012. Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *PNAS* 109:3434–39
12. Gavrilets S, Losos JB. 2009. Adaptive radiation: contrasting theory with data. *Science* 323:732–37
13. Eastman JT. 2019. An analysis of maximum body size and designation of size categories for notothenioid fishes. *Polar Biol*. 42:1131–45
14. Eastman JT. 2020. The buoyancy-based biotope axis of the evolutionary radiation of Antarctic cryonotothenioid fishes. *Polar Biol*. 43:1217–31
15. Eastman JT. 2017. Bathymetric distributions of notothenioid fishes. *Polar Biol*. 40:2077–95
16. Colombo M, Damerau M, Hanel R, Salzburger W, Matschiner M. 2015. Diversity and disparity through time in the adaptive radiation of Antarctic notothenioid fishes. *J. Evol. Biol.* 28:376–94
17. Fryer G, Iles TD. 1972. *The Cichlid Fishes of the Great Lakes of Africa*. London: Oliver & Boyd
18. La Mesa M, Llompart F, Riginella E, Eastman JT. 2021. Parental care and reproductive strategies in notothenioid fishes. *Fish Fish*. 22:356–76
19. Eastman JT, Eakin RR. 2021. Checklist of the species of notothenioid fishes. *Antarct. Sci.* 33:273–80
20. Shin SC, Ahn DH, Kim SJ, Pyo CW, Lee H, et al. 2014. The genome sequence of the Antarctic bullhead notothen reveals evolutionary adaptations to a cold environment. *Genome Biol*. 15:468
21. Bargelloni L, Babbucci M, Ferraresso S, Papetti C, Vitulo N, et al. 2019. Draft genome assembly and transcriptome data of the icefish *Chionodraco myersi* reveal the key role of mitochondria for a life without hemoglobin at subzero temperatures. *Commun. Biol.* 2:443
22. Kim B-M, Amores A, Kang S, Ahn DH, Kim JH, et al. 2019. Antarctic blackfin icefish genome reveals adaptations to extreme environments. *Nat. Ecol. Evol.* 3:469–78
23. Ahn D-H, Shin SC, Kim B-M, Kang S, Kim J-H, et al. 2017. Draft genome of the Antarctic dragonfish, *Parachaenichthys charcoti*. *Gigascience* 6:gix060
24. Chen L, Lu Y, Li W, Ren Y, Yu M, et al. 2019. The genomic basis for colonizing the freezing Southern Ocean revealed by Antarctic toothfish and Patagonian robalo genomes. *Gigascience* 8:giz016
25. Lee SJ, Kim JH, Jo E, Choi E, Kim J, et al. 2021. Chromosomal assembly of the Antarctic toothfish (*Dissostichus mawsoni*) genome using third-generation DNA sequencing and Hi-C technology. *Zool. Res.* 42:124–29
26. Baalsrud HT, Tørresen OK, Solbakken MH, Salzburger W, Hanel R, et al. 2018. De novo gene evolution of antifreeze glycoproteins in codfishes revealed by whole genome sequence data. *Mol. Biol. Evol.* 35:593–606
27. Bista I, McCarthy SA, Wood J, Ning Z, Detrich HW III, et al. 2020. The genome sequence of the channel bull blenny, *Cottoperca gobio* (Günther, 1861). *Wellcome Open Res*. 5:148

28. Near TJ, Dornburg A, Harrington RC, Oliveira C, Pietsch TW, et al. 2015. Identification of the notothenioid sister lineage illuminates the biogeographic history of an Antarctic adaptive radiation. *BMC Evol. Biol.* 15:109
29. Daane JM, Dornburg A, Smits P, MacGuigan DJ, Hawkins MB, et al. 2019. Historical contingency shapes adaptive radiation in Antarctic fishes. *Nat. Ecol. Evol.* 3:1102–9
30. Feulner PGD, De-Kayne R. 2017. Genome evolution, structural rearrangements and speciation. *J. Evol. Biol.* 30:1488–90
31. Mazzei F, Ghigliotti L, Lecointre G, Ozouf-Costaz C, Coutanceau J-P, et al. 2006. Karyotypes of basal lineages in notothenioid fishes: the genus *Bovichtus*. *Polar Biol*. 29:1071–76
32. Pisano E, Ozouf-Costaz C, Hureau J-C, Williams R. 1995. Chromosome differentiation in the subantarctic Bovichtidae species *Cottoperca gobio* (Günther, 1861) and *Pseudaphritis urvillii* (Valenciennes, 1832) (Pisces, Perciformes). *Antarct. Sci.* 7:381–86
33. Auvinet J, Graça P, Dettai A, Amores A, Postlethwait JH, et al. 2020. Multiple independent chromosomal fusions accompanied the radiation of the Antarctic teleost genus *Trematomus* (Notothenioidei:Nototheniidae). *BMC Evol. Biol.* 20:39
34. Amores A, Wilson CA, Allard CAH, Detrich HW III, Postlethwait JH. 2017. Cold fusion: massive karyotype evolution in the Antarctic bullhead notothen *Notothenia coriiceps*. *G3* 7:2195–207
35. Auvinet J, Graça P, Belkadi L, Petit L, Bonnivard E, et al. 2018. Mobilization of retrotransposons as a cause of chromosomal diversification and rapid speciation: the case for the Antarctic teleost genus *Trematomus*. *BMC Genom*. 19:339
36. Ghigliotti L, Cheng CHC, Pisano E. 2016. Sex determination in Antarctic notothenioid fish: chromosomal clues and evolutionary hypotheses. *Polar Biol*. 39:11–22
37. Oliver KR, Greene WK. 2009. Transposable elements: powerful facilitators of evolution. *BioEssays* 31:703–14
38. Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJE, et al. 2013. Hybridization and speciation. *J. Evol. Biol.* 26:229–46
39. Poletto AB, Ferreira IA, Cabral-de-Mello DC, Nakajima RT, Mazzuchelli J, et al. 2010. Chromosome differentiation patterns during cichlid fish evolution. *BMC Genet*. 11:50. Erratum. 2012. *BMC Genet*. 13:2
40. Svardal H, Salzburger W, Malinsky M. 2021. Genetic variation and hybridization in evolutionary radiations of cichlid fishes. *Annu. Rev. Anim. Biosci.* 9:55–79
41. Conte MA, Joshi R, Moore EC, Nandamuri SP, Gammerdinger WJ, et al. 2019. Chromosome-scale assemblies reveal the structural evolution of African cichlid genomes. *Gigascience* 8:giz030
42. Salzburger W. 2018. Understanding explosive diversification through cichlid fish genomics. *Nat. Rev. Genet.* 19:705–17
43. Marques DA, Meier JI, Seehausen O. 2019. A combinatorial view on speciation and adaptive radiation. *Trends Ecol. Evol.* 34:531–44
44. McGee MD, Borstein SR, Meier JI, Marques DA, Mwaiko S, et al. 2020. The ecological and genomic basis of explosive adaptive radiation. *Nature* 586:75–79
45. Ohno S. 1970. *Evolution by Gene Duplication*. New York: Springer-Verlag
46. Detrich HW, Stuart A, Schoenborn M, Parker SK, et al. 2010. Genome enablement of the Notothenioidei: genome size estimates from 11 species and BAC libraries from 2 representative taxa. *J. Exp. Zool. B* 314:369–81
47. Chen L, DeVries A, Cheng C. 1997. Evolution of antifreeze glycoprotein gene from a trypsinogen gene in Antarctic notothenioid fish. *PNAS* 94:3811–16
48. Miya T, Gon O, Mwale M, Cheng C-HC. 2015. Multiple independent reduction or loss of antifreeze trait in low Antarctic and sub-Antarctic notothenioid fishes. *Antarct. Sci.* 28:17–28
49. Cziko PA, Evans CW, Cheng C-HC, DeVries AL. 2006. Freezing resistance of antifreeze-deficient larval Antarctic fish. *J. Exp. Biol.* 209:407–20
50. Cao L, Huang Q, Wu Z, Cao D, Ma Z, et al. 2016. Neofunctionalization of zona pellucida proteins enhances freeze-prevention in the eggs of Antarctic notothenioids. *Nat. Commun.* 7:12987
51. Halliwell B, Gutteridge JMC. 2015. *Free Radicals in Biology and Medicine*. Oxford, UK: Oxford Univ. Press

52. Steinbrenner H, Sies H. 2009. Protection against reactive oxygen species by selenoproteins. *Biochim. Biophys. Acta* 1790:1478–85
53. Xiong P, Hulsey CD, Fruciano C, Wong WY, Nater A, et al. 2020. The comparative genomic landscape of adaptive radiation in crater lake cichlid fishes. *Mol. Ecol.* 30:955–72
54. Ronco F, Matschiner M, Böhne A, Boila A, Büscher HH, et al. 2021. Drivers and dynamics of a massive adaptive radiation in cichlid fishes. *Nature* 589:76–81
55. Brawand D, Wagner CE, Li YI, Malinsky M, Keller I, et al. 2014. The genomic substrate for adaptive radiation in African cichlid fish. *Nature* 513:375–81
56. Schrader L, Schmitz J. 2019. The impact of transposable elements in adaptive evolution. *Mol. Ecol.* 28:1537–49
57. Serrato-Capuchina A, Matute D. 2018. The role of transposable elements in speciation. *Genes* 9:254
58. Ricci M, Peona V, Guichard E, Taccioli C, Boattini A. 2018. Transposable elements activity is positively related to rate of speciation in mammals. *J. Mol. Evol.* 86:303–10
59. Chen Z, Cheng C-HC, Zhang J, Cao L, Chen L, et al. 2008. Transcriptomic and genomic evolution under constant cold in Antarctic notothenioid fish. *PNAS* 105:12944–49
60. Chen S, Yu M, Chu X, Li W, Yin X, Chen L. 2017. Cold-induced retrotransposition of fish LINEs. *J. Genet. Genom.* 44:385–94
61. Santos ME, Braasch I, Boileau N, Meyer BS, Sauteur L, Böhne A, et al. 2014. The evolution of cichlid fish egg-spots is linked with a cis-regulatory change. *Nat. Commun.* 5:5149
62. Carleton KL, Conte MA, Malinsky M, Nandamuri SP, Sandkam BA, Meier JI, et al. 2020. Movement of transposable elements contributes to cichlid diversity. *Mol. Ecol.* 29:4956–69
63. Carroll SB. 2008. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* 134:25–36
64. Heimberg AM, Sempere LF, Moy VN, Donoghue PCJ, Peterson KJ. 2008. MicroRNAs and the advent of vertebrate morphological complexity. *PNAS* 105:2946–50
65. Daane JM, Giordano D, Coppola D, di Prisco G, Detrich HW, Verde C. 2019. Adaptations to environmental change: globin superfamily evolution in Antarctic fishes. *Mar. Genom.* 49:100724
66. Lau Y, Parker SK, Near TJ, Detrich HW III. 2012. Evolution and function of the globin intergenic regulatory regions of the Antarctic dragonfishes (Notothenioidei: Bathydraconidae). *Mol. Biol. Evol.* 29:1071–80
67. Lau DT, Saeed-Kothe A, Parker SK, Detrich HW III. 2001. Adaptive evolution of gene expression in Antarctic fishes: Divergent transcription of the 5′-to-5′ linked adult α1- and β-globin genes of the Antarctic teleost *Notothenia coriiceps* is controlled by dual promoters and intergenic enhancers. *Am. Zool.* 132:113–32
68. Polychronopoulos D, King JWD, Nash AJ, Tan G, Lenhard B. 2017. Conserved non-coding elements: Developmental gene regulation meets genome organization. *Nucleic Acids Res.* 45:12611–24
69. Daane JM, Auvinet J, Stoebenau A, Yergeau D, Harris MP, Detrich HW III. 2020. Developmental constraint shaped genome evolution and erythrocyte loss in Antarctic fishes following paleoclimate change. *PLOS Genet.* 16:e1009173
70. Privalov PL. 1990. Cold denaturation of protein. *Crit. Rev. Biochem. Mol. Biol.* 25:281–306
71. Bilyk KT, Vargas-Chacoff L, Cheng C-HC. 2018. Evolution in chronic cold: varied loss of cellular response to heat in Antarctic notothenioid fish. *BMC Evol. Biol.* 18:143
72. Lindquist S. 1986. The heat-shock response. *Annu. Rev. Biochem.* 55:1151–91
73. Bogan SN, Place SP. 2019. Accelerated evolution at chaperone promoters among Antarctic notothenioid fishes. *BMC Evol. Biol.* 19:205
74. Bilyk KT, Zhuang X, Vargas-Chacoff L, Cheng C-HC. 2021. Evolution of chaperome gene expression and regulatory elements in the Antarctic notothenioid fishes. *Heredity* 126:424–41
75. Sato A. 2018. Chaperones, canalization, and evolution of animal forms. *Int. J. Mol. Sci.* 19:3029
76. Specchia V, Piacentini L, Tritto P, Fanti L, Dalessandro R, et al. 2010. Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. *Nature* 463:662–65
77. Desvignes T, Detrich HW III, Postlethwait JH. 2016. Genomic conservation of erythropoietic microRNAs (erythromiRs) in white-blooded Antarctic icefish. *Mar. Genom.* 30:27–34

78. Xu Q, Cai C, Hu X, Liu Y, Guo Y, et al. 2015. Evolutionary suppression of erythropoiesis via the modulation of TGF-β signalling in an Antarctic icefish. *Mol. Ecol.* 24:4664–78
79. Huth TJ, Place SP. 2016. Transcriptome wide analyses reveal a sustained cellular stress response in the gill tissue of *Trematomus bernacchii* after acclimation to multiple stressors. *BMC Genom.* 17:127
80. Vasadia DJ, Zippay ML, Place SP. 2019. Characterization of thermally sensitive miRNAs reveals a central role of the FoxO signaling pathway in regulating the cellular stress response of an extreme stenotherm, *Trematomus bernacchii*. *Mar. Genom.* 48:100698
81. Xiong P, Schneider RF, Hulsey CD, Meyer A, Franchini P. 2019. Conservation and novelty in the microRNA genomic landscape of hyperdiverse cichlid fishes. *Sci. Rep.* 9:13848
82. Loh YHE, Yi SV, Streelman JT. 2011. Evolution of microRNAs and the diversification of species. *Genome Biol. Evol.* 3:55–65
83. Franchini P, Xiong P, Fruciano C, Schneider RF, Woltering JM, et al. 2019. MicroRNA gene regulation in extremely young and parallel adaptive radiations of crater lake cichlid fish. *Mol. Biol. Evol.* 36:2498–511
84. Varriale A, Bernardi G. 2006. DNA methylation and body temperature in fishes. *Gene* 385:111–21
85. Lynch M, Ackerman MS, Gout J-F, Long H, Sung W, et al. 2016. Genetic drift, selection and the evolution of the mutation rate. *Nat. Rev. Genet.* 17:704–14
86. Hedrick PW. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Mol. Ecol.* 22:4606–18
87. Malinsky M, Svardal H, Tyers AM, Miska EA, Genner MJ, et al. 2018. Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. *Nat. Ecol. Evol.* 2:1940–55
88. Nelson TC, Cresko WA. 2018. Ancient genomic variation underlies repeated ecological adaptation in young stickleback populations. *Evol. Lett.* 2:9–21
89. Meier JI, Marques DA, Mwaiko S, Wagner CE, Excoffier L, Seehausen O. 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nat. Commun.* 8:14363
90. Lanfear R, Ho SYW, Love D, Bromham L. 2010. Mutation rate is linked to diversification in birds. *PNAS* 107:20423–28
91. Goldie X, Lanfear R, Bromham L. 2011. Diversification and the rate of molecular evolution: no evidence of a link in mammals. *BMC Evol. Biol.* 11:286
92. Gillman LN, Keeling DJ, Ross HA, Wright SD. 2009. Latitude, elevation and the tempo of molecular evolution in mammals. *Proc. R. Soc. B* 276:3353–59
93. Eastman JT, Witmer LM, Ridgely RC, Kuhn KL. 2014. Divergence in skeletal mass and bone morphology in antarctic notothenioid fishes. *J. Morphol.* 275:841–61
94. Janko K, Lecointre G, DeVries A, Couloux A, Cruaud C, Marshall C. 2007. Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. *BMC Evol. Biol.* 7:220
95. Kašparová E, Van de Putte AP, Marshall C, Janko K. 2015. Lifestyle and ice: the relationship between ecological specialization and response to Pleistocene climate change. *PLOS ONE* 10:e0138766
96. Ceballos SG, Lessa EP, Victorio MF, Fernández DA. 2012. Phylogeography of the sub-Antarctic notothenioid fish *Eleginops maclovinus*: evidence of population expansion. *Mar. Biol.* 159:499–505
97. Kuhn KL, Near TJ, Jones CD, Eastman JT. 2009. Aspects of the biology and population genetics of the Antarctic nototheniid fish *Trematomus nicolai*. *Copeia* 2009:320–27
98. Damerau M, Salzburger W, Hanel R. 2014. Population genetic structure of *Lepidonotothen larseni* revisited: *cyb* and microsatellites suggest limited connectivity in the Southern Ocean. *Mar. Ecol. Prog. Ser.* 517:251–63
99. Damerau M, Matschiner M, Salzburger W, Hanel R. 2012. Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. *Polar Biol.* 35:1073–86
100. Malinsky M, Challis RJ, Tyers AM, Schiffels S, Terai Y, et al. 2015. Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake. *Science* 350:1493–98
101. Dion-Côté AM, Renaut S, Normandeau E, Bernatchez L. 2014. RNA-seq reveals transcriptomic shock involving transposable elements reactivation in hybrids of young lake whitefish species. *Mol. Biol. Evol.* 31:1188–99

102. Matschiner M, Hanel R, Salzburger W. 2009. Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. *Mol. Ecol.* 18:2574–87
103. Kuhn KL, Gaffney PM. 2006. Preliminary assessment of population structure in the mackerel icefish (*Champsocephalus gunnari*). *Polar Biol.* 29:927–35
104. Damerau M, Matschiner M, Salzburger W, Hanel R. 2014. Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae). *Mol. Ecol.* 23:284–99
105. Van de Putte AP, Janko K, Kasparova E, Maes GE, Rock J, et al. 2012. Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species. *Mar. Genom.* 8:23–34
106. Papetti C, Susana E, Patarnello T, Zane L. 2009. Spatial and temporal boundaries to gene flow between *Chaenocephalus aceratus* populations at South Orkney and South Shetlands. *Mar. Ecol. Prog. Ser.* 376:269–81
107. North AW. 1991. Review of the early life history of Antarctic notothenioid fish. In *Biology of Antarctic Fish*, ed. G di Prisco, B Maresca, B Tota, pp. 70–86. Berlin, Heidelberg: Springer
108. Deli Antoni MY, Delpiani SM, González-Castro M, Blasina GE, Spath MC, et al. 2019. Comparative populational study of *Lepidonotothen larseni* and *L. nudifrons* (Teleostei: Nototheniidae) from the Antarctic Peninsula and the South Shetland Islands, Antarctica. *Polar Biol.* 42:1537–47
109. Jones CD, Anderson ME, Balushkin AV, Duhamel G, Eakin RR, et al. 2008. Diversity, relative abundance, new locality records and population structure of Antarctic demersal fishes from the northern Scotia Arc islands and Bouvetøya. *Polar Biol.* 31:1481–97
110. Miya T, Gon O, Mwale M, Poulin E. 2016. Molecular systematics and taxonomic status of three latitudinally widespread nototheniid (Perciformes: Notothenioidei) fishes from the Southern Ocean. *Zootaxa* 4061:381–96
111. Dornburg A, Federman S, Eytan RI, Near TJ. 2016. Cryptic species diversity in sub-Antarctic islands: a case study of *Lepidonotothen*. *Mol. Phylogenet. Evol.* 104:32–43
112. Marino IAM, Benazzo A, Agostini C, Mezzavilla M, Hoban SM, et al. 2013. Evidence for past and present hybridization in three Antarctic icefish species provides new perspectives on an evolutionary radiation. *Mol. Ecol.* 22:5148–61
113. Desvignes T, Le François NR, Goetz LC, Smith SS, Shusdock KA, et al. 2019. Intergeneric hybrids inform reproductive isolating barriers in the Antarctic icefish radiation. *Sci. Rep.* 9:5989
114. Litsios G, Salamin N. 2014. Hybridisation and diversification in the adaptive radiation of clownfishes. *BMC Evol. Biol.* 14:245
115. Richards EJ, Martin CH. 2017. Adaptive introgression from distant Caribbean islands contributed to the diversification of a microendemic adaptive radiation of trophic specialist pupfishes. *PLOS Genet.* 13:e1006919
116. Stelkens RB, Schmid C, Seehausen O. 2015. Hybrid breakdown in cichlid fish. *PLOS ONE* 10:e0127207
117. Franchini P, Fruciano C, Spreitzer ML, Jones JC, Elmer KR, et al. 2014. Genomic architecture of ecologically divergent body shape in a pair of sympatric crater lake cichlid fishes. *Mol. Ecol.* 23:1828–45
118. Stelkens RB, Schmid C, Selz O, Seehausen O. 2009. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. *BMC Evol. Biol.* 9:283
119. Nichols P, Genner MJ, van Oosterhout C, Smith A, Parsons P, et al. 2015. Secondary contact seeds phenotypic novelty in cichlid fishes. *Proc. R. Soc. B* 282:20142272
120. Selz OM, Seehausen O. 2019. Interspecific hybridization can generate functional novelty in cichlid fish. *Proc. R. Soc. B* 286:20191621
121. Feller AF, Selz OM, McGee MD, Meier JI, Mwaiko S, Seehausen O. 2020. Rapid generation of ecologically relevant behavioral novelty in experimental cichlid hybrids. *Ecol. Evol.* 10:7445–62
122. Monroe JG, McKay JK, Weigel D, Flood PJ. 2021. The population genomics of adaptive loss of function. *Heredity* 126:383–95
123. Murray AW. 2020. Can gene-inactivating mutations lead to evolutionary novelty? *Curr. Biol.* 30:R465–71
124. Albalat R, Cañestro C. 2016. Evolution by gene loss. *Nat. Rev. Genet.* 17:379–91
125. Helsen J, Voordeckers K, Vanderwaeren L, Santermans T, Tsontaki M, et al. 2020. Gene loss predictably drives evolutionary adaptation. *Mol. Biol. Evol.* 37:2989–3002

126. Goldschmidt R. 1940. *The Material Basis of Evolution*. New Haven, CT: Yale Univ. Press
127. Bilyk KT, Zhuang X, Murphy KR, Cheng C-HC. 2019. A tale of two genes: divergent evolutionary fate of haptoglobin and hemopexin in hemoglobinless Antarctic icefishes. *J. Exp. Biol.* 222:jeb188573
128. Peters MJ, Parker SK, Grim J, Allard CAH, Levin J, Detrich HW III. 2018. Divergent *Hemogen* genes of teleosts and mammals share conserved roles in erythropoiesis: analysis using transgenic and mutant zebrafish. *Biol. Open* 7:bio035576
129. Sidell BD, O'Brien KM. 2006. When bad things happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes. *J. Exp. Biol.* 209:1791–802
130. Laptikhovsky V. 2019. White blood of the Antarctic icefish: Why? *Mar. Ecol. Prog. Ser.* 626:227–31
131. Xie KT, Wang G, Thompson AC, Wucherpfennig JI, Reimchen TE, et al. 2019. DNA fragility in the parallel evolution of pelvic reduction in stickleback fish. *Science* 363:81–84
132. Urban S, Nater A, Meyer A, Kratochwil CF. 2021. Different sources of allelic variation drove repeated color pattern divergence in cichlid fishes. *Mol. Biol. Evol.* 38:465–77
133. Brakefield PM. 2011. Evo-devo and accounting for Darwin's endless forms. *Philos. Trans. R. Soc. Lond. B* 366:2069–75
134. Gibert J. 2017. The flexible stem hypothesis: evidence from genetic data. *Dev. Genes Evol.* 227:297–307
135. Greenwood PH. 1965. Environmental effects on the pharyngeal mill of a cichlid fish, *Astatoreochromis alluaudi*, and their taxonomic implications. *Proc. Linn. Soc.* 176:1–10
136. Muschick M, Barluenga M, Salzburger W, Meyer A. 2011. Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol. Biol.* 11:116
137. Gunter HM, Fan S, Xiong F, Franchini P, Fruciano C, Meyer A. 2013. Shaping development through mechanical strain: the transcriptional basis of diet-induced phenotypic plasticity in a cichlid fish. *Mol. Ecol.* 22:4516–31
138. Schneider RF, Meyer A. 2017. How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Mol. Ecol.* 26:330–50
139. Barrera-Oro E, Eastman JT, Moreira E. 2012. Phenotypic plasticity in the Antarctic nototheniid fish *Trematomus newnesi*: a guide to the identification of typical, large mouth and intermediate morphs. *Polar Biol.* 35:1047–56
140. Landaeta MF, Villegas A, Hüne M. 2021. Shape, condition and diet of the pike icefish *Champsocephalus esox* (Teleostei: Channichthyidae): Evidence of phenotypic plasticity? *Antarct. Sci.* 33:10–16
141. Kautt AF, Kratochwil CF, Nater A, Machado-Schiaffino G, Olave M, et al. 2020. Contrasting signatures of genomic divergence during sympatric speciation. *Nature* 588:106–11
142. Pankhurst NW, Sideleva VG, Pankhurst PM, Smirnova O, Janssen J. 1994. Ocular morphology of the Baikal sculpin-oilfishes, *Comephorus baicalensis* and *C. dybowskii* (Comephoridae). *Environ. Biol. Fishes* 39:51–58
143. Sideleva VG. 1996. Comparative character of the deep-water and inshore cottoid fishes endemic to Lake Baikal. *J. Fish Biol.* 49:192–206
144. Sideleva VG. 2016. Communities of the cottoid fish (Cottoidei) in the areas of hydrothermal vents and cold seeps of the abyssal zone of Baikal Lake. *J. Ichthyol.* 56:694–701
145. Goto A, Yokoyama R, Sideleva VG. 2015. Evolutionary diversification in freshwater sculpins (Cottoidea): a review of two major adaptive radiations. *Environ. Biol. Fish.* 98:307–35
146. Nishizaki SS, Boyle AP. 2017. Mining the unknown: assigning function to noncoding single nucleotide polymorphisms toward the goal of understanding variation. *Trends Genet.* 33:34–45
147. Eastman JT. 1993. *Antarctic Fish Biology: Evolution in a Unique Environment*. San Diego, CA: Academic
148. Hiller M, Schaar BT, Indjeian VB, Kingsley DM, Hagey LR, Bejerano G. 2012. A "forward genomics" approach links genotype to phenotype using independent phenotypic losses among related species. *Cell Rep.* 2:817–23
149. Tse W, Lux SE. 1999. Red blood cell membrane disorders. *Br. J. Haematol.* 104:2–13
150. Stefen C, Wagner F, Asztalos M, Giere P, Grobe P, et al. 2021. Phenotyping in the era of genomics: MaTrics—a digital character matrix to document mammalian phenotypic traits coded numerically. bioRxiv 426960. **https://doi.org/10.1101/2021.01.17.426960**
151. Yergeau DA, Cornell CN, Parker SK, Zhou Y, Detrich HW III. 2005. *bloodthirsty*, an RBCC/TRIM gene required for erythropoiesis in zebrafish. *Dev. Biol.* 283:97–112

152. Scotese C. 2016. *PALEOMAP PaleoAtlas for GPlates and the PaleoData Plotter Program*. Rep., PALEOMAP Project, Evanston, IL
153. Martínez D, Pontigo J, Morera F, Yañéz A, Vargas-Chacoff L. 2018. Head kidney transcriptome analysis and characterization for the sub-Antarctic notothenioid fish *Eleginops maclovinus*. *Fishes* 3:8
154. Zhang D, Hu P, Liu T, Wang J, Jiang S, et al. 2018. GC bias lead to increased small amino acids and random coils of proteins in cold-water fishes. *BMC Genom*. 19:315
155. Touma J, García KK, Bravo S, Leiva F, Moya J, et al. 2019. *De novo* assembly and characterization of Patagonian toothfish transcriptome and develop of EST-SSR markers for population genetics. *Front. Mar. Sci.* 6:720
156. Shin SC, Kim SJ, Lee JK, Ahn DH, Kim MG, et al. 2012. Transcriptomics and comparative analysis of three Antarctic notothenioid fishes. *PLOS ONE*. 7:e43762
157. Papetti C, Harms L, Windisch HS, Frickenhaus S, Sandersfeld T, et al. 2015. A first insight into the spleen transcriptome of the notothenioid fish *Lepidonotothen nudifrons*: resource description and functional overview. *Mar. Genom*. 24:237–39
158. Gerdol M, Buonocore F, Scapigliati G, Pallavicini A. 2015. Analysis and characterization of the head kidney transcriptome from the Antarctic fish *Trematomus bernacchii* (Teleostea, Notothenioidea): a source for immune relevant genes. *Mar. Genom*. 20:13–15
159. Kim B-M, Ahn D-H, Kang S, Jeong J, Jo E, et al. 2019. Skin transcriptome profiling reveals the distinctive molecular effects of temperature changes on Antarctic bullhead notothen. *Mol. Cell. Toxicol.* 15:163–72
160. Berthelot C, Clarke J, Desvignes T, Detrich HW, Flicek P, et al. 2019. Adaptation of proteins to the cold in Antarctic fish: A role for methionine? *Genome Biol. Evol.* 11:220–31
161. Coppe A, Agostini C, Marino IAM, Zane L, Bargelloni L, et al. 2013. Genome evolution in the cold: Antarctic icefish muscle transcriptome reveals selective duplications increasing mitochondrial function. *Genome Biol. Evol.* 5:45–60
162. Ansaloni F, Gerdol M, Torboli V, Fornaini NR, Greco S, et al. 2021. Cold adaptation in Antarctic notothenioids: Comparative transcriptomics reveals novel insights in the peculiar role of gills and highlights signatures of cobalamin deficiency. *Int. J. Mol. Sci.* 22:1812

Annual Review of
Animal Biosciences

Volume 10, 2022

Contents

Errata

An online log of corrections to *Annual Review of Animal Biosciences* articles may be found at http://www.annualreviews.org/errata/animal