Cold Fusion: Massive Karyotype Evolution in the Antarctic Bullhead Notothen *Notothenia coriiceps*

Angel Amores,* Catherine A. Wilson,* Corey A. H. Allard,[†,‡,1] H. William Detrich, III,[†,‡] and John H. Postlethwait*,[2]

*Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403 and [†]Department of Marine and Environmental Sciences and [‡]Department of Biology, Marine Science Center, Northeastern University, Nahant, Massachusetts 01908

ORCID ID: 0000-0002-5476-2137 (J.H.P.)

ABSTRACT Half of all vertebrate species share a series of chromosome fusions that preceded the teleost genome duplication (TGD), but we do not understand the causative evolutionary mechanisms. The "Robertsonian-translocation hypothesis" suggests a regular fusion of each ancestral acro- or telocentric chromosome to just one other by centromere fusions, thus halving the karyotype. An alternative "genome-stirring hypothesis" posits haphazard and repeated fusions, inversions, and reciprocal and nonreciprocal translocations. To study large-scale karyotype reduction, we investigated the decrease of chromosome numbers in Antarctic notothenioid fish. Most notothenioids have 24 haploid chromosomes, but bullhead notothen (*Notothenia coriiceps*) has 11. To understand mechanisms, we made a RAD-tag meiotic map with ~10,000 polymorphic markers. Comparative genomics aligned about a thousand orthologs of platyfish and stickleback genes along bullhead chromosomes. Results revealed that 9 of 11 bullhead chromosomes arose by fusion of just two ancestral chromosomes and two others by fusion of three ancestral chromosomes. All markers from each ancestral chromosome remained contiguous, implying no inversions across fusion borders. Karyotype comparisons support a history of: (1) Robertsonian fusions of 22 ancestral chromosomes in pairs to yield 11 fused plus two small unfused chromosomes, like *N. angustata*; (2) fusion of one of the remaining two ancestral chromosomes to a preexisting fused pair, giving 12 chromosomes like *N. rossii*; and (3) fusion of the remaining ancestral chromosome to another fused pair, giving 11 chromosomes in *N. coriiceps*. These results raise the question of what selective forces promoted the systematic fusion of chromosomes in pairs and the suppression of pericentric inversions in this lineage, and provide a model for chromosome fusions in stem teleosts.

Compared to placental mammals, teleost fish have remarkably similar chromosome numbers: 58% of 580 teleost species studied have 24 or 25 haploid chromosomes (Naruse *et al.* 2004). The distribution shows a

small secondary peak at ~50 chromosomes (25 of 580 species, 0.4%) (Naruse *et al.* 2004), which mostly involves species like salmonids and carps, whose lineages experienced a relatively recent genome duplication event (Ohno *et al.* 1967; Allendorf and Thorgaard 1984). Placental mammals, on the other hand, have wildly variable karyotypes with four small peaks at 19 (4.7% of mammals), 21 (8.5%), 22 (8.1%), and 24 (8.8%) haploid chromosomes, with the remaining 70% distributed between 3 and > 50 chromosomes (Naruse *et al.* 2004). Even within a single order, different placental mammals can have dramatically different karyotypes; for example, Cervidae (deer) vary between 3 and 40 chromosomes in the haploid set and Rodentia (rodents) range between 5 and 51 (Scherthan 2012). Teleosts with a sequenced genome generally preserve chromosome numbers similar to the ancestral karyotype: zebrafish (*Danio rerio*) with 25 chromosomes (Amores and Postlethwait 1999), medaka (*Oryzias latipes*) and platyfish (*Xiphophorus maculatus*) both with 24 (Ocalewicz 2004; Kasahara *et al.* 2007), cod

(*Gadus morhua*) with 23 (Ghigliotti *et al.* 2012), fugu pufferfish (*Takifugu rubripes*) having 22 (Miyaki *et al.* 1995), and both stickleback (*Gasterosteus aculeatus*) and green pufferfish (*Tetraodon nigroviridis*) with 21 haploid chromosomes (Grutzner *et al.* 1999; Urton *et al.* 2011). Karyotypic stability in teleosts *vs.* variability in mammals is even more notable given that teleosts have been diverging for more than twice as long as placental mammals, ~230 *vs.* ~65 MY (Hurley *et al.* 2007; Wible *et al.* 2007; Santini *et al.* 2009). These observations pose questions concerning evolutionary mechanisms that appear to have constrained karyotypic evolution in teleosts or to have permitted variable karyotypes in mammals.

Karyotypic stability among teleosts is even more surprising given the TGD, which occurred ~226–316 MYA (Amores *et al.* 1998; Taylor *et al.* 2003; Jaillon *et al.* 2004; Hurley *et al.* 2007; Santini *et al.* 2009). The TGD initially doubled the chromosome number, and the return to functional diploidy involved a substantial number of chromosomal rearrangements after the TGD (Nakatani *et al.* 2007). The TGD occurred after the divergence of the teleost lineage from the Holostean lineage, which includes spotted gar (*Lepisosteus occulatus*) and bowfin (*Amia calva*), ~350 MYA (Hoegg *et al.* 2004; Amores *et al.* 2011). Surviving holosteans have slowly evolving genomes (Braasch *et al.* 2016) and, remarkably, many entire gar and chicken chromosomes have orthologous gene content and even chromosome size (Braasch *et al.* 2016), suggesting that few interchromosomal exchanges occurred in these lineages since gar and chicken last shared a common ancestor ~450 MYA. Because the nonteleost fish spotted gar has 29 chromosomes in its haploid set (Braasch *et al.* 2016), extensive chromosome fusion events must have occurred in the teleost lineage to achieve the 25 haploid chromosomes found in most of today's teleosts despite the TGD. Comparative genomic analyses showed that pairwise chromosome fusions occurred in the teleost lineage before the TGD (Braasch *et al.* 2016). Understanding the mechanisms of such massive fusion events would help us to understand genome evolution for half of all vertebrates.

Although teleost genomes retained extraordinarily stable chromosome numbers with few interchromosomal exchanges (translocations) after the chromosomal fusion events that preceded the TGD, comparative genomic analyses have shown that teleosts experienced frequent intrachromosomal rearrangements (inversions and transpositions) (Postlethwait *et al.* 1998; Naruse *et al.* 2004). For example, platyfish and medaka karyotypes remained remarkably similar with few translocations between chromosomes but with numerous transpositions and inversions since their lineages diverged ~120 MYA (Amores *et al.* 2011, 2014). An example is linkage group (LG) 9 of the platyfish, which, although sharing reciprocally exclusive conserved synteny with LG4 of medaka, experienced > 30 inversions and transpositions with respect to the latter (Amores *et al.* 2014). These observations pose a problem: what forces permitted intrachromosomal rearrangements but restricted interchromosomal exchanges in teleosts? Solutions could come from exploring situations that counter these rules, situations in which translocations are many but perhaps inversions might be few. To probe these issues, we investigated a case of extensive chromosome number reduction in Antarctic notothenioid fishes.

Within teleosts, a single suborder of percomorph fish, the notothenioids, currently dominates the subzero (−1.9°) water of the Southern Ocean (Eastman 2005). After the separation of Antarctica, South America, and Australia ~35 MYA (Near *et al.* 2015), the Drake Passage opened and the Circumpolar Currents formed, thereby isolating the Southern Ocean and allowing it to gradually chill to its current frigid condition ~10–14 MYA (Kennett 1977). During this prolonged cooling period, many taxa became locally extinct, liberating numerous

ecological niches into which notothenioids could radiate (Eastman 1993; Eastman and McCune 2000). As notothenioids conquered Antarctic waters, they evolved key innovations, including the origin of antifreeze glycoproteins (AFGPs) (DeVries 1988; Chen *et al.* 1997; Cheng and Chen 1999; Cheng and Detrich 2007) and a greatly altered inducible heat shock response (Hofmann *et al.* 2000; Place and Hofmann 2005; Huth and Place 2013; Buckley *et al.* 2004; Buckley and Somero 2009; Detrich *et al.* 2012).

Like most teleosts, Antarctic notothenioids generally have 24 chromosome pairs (see Figure 1). Even icefish (Channichthyidae, Figure 1) retain this ancestral karyotype despite their highly derived features: delayed and reduced bone and scale mineralization, substantial deposition of lipids, the loss of myoglobin and/or hemoglobin genes, the diffusion of oxygen directly through scaleless skin, decreased oxygen demand associated with changes in the density and morphology of mitochondria, and increased relative heart size and pumping volume (Koch 2005; Albertson *et al.* 2010; Friedrich and Hagen 1994; Hagen *et al.* 2000; Hemmingsen 1991; Egginton *et al.* 2002; Sidell *et al.* 1997; O'Brien *et al.* 2000).

However, several notothenioid lineages retained ancestral morphologies but evolved karyotypes with a dramatically reduced chromosome number (Figure 1), including *Lepidonotothen nudifons* (1*n* = 14), *Trematomus loennbergii* (1*n* = 14), and the *Bathydraco marri* and *Rakovitzia glacialis* lineages (1*n* = 19 and 18, respectively) (Tomaszkiewicz *et al.* 2011; Morescalchi *et al.* 1992b; Near and Cheng 2008; Ozouf-Costaz *et al.* 1991). However, the most dramatic karyotype evolution among notothenioid fish involves the lineage of the genus *Notothenia*. Fish in the monophyletic group *Notothenia angustata*, *N. microlepidota*, and *N. magellanica* (Dettai *et al.* 2012) have 13 chromosomes as a haploid set, *N. rossii* has 12, and *N. coriiceps* has the further reduced set of 11 haploid chromosomes (Prirodina and Neyelov 1984; Doussau de Bazignan and Ozouf-Costaz 1985; Van *et al.* 1987; Ozouf-Costaz and Doussau de Bazignan 1987; Ozouf-Costaz *et al.* 1991; Pisano *et al.* 2003b) (Figure 1). These observations raise the question: what evolutionary processes resulted in the *N. coriiceps* genome possessing fewer than half the number of chromosomes that is found in most other notothenioids and indeed, most other teleost fish?

N. coriiceps could have evolved a greatly reduced karyotype by any of several mechanisms. Under a simple "Robertsonian-translocation hypothesis," each of the 24 ancestral haploid acro- or telocentric chromosomes fused with just one other chromosome at their centromeres (Robertsonian translocations) (Robertson 1916) to produce 12 chromosomes (as is now found in *N. rossii*), followed by the fusion of the last two single chromosomes to two previously-fused chromosomes to give the 11 haploid chromosomes of today's bullhead *N. coriiceps*. In contrast to this hypothesized regular, one-to-one, mostly centromere-to-centromere (Robertsonian translocation) mechanism, one could imagine an alternative "genome stirring hypothesis" that involves many seemingly haphazard events involving multiple and repeated chromosome fusions, fissions, transpositions, inversions, and reciprocal or nonreciprocal translocations, as in some grasses (The International Brachypodium Initiative 2010). Hypotheses intermediate between these two extremes are also possible. These two hypotheses make different predictions regarding the historical origin of parts of each bullhead chromosome. The Robertsonian-translocation hypothesis predicts that nearly all bullhead chromosomes would correspond to all of just two ancestral chromosomes fused at their centromeres. In contrast, the genome-stirring hypothesis predicts that each bullhead chromosome would be a haphazard mingling of parts of several ancestral chromosomes, with different segments of each ancestral chromosome appearing on several bullhead chromosomes, and each
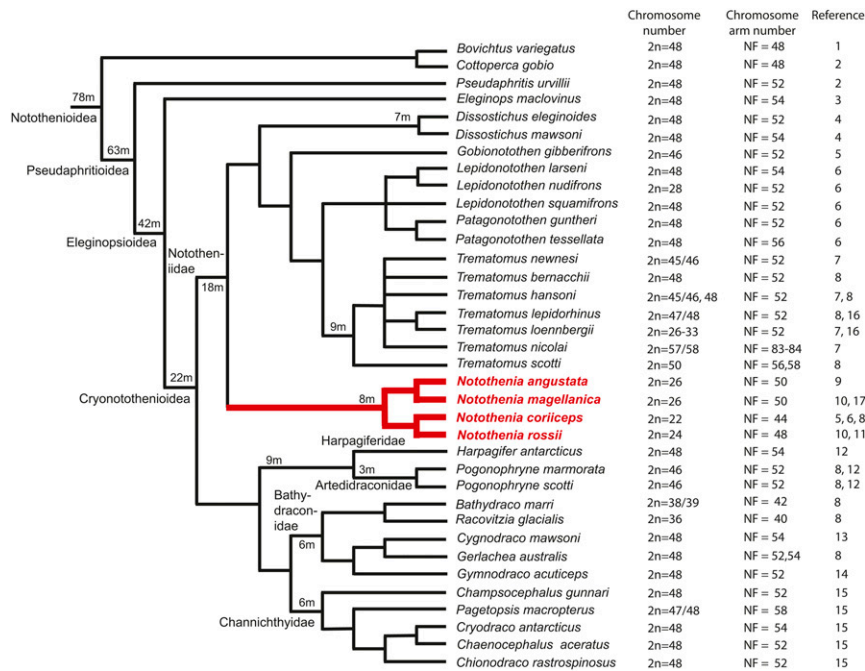
Species	Chromosome number	Chromosome arm number	Reference
Bovichtus variegatus	2n=48	NF = 48	1
Cottoperca gobio	2n=48	NF = 48	2
Pseudaphritis urvillii	2n=48	NF = 52	2
Eleginops maclovinus	2n=48	NF = 54	3
Dissostichus eleginoides	2n=48	NF = 52	4
Dissostichus mawsoni	2n=48	NF = 54	4
Gobionotothen gibberifrons	2n=46	NF = 52	5
Lepidonotothen larseni	2n=48	NF = 54	6
Lepidonotothen nudifrons	2n=28	NF = 52	6
Lepidonotothen squamifrons	2n=48	NF = 52	6
Patagonotothen guntheri	2n=48	NF = 52	6
Patagonotothen tessellata	2n=48	NF = 56	6
Trematomus newnesi	2n=45/46	NF = 52	7
Trematomus bernacchii	2n=48	NF = 52	8
Trematomus hansoni	2n=45/46, 48	NF = 52	7, 8
Trematomus lepidorhinus	2n=47/48	NF = 52	8, 16
Trematomus loennbergii	2n=26-33	NF = 52	7, 16
Trematomus nicolai	2n=57/58	NF = 83-84	7
Trematomus scotti	2n=50	NF = 56,58	8
Notothenia angustata	2n=26	NF = 50	9
Notothenia magellanica	2n=26	NF = 50	10, 17
Notothenia coriiceps	2n=22	NF = 44	5, 6, 8
Notothenia rossii	2n=24	NF = 48	10, 11
Harpagifer antarcticus	2n=48	NF = 54	12
Pogonophryne marmorata	2n=46	NF = 52	8, 12
Pogonophryne scotti	2n=46	NF = 52	8, 12
Bathydraco marri	2n=38/39	NF = 42	8
Racovitzia glacialis	2n=36	NF = 40	8
Cygnodraco mawsoni	2n=48	NF = 54	13
Gerlachea australis	2n=48	NF = 52,54	8
Gymnodraco acuticeps	2n=48	NF = 52	14
Champsocephalus gunnari	2n=48	NF = 52	15
Pagetopsis macropterus	2n=47/48	NF = 58	15
Cryodraco antarcticus	2n=48	NF = 54	15
Chaenocephalus aceratus	2n=48	NF = 52	15
Chionodraco rastrospinosus	2n=48	NF = 52	15

Figure 1 Cladogram and chromosome numbers for Antarctic fish. The phylogeny is modified from (Near 2008; Near 2015; Near 2004; Papetti 2016). Abbreviations: 2N, diploid chromosome number; NF, the number of chromosome arms (nombre fundamental). References: 1: (Mazzei *et al.* 2006). 2: (Pisano *et al.* 1995). 3: (Mazzei *et al.* 2008). 4: (Ghigliotti *et al.* 2007). 5: (Phan *et al.* 1987). 6: (Tomaszkiewicz *et al.* 2011). 7: (Morescalchi *et al.* 1992a). 8: (Ozouf-Costaz *et al.* 1991). 9: (Pisano *et al.* 2003b). 10: (Doussau de Bazignan and ozouf-Costaz 1985). 11: (Prirodina and Neyelov 1984). 12: (Prirodina 1997). 13: (Morescalchi *et al.* 1996). 14: (Pisano *et al.* 2001). 15: (Morescalchi *et al.* 1992a,b). 16: (Ghigliotti *et al.* 2015).

bullhead chromosome consisting of segments from several ancestral chromosomes.

To distinguish these two hypotheses, we made sex-specific meiotic maps for *N. coriiceps* using sequence-based markers produced by RAD-seq (Baird *et al.* 2008; Miller *et al.* 2007a,b; Amores *et al.* 2011) and identified ancestral chromosomes by comparative genomics with out-groups. For comparative genomics, we identified coding sequences on RAD-tag markers mapped on the *N. coriiceps* map to stickleback and platyfish genome sequences (Schartl *et al.* 2013; Amores *et al.* 2014; Jones *et al.* 2012). Because notothenioids and sticklebacks both occupy Near's clade XIII of spiny-ray fishes, DNA sequences of notothenioids and stickleback should be more similar than DNA sequences of noto-thenioids and platyfish, which occupies clade VII (Near *et al.* 2012b). Nevertheless, the karyotype of stickleback with 21 chromosomes is more derived than platyfish with 24. The Robertsonian fusion hypothesis predicts that ancestral chromosomes would mostly be fused one-to-one. In contrast, the genome stirring hypothesis predicts a disorganized, rather haphazard arrangement of parts of ancestral chromosomes. Results showed that 22 of the 24 chromosomes present in the ancestral Notothenioid (as inferred from comparative genomics with stickleback and platyfish) fused in pairs, and then the remaining two unfused chromosomes joined two previously merged chromosomes to form today's *N. coriiceps* karyotype of 11 haploid chromosomes. In contrast to the dramatic, extensive, and concerted evolution of Robert-sonian translocations experienced in the *N. coriiceps* lineage, the current karyotype shows no inversions across centromeres since the flurry of fusions. Coupling the *N. coriiceps* genome sequence (Shin 2014) to the extensive meiotic map presented here should illuminate the molecular genetic mechanisms responsible for the trend of karyotype evolution experienced in the lineage of genus *Notothenia*.

MATERIALS AND METHODS

Animals

Adult *N. coriiceps* were collected by bottom trawls or baited traps deployed from the *ARSV Laurence M. Gould* southwest of Low Island [Antarctic Specially Protected Area (ASPA) 152, Western Bransfield Strait; latitudes 63°15′S–63°30′S, longitudes 62°00′W–62°45′W, bounded on northeast by Low Island)] or west of Brabant Island (ASPA 153, Eastern Dallmann Bay; latitudes 63°53′S–64°20′S and longitudes 62°16′W–62°45′W, bounded on the east by the shoreline of Brabant Island) in the Palmer Archipelago in May 2008. Fish were transported alive to Palmer Station, Antarctica, where they were maintained in seawater aquaria at −1° to 0°. Eggs from a single female and sperm from a single male were stripped, gametes were mixed, and progeny from this single *in vitro* fertilization event were maintained at ~−1C until they were about a month old, in midem-bryo stages [see Postlethwait *et al.* (2016)], and then were stored in 100% EtOH at −20°. The male and female parents were killed by MS-222 overdose and samples of muscle, liver, fin, and spleen were stored in 100% EtOH at −20° until further use. Genomic DNA was isolated using the DNeasy Blood & Tissue Kit (QIAGEN). The University of Oregon IACUC approved all protocols (13-27RR).

Creation of RAD-tag libraries

Genomic DNA was purified from the male and female parents and 244 of their progeny. RAD-tag libraries were created as described (Amores *et al.* 2011). DNA from each fish was digested with high-fidelity *Sbf*1 (New England Biolabs) restriction enzyme overnight and each sample was separately barcoded with P1 and P2 adaptor ligations overnight, using 96 different five-nucleotide barcodes. Fifty nanograms of pooled, size-selected DNA was amplified by PCR for 12 cycles and the PCR product was gel purified by excising a 200–500 bp fraction. Libraries were sequenced on an Illumina GAII or HiSeq2000 to obtain 100-nucleotide single-end reads.

Marker genotyping

Sequenced reads from the Illumina runs were sorted by barcode, allowing up to one mismatched nucleotide in the barcode sequence. Reads containing uncalled bases and those that had an average *phred* quality score that fell below 10 over 15% of the read length were discarded. Retained reads were genotyped using the software Stacks (Catchen *et al.* 2011). Progeny with < 800,000 reads were excluded

■ Table 1 Total genetic length in centimorgans (cM) and total number of markers for each linkage group

LG	Female Map cM	Male Map cM	Female Map Markers	Male Map Markers	Total Markers	ab × cd	ef × eg	hk × hk	lm × ll	nn × np	Synteny to Gac	Synteny to Xma
1	104.3	106.4	457	468	896	34	112	114	310	326	176	136
2	100	103.3	487	503	976	18	100	106	367	385	167	122
3	92.5	107.6	454	443	862	27	106	100	319	310	197	128
4	111.5	96.2	441	487	894	35	118	110	296	335	184	120
5	101.3	91.1	408	439	841	23	91	107	294	326	199	138
6	105	102.2	415	456	853	28	99	108	288	330	178	124
7	101.7	104.5	421	438	831	23	95	89	303	321	165	125
8	105.5	86.9	446	406	801	21	116	84	309	271	149	102
9	113.5	96.2	384	396	764	17	95	97	272	283	197	131
10	105.1	108.5	367	405	740	15	95	81	253	296	147	101
11	95.6	98	346	359	680	15	78	68	253	266	126	93
Total	1136	1100.9	4626	4800	9138	256	1105	1064	3264	3449	1885	1320

Markers of type ab × cd (segregating 1:1:1:1) were heterozygous in both parents with two male-specific alleles and two female-specific alleles; markers of type ef × eg (segregating 1:1:1:1) were heterozygous in both parents with one male-specific allele, one female-specific allele, and one shared allele; markers of type hk × hk (segregating 1:2:1) were heterozygous in both parents with two shared alleles; markers of type lm × ll (segregating 1:1) were heterozygous in the female parent and homozygous in the male parent; and markers of type nn × np (segregating 1:1) were homozygous in the female parent and heterozygous in the male parent. LG, linkage group; Gac, conserved markers for stickleback; Xma, conserved markers for platyfish.

from Stacks analysis. The parameters for Stacks provided to denovo_map.pl were: a minimum of five reads for a stack (-m 5), up to four differences when merging stacks into loci (-M 4), and up to two fixed differences when merging loci from the parents into the catalog (-n 2). Stacks exported data into JoinMap 4.1 (Van Ooijen 2006) for linkage analysis.

Map construction

Of the 244 F1 individual progeny sequenced, 52 fish did not have enough coverage or had too many missing genotypes (> 20%) to use for mapping. Linkage analysis was performed with JoinMap 4.1 (Van Ooijen 2006) with markers present in at least 150 of the 192 remaining individuals. Because JoinMap 4.1 could not work with our large number of markers, we partitioned the original dataset into two sets of markers. Markers were initially grouped in JoinMap 4.1 using the "independence LOD" parameter under "population grouping" with a minimum LOD value of 23.0. After one dataset was analyzed, some markers from each LG were added as anchors to the second dataset, and LGs were established. All markers from the same LG in both data subsets were combined and analysis of individual LGs was performed at LOD of 25.0. Markers that remained unlinked at LOD < 25 were excluded. Markers segregated according to one of five different patterns: (1) type ab × cd markers (segregating 1:1:1:1) were heterozygous in both parents with two male specific alleles and two female specific alleles; (2) type ef × eg markers (segregating 1:1:1:1) were heterozygous in both parents with one male specific allele, one female specific allele, and one shared allele; (3) type hk × hk markers (segregating 1:2:1) were heterozygous in both parents with shared alleles; (4) type lm × ll markers (segregating 1:1) were heterozygous in the female parent and homozygous in the male parent; and (5) type nn × np markers (segregating 1:1) were homozygous in the female parent and heterozygous in the male parent. After obtaining a consensus map, marker sets were partitioned into paternal and maternal markers to enable the construction of sex-specific linkage maps using the "Create Maternal and Paternal Population Nodes" feature in JoinMap. Marker ordering was performed using the Maximum Likelihood algorithm in JoinMap 4.1 with default parameters. Putative double recombinants were identified using the "genotype probabilities" feature in JoinMap 4.1 and by visual inspection of the colorized graphical genotypes. After visual inspection of the individual sequences in Stacks, markers were

corrected as needed. For example, if a double recombinant was a homozygote with a small number of reads, the genotype was eliminated because it might represent a heterozygote that lacked sufficient depth, by chance, to obtain a sequence for the second allele; likewise, if a double recombinant was a heterozygote with only one sequence for the second allele, the genotype was eliminated because the second sequence could be a sequencing error. The new dataset with corrected genotypes was loaded again into JoinMap 4.1 and linkage analysis was repeated until Joinmap identified no suspicious genotypes. The "expected recombination count" feature in JoinMap 4.1 was used to identify individuals with more recombination events than expected. Visual inspection of marker order was performed and, when necessary, marker order was manually optimized by moving a marker or group of markers to a new position that reduced the total number of recombination events. Segregation distortion was determined by calculating the χ^2 values for each marker using JoinMap 4.1. Relative segregation distortion along each LG was determined by plotting the χ^2 values against position along each LG using only markers with no more than five missing genotypes. Significance thresholds of $P = 0.01$ and 0.001 were plotted as horizontal lines on the graphs.

Analysis of conserved syntenies

The sequence from each RAD marker was used as a search query against the platyfish (Amores et al. 2014) and stickleback (Jones et al. 2012) genome databases using an e-value cutoff of 1e−12 and a minimum sequence alignment overlap of 75% of the RAD-tag length. Markers with sequence conservation to multiple locations were eliminated from the analysis.

Genome size and coverage

Genome length was estimated using Method-4 of Chakravarti et al. (1991) in which each LG length is adjusted by the factor $m+1/m-1$, where m is the number of markers on the LG, and by the method described in (Fishman et al. 2001 #70), in which twice the value of marker spacing, s, is added to the length of each LG. Total map coverage was calculated as the ratio between observed and estimated genome length.

Data availability

RAD-tag sequences of all mapped individuals are available online at the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra; accession number SRP047484).

RESULTS

Making the map

Sequencing produced 15.3 million sequences for both the male and female parents of the map cross and an average of 2.2 million sequences for individual progeny. Stacks software yielded a total of 74,508 RAD-tags, of which 60,349 were present in at least 10 progeny individuals. We identified SNPs segregating in 14,396 RAD-tags, and of those, 11,808 were present in at least 150 progeny fish. (Supplemental Material Table S1.)

Of the 11,808 mapped RAD-tag markers present in at least 150 of 192 progeny, 9138 markers mapped to the male map, or to the female map, or to both (Table 1), at the minimum required LOD of 25. The dataset included 1,654,303 total genotypes, 96,038 (5.5%) missing genotypes, and 1853 (0.1%) manually corrected genotypes. JoinMap 4.1 assigned markers to 11 LGs, which equals the number of cytogenetically described chromosomes (Phan *et al.* 1987; Ozouf-Costaz *et al.* 1991; Tomaszkiewicz *et al.* 2011). The total number of markers belonging to each LG ranged from a high of 976 in *N. coriiceps* LG2 (Nco2) to a low of 680 markers in Nco11. (Figure S1.)

Map lengths were similar for both sexes: 1136 cM for the female map and 1100.9 cM for the male map; the combined consensus map was 1300 cM long. LG sizes ranged between 92.5 and 113.5 cM for the female map and between 86.9 and 108.5 cM for the male map. The estimated genome length based on genetic map data showed that the RAD-tag map covers from 99.1 to 99.4% of the total genome length (Chakravarti *et al.* 1991; Fishman *et al.* 2001). Because the bullhead notothen genome is ~637 Mb (Shin 2014), the genome has a density of ~14.3 mapped markers per Mb.

Sex-specific recombination rates

Although the sex-specific linkage maps were similar in total length, the two maps differed substantially in the distribution of recombination events along the chromosomes. In the female map, recombination was more frequent near the centromeres, but in the male map, the recombination rate was higher near the telomeres; Figure 2 shows an example for LG1 (Nco1, *N. coriiceps* LG 1). Centromere position was estimated from cytogenetic data (Ozouf-Costaz *et al.* 1991; Phan *et al.* 1987; Tomaszkiewicz *et al.* 2011) as the block of markers with greatly reduced recombination near the middle of LGs for these metacentric chromosomes.

Segregation distortion

Most markers showed expected Mendelian segregation ratios. However, the segregation of some markers, fell outside expected Mendelian segregation ratios, a phenomenon that can occur if gametes or embryos have viability defects due to linked loci. For example, Nco11 showed little segregation distortion across most of the chromosome (Figure 3, A and B). In contrast, 464 markers (4.7%) showed significant segregation distortion ($P < 0.01$), nearly all (426/464) of which localized in two large sex-specific blocks. One block of segregation distortion included 244 markers distributed between 39.1 and 60.7 cM in the Nco1 male map (Figure 3, C and D), and the other large block of segregation distortion included 182 markers located between 39.8 and 65.7 cM in the Nco4 female map (Figure 3, E and F). For example, lm × ll markers in the female LG4 map showed 72 homozygous individuals and 120 heterozygous individuals, a ratio of 0.75: 1.25 [or, for markers with the opposite polarity, 120 homozygotes to 72 heterozygotes (a ratio of 1.25: 0.75)] rather than the expected 1: 1 (96: 96) segregation ratio. Some ab × cd markers in the same region of LG4 showed a segregation ratio of 1.33: 1.17: 0.73: 0.77 (64 ac genotypes: 56 ad: 35 bc: 37 bd) rather than
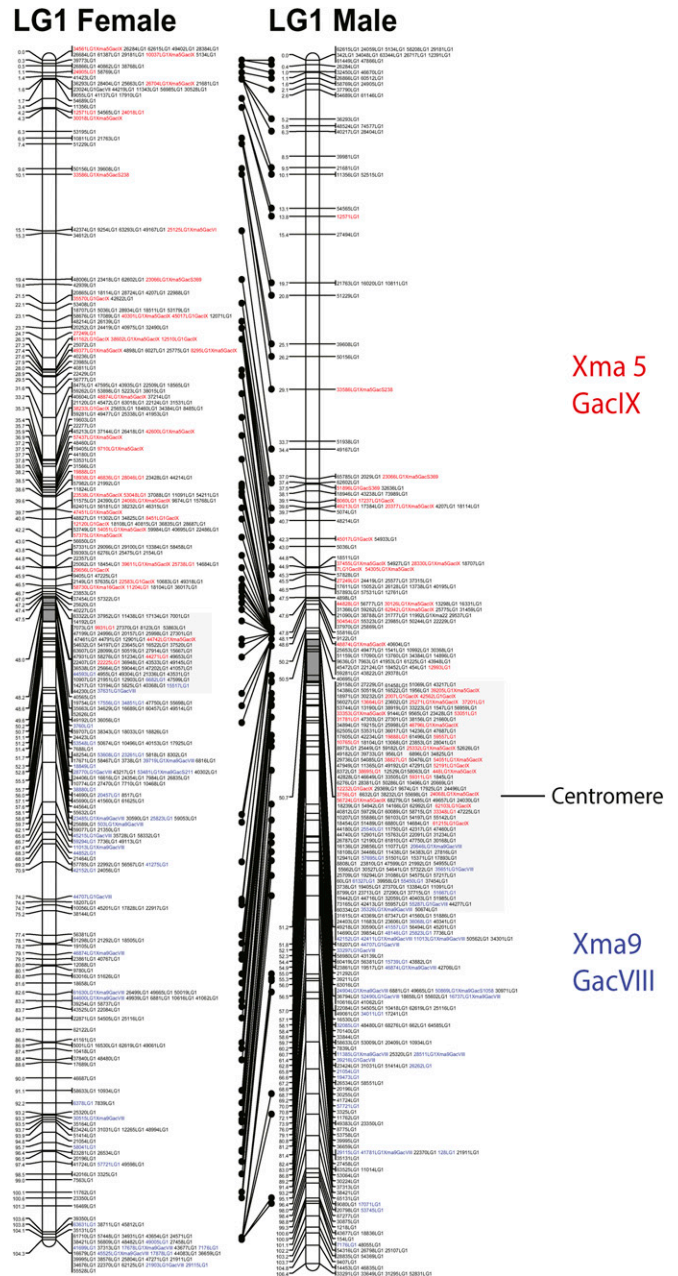

Figure 2 Male and female linkage maps for *N. coriiceps* chromosomes Nco1. The position of the centromere (Cen, gray box) is estimated based on published karyotype information and greatly reduced recombination rates. Connecting lines indicate the position of markers present in both the male and female chromosomes. Markers with sequence conservation to stickleback (Gac) or platyfish (Xma) sequences are color coded based on chromosome origin within these two species. For clarity, some loci do not list all polymorphic RAD-tags that mapped to the indicated position. Note sex-specific recombination rates across the chromosome. RAD, restriction site-associated DNA.

the expected 1: 1: 1: 1 ratio (48: 48: 48: 48 individual genotypes). When scoring female and male alleles for such markers, the segregation of the female a:b alleles was distorted (120:72) and that of the male b:c alleles was normal (99:93), in agreement with the pattern of the surrounding male and female markers in the region. Three additional small blocks of significant segregation distortion were also identified: (1) one block
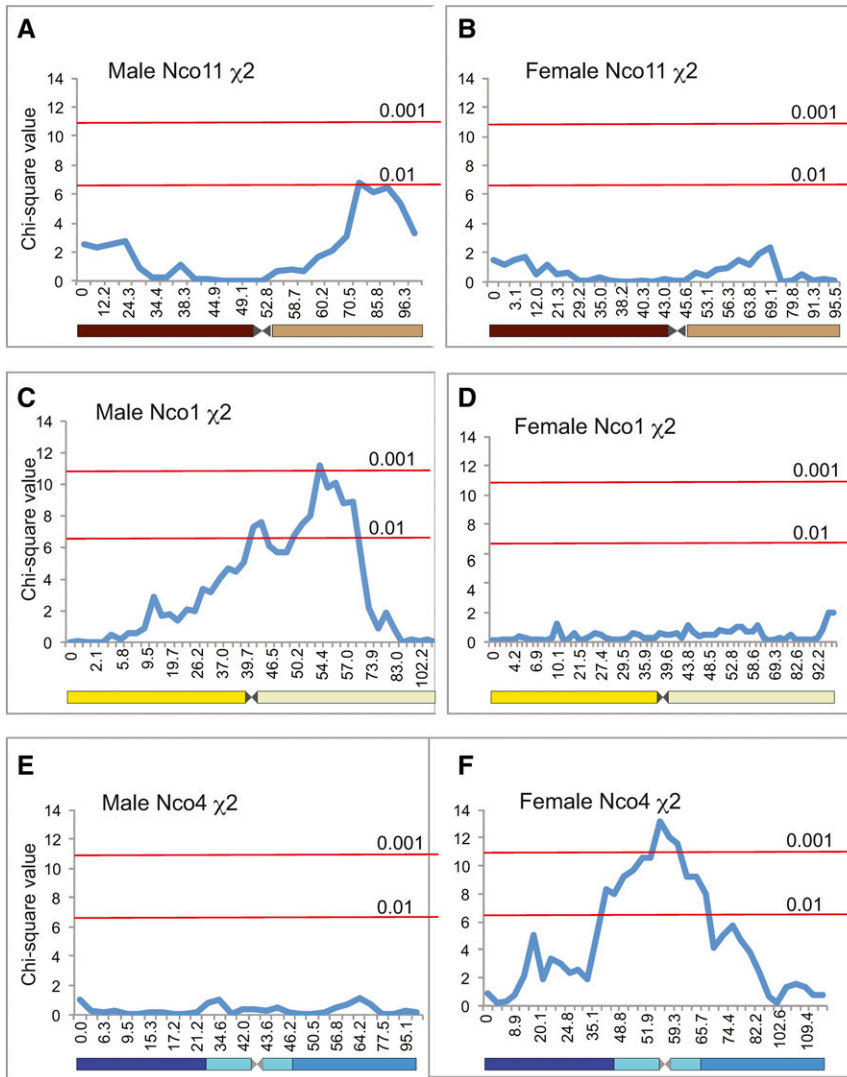
Figure 3 Patterns of sex-specific segregation distortion in bullhead chromosomes. (A and B), Nco11; (C and D), Nco1; and (E and F), Nco4. (A, C, and E) male map; (B, D, and F), female map. Distortion is plotted as a function of χ^2-squared values for the hypothesis of Mendelian segregation *vs.* marker position along the linkage group. Horizontal lines represent levels of significance of $P = 0.01$ and $P = 0.001$. Fused chromosomes are displayed across the horizontal axis, color key in Figure 6. Nco11 has a small region that barely reaches significance; Nco1 has a large region of segregation distortion for meiosis in the male parent and Nco4 has a large region of segregation distortion for meiosis in the female parent.

involved two widely separated markers and was barely statistically significant at position 70.5 in the male Nco11 map (Figure 3A); (2) another block involved four markers, two in each of two widely separated regions on Nco3; (3) and the final block involved 32 markers at position 52.2–56.9 cM in the female Nco5 map (Figure S2).

Analysis of conserved syntenies

Comparative genomic analyses are necessary to test whether the Robertsonian translocation hypothesis or the genome stirring hypothesis better predict the mechanism giving rise to the greatly reduced bullhead notothen karyotype. To obtain comparative data, we identified conserved sequences in our mapped RAD-tag sequences by BLAST comparisons of the *N. coriiceps* RAD-tag sequences to the stickleback and platyfish reference genome sequences. Fortunately, the restriction enzyme we used for making RAD-tags cuts preferentially at exon–intron borders, which makes protein-coding sequences appear more frequently than by chance (McCluskey and Postlethwait 2015). Among the 9138 mapped markers, 1320 (14.4%) had significant sequence similarity to sequences in the platyfish genome (Schartl *et al.* 2013) and 1885 (20.6%) had significant similarity to sequences in the genome of stickleback (Jones *et al.* 2012), to which bullhead notothen is more

closely related than it is to platyfish (Near *et al.* 2012a,b). A total of 81 RAD-tags with conserved sequence were located on unmapped platyfish scaffolds, and 197 RAD-tags with conserved sequence were located on unmapped stickleback scaffolds. A display of markers with sequence homology along the genetic map allowed us to conduct comparative genomic analyses.

Although platyfish is an outgroup to the stickleback + notothenioid clade (Near *et al.* 2012), the platyfish haploid karyotype of 24 chromosome pairs containing 48 chromosome arms [NF (nombre fundamental, the number of chromosome arms) = 48] reflects more closely the ancestral condition than the stickleback karyotype with 21 chromosome pairs (NF = 58) (see summary Figure 6) due to chromosome rearrangements in the stickleback lineage (Urton *et al.* 2011). Bullhead *vs.* platyfish and stickleback comparisons showed that 9 of the 11 bullhead chromosomes each have homology to two platyfish or stickleback chromosomes. Figure 2, for example, shows male and female maps for Nco1 with conserved markers for platyfish LG5 (Xma5) and stickleback LG IX (GacIX) on the top arm and platyfish Xma9 and stickleback GacVIII on the lower arm [for nomenclature, consider that the example marker called 44742Nco1Xma5GacIX means marker number 44742 on bullhead Nco1, which has sequence similarity to a locus on platyfish
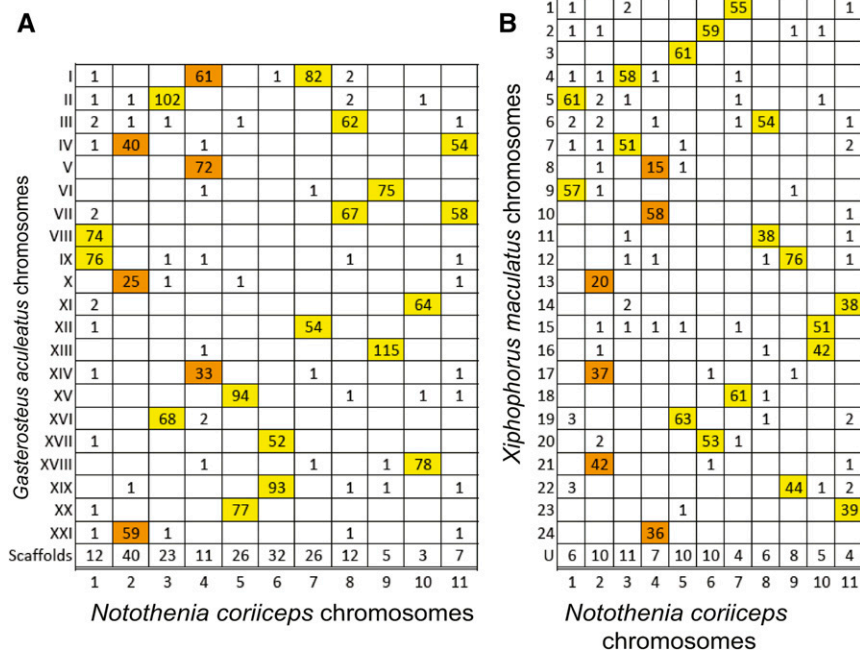
A

Gasterosteus aculeatus chromosomes

	1	2	3	4	5	6	7	8	9	10	11
I	1			61		1	82	2			
II	1	1	102					2		1	
III	2	1	1		1		62				1
IV	1	40		1							54
V				72							
VI				1		1		75			
VII	2					67				58	
VIII	74										
IX	76		1	1			1			1	
X		25	1		1						1
XI	2									64	
XII	1				54						
XIII				1			115				
XIV	1		33			1					1
XV				94			1		1	1	
XVI			68	2							
XVII	1				52						
XVIII				1		1			1	78	
XIX		1			93		1	1		1	
XX	1			77							
XXI	1	59	1				1				1
Scaffolds	12	40	23	11	26	32	26	12	5	3	7

Notothenia coriiceps chromosomes

B

Xiphophorus maculatus chromosomes

	1	2	3	4	5	6	7	8	9	10	11
1	1		2			55					1
2	1	1			59			1	1		
3				61							
4	1	1	58	1		1					
5	61	1			1			1			1
6	2	2		1		1	54				1
7	1	1	51		1						2
8		1		15	1						
9	57	1					1				
10				58							1
11		1				38					1
12		1	1			1	76				1
13	20										
14		2								38	
15	1	1	1	1		1		51			
16	1						42				
17	37			1		1					
18				61	1						
19	3			63		1				2	
20		2		53	1						
21	42			1						1	
22	3						44	1	2		
23			1							39	
24				36							
U	6	10	11	7	10	10	4	6	8	5	4

Notothenia coriiceps chromosomes

Figure 4 Oxford grids showing conservation of synteny between (A) bullhead and stickleback (G. aculeatus) and (B) between bullhead and platyfish (X. maculatus). Numbers in boxes indicate the number of mapped bullhead RAD-tag markers with sequence conservation to the genomes of stickleback and platyfish. Bullhead chromosomes Nco2 and Nco4 each have conserved synteny to three stickleback and three platyfish chromosomes (red boxes). Each of the remaining bullhead linkage groups conserve synteny to two stickleback and the orthologous two platyfish chromosomes (yellow boxes). "Scaffolds" and "U" represent contigs not assembled into the official genome assemblies of stickleback and platyfish, respectively. RAD, restriction site-associated DNA.

X. maculatus LG5 (Xma5) and stickleback G. aculeatus LGIX (GacIX)]. In all bullhead chromosomes except Nco2 and Nco4, markers homologous to one platyfish chromosome occupied one arm of the bullhead chromosome and markers homologous to another platyfish chromosome occupied the other arm (Figure S1). Figure 4 shows the conserved synteny data in Oxford Grid format. We conclude that 9 of the 11 bullhead chromosomes formed by the fusion of two and only two ancestral chromosomes. Figure 6 summarizes the comparative genomic analyses at the whole-chromosome level.

While markers on most of the bullhead chromosomes were conserved on two platyfish or two stickleback chromosomes, two bullhead chromosomes, Nco2 and Nco4, had sequences homologous to three platyfish or stickleback chromosomes (see Figure 5 for Nco4 and Figure S1 for Nco2). For example, bullhead Nco4 was orthologous to the platyfish chromosomes Xma10, Xma8, and Xma24 (Figure 5). In both Nco2 and Nco4, homologous markers belonging to the three platyfish chromosomes were located in three consecutive segments with no mixing of markers homologous to different platyfish chromosomes (Figure 5). We conclude that bullhead chromosomes Nco2 and Nco4 originated from the fusion of three ancestral chromosomes (Figure 6).

For all 11 bullhead chromosomes, markers homologous to a given platyfish chromosome were contiguous along the LG, with no mixing of markers homologous to one platyfish chromosome intermingled with markers homologous to the other platyfish chromosome across the region of contact (Figure 2, Figure 5, and Figure S1). This result indicates that the bullhead lineage experienced few, or maybe no, inversions or transpositions across the centromere following the chromosome fusion events.

DISCUSSION

Here, we report the first dense genetic linkage map for any species of Antarctic fish. The 11 bullhead LGs correspond to the number of chromosomes detected cytogenetically (Phan et al. 1987; Ozouf-Costaz et al. 1991; Tomaszkiewicz et al. 2011). We conclude that the linkage map spans the entire bullhead genome.

Analysis of chromosome numbers and arm numbers, coupled with the bullhead genetic map, shows the pattern of karyotype evolution in notothenioids and their relatives. Basally diverging notothenioids, like Cottoperca gobio and Bovichtus variegatus, have a diploid number of 48 and a chromosome arm number of 48 (Figure 1 and references therein), indicating that all ancestral chromosomes were likely acro- or telocentric. The next most recently branching lineage, represented by Pseudaphritis urvillii, still has $2n = 48$, but has NF = 52 rather than NF = 48, indicating that two pericentric inversions brought the centromere toward the middle of the chromosome for two ancestral chromosomes, a condition shared by all of the Cryonotothenioidea (Figure 1 and references therein). Within the Harpagiferidae–Artedidraconidae–Bathydraconidae–Channichthyidae clade, chromosome counts and arm numbers are mostly rather stable, although some lineages experienced a few chromosome fusions and pericentric inversions (Figure 1). Within the Nototheniidae, some lineages show chromosome fusions, fissions, and pericentric inversions, especially in the Trematomus genus, for example T. loennbergii with a diploid set of 28 or 30 chromosomes having 52 chromosome arms and T. nicolai with a diploid set of 57 or 58 chromosomes and 83–84 chromosome arms (Figure 1). However, the most dramatic karyotype evolution is in the Notothenia–Paranotothenia clade, the mechanisms of which our comparative genetic map elucidates.

A model for karyotype reduction in the Notothenia–Paranotothenia clade

Comparative genomic analyses showed that 9 of the 11 bullhead notothen chromosomes originated by fusion of two ancestral chromosomes, two bullhead chromosomes arose from the fusion of three ancestral chromosomes, and that no chromosome inversions or transpositions were detected across the centromeres (Figure 6). These comparative genomic data rule out the genome-stirring hypothesis for the reduction of the bullhead karyotype compared to outgroups (Figure 1) and are what would be expected from the hypothesis of an organized series of mostly one-to-one Robertsonian translocations.
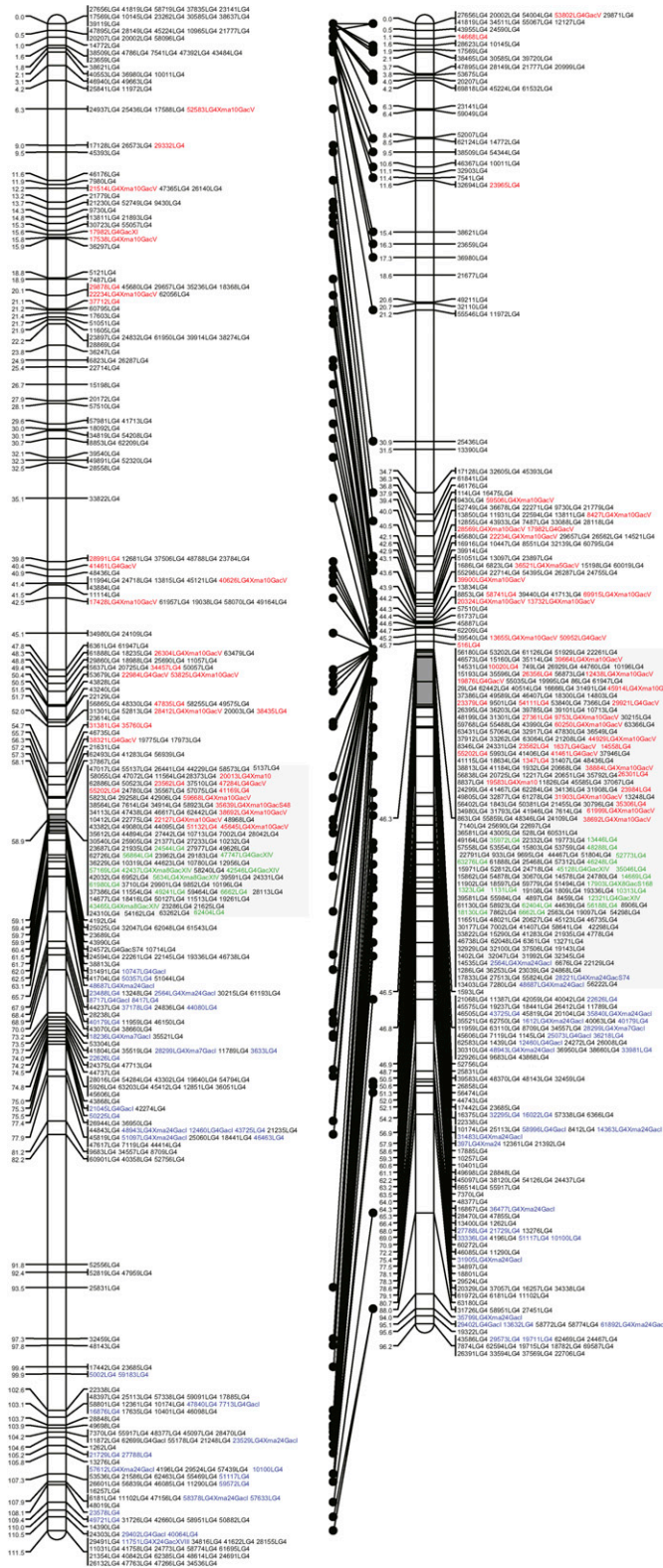
Figure 5 Comparison of male and female linkage maps for bullhead chromosome Nco4. Markers with sequence conservation to the genome of stickleback and platyfish are color coded according to chromosomes with conserved synteny. The position of the centromere (Cen) is estimated based on published chromosome morphology (Ozouf-Costaz *et al.* 1991; Tomaszkiewicz *et al.* 2011). RAD markers with sequence conservation to chromosomes GacV or Xma10 are in red; RAD markers with sequence conservation to GacXIV or Xma8 are colored in green; and RAD markers with sequence conservation to GacI or Xma24 are colored in blue. Lines between the female and male linkage groups represent shared markers present in meiosis of both sexes. RAD, restriction site-associated DNA.

Coupling our bullhead map data with published chromosome numbers for notothenioids and their nearest outgroups (Figure 1) provides a model to explain the massive reduction of chromosome number in the *Notothenia* species group (Figure 7). First, the data suggest that the last common ancestor of the *Notothenia* species group had 24 chromosomes in its haploid set (Figure 7, step 1) because the nearest
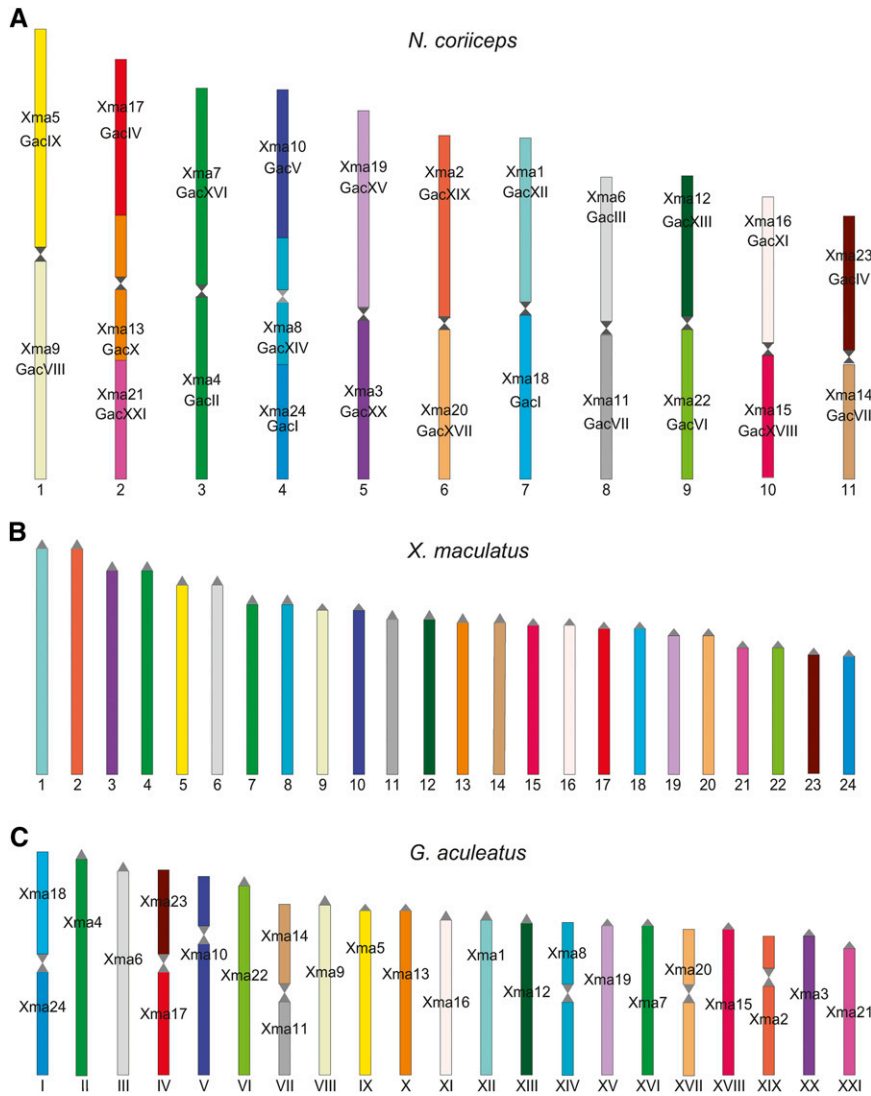
Figure 6 Conserved synteny relationship among chromosomes of (A) bullhead (Nco), (B) platyfish (Xma), and (C) stickleback (Gac). Note that stickleback LGs I, IV, and VII consist of fusions of two platyfish chromosomes.

outgroup, the Nototheniidae including *Dissostichus mawsoni*, *L. squamifrons*, and *T. lepidorhinus*, has mainly 24 haploid chromosomes, as do most fish in the sister lineage, which includes *Harpagifer antarcticus* and icefish (Channichthyidae) like *Chionodraco rastrospinosus* and *C. aceratus* (Figure 1). Second, a pericentric inversion in one of the smallest chromosomes created a small metacentric chromosome (the smallest, blue chromosome in Figure 7B). This small metacentric chromosome is still present in *N. angustata*, *N. magellanica*, and *N. rossii* (Doussau de Bazignan and Ozouf-Costaz 1985; Pisano *et al.* 2003b). Third, 22 ancestral chromosomes in the haploid karyotype fused together one-to-one, reducing the chromosome number from 24 pairs of acrocentric chromosomes to 11 large metacentric chromosomes and two small unfused chromosomes, giving a haploid karyotype with 13 chromosomes. *N. angustata* and *N. magellanica* possess such a karyotype today (Figure 1 and Figure 7 step 3). Because the lineage of *N. coriiceps* + *N. rossii* diverged from *N. angustata* + *P. magellanica* ~8 MYA (Near *et al.* 2012), this massive one-to-one chromosome fusion event is either older than 8 MY or, less parsimoniously, happened more recently but twice, and independently in the *N. angustata* + *N. magellanica* lineage. Fourth, after the divergence of the *N. coriiceps* + *N. rossii* clade from the *N. angustata* + *N. magellanica* clade, one of the

small, previously unfused chromosomes became fused to one of the existing, already-fused metacentric chromosomes, creating the current Nco2 or Nco4 chromosomes. *N. rossii* shows a karyotype today that would fit this model (Figure 7, step 4). In a final step, the last small, previously unfused metacentric chromosome joined another existing metacentric chromosome to create the current Nco2 or Nco4 in *N. coriiceps* (Figure 7, Step 5). An alternative, less parsimonious possibility not ruled out by our data on bullhead notothen is that some of the predicted chromosome fusions in the model originated independently in some of the four species in the *Notothenia* clade. Experiments similar to the ones we report here for bullhead notothen are required to test the hypothesis of evolution to similar chromosome numbers but independent chromosome fusion events.

Inversions

Because all conserved synteny markers were contiguous with no intermixing of markers belonging to one of the individual ancestral chromosomes with markers from the other ancestral chromosome across their position of contact in each fused bullhead chromosome, we conclude that no pericentric inversions occurred in the bullhead lineage after the chromosomal fusion events that led to the reduction in
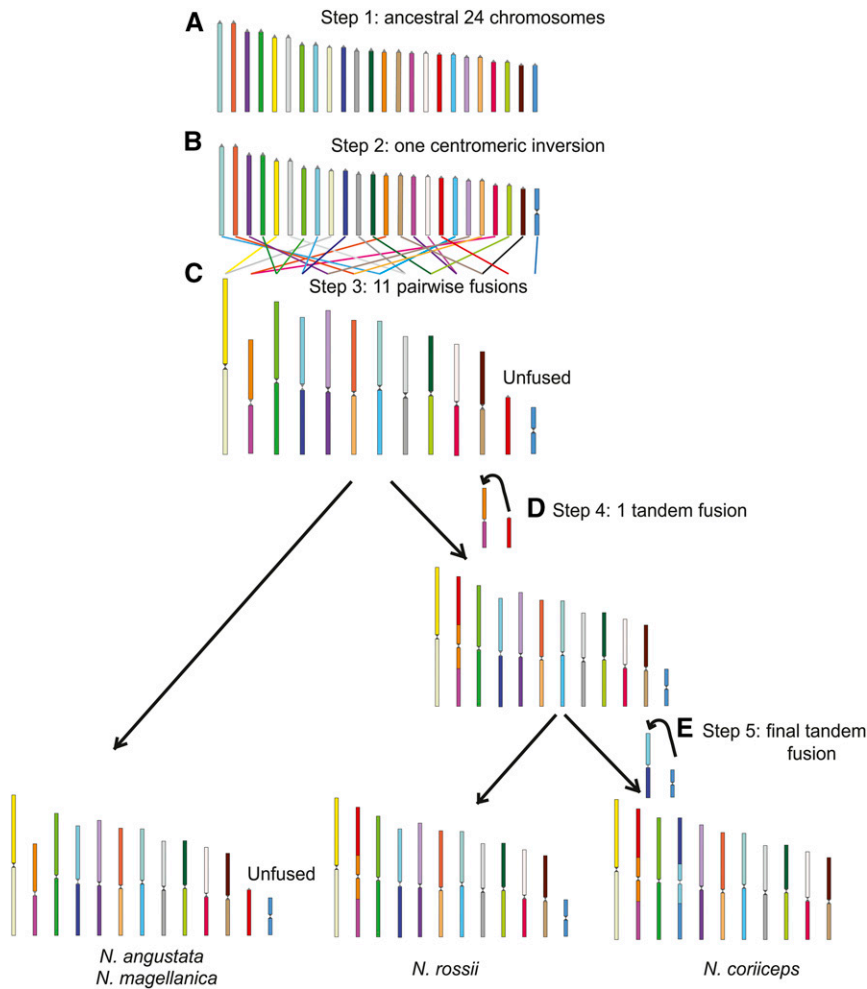
Figure 7 A model for the history of chromosome fusions in bullhead notothen *N. coriiceps*. (A) Presumed ancestral state. (B) In step 2, a pericentromeric inversion of a small acrocentric chromosome created a small metacentric. (C) After step 2, the fusion of 22 chromosomes one-to-one gave 11 large metacentrics, one unfused acrocentric, and one small unfused metacentric, as in today's *N. angustata* and *N. magellanica*. (D) Fusion of first one (step 4, as in *N. rossii*) and then the other (step 5, as in *N. coriiceps*) of the unfused chromosomes from (C) to form Nco2 and Nco4.

chromosome number. This is notable because the chromosome fusions do not appear to be recent; they are shared by other members of the *Notothenia* clade, including *N. magellanica* (n = 13), *N. angustata*, (n = 13), and *N. rossii* (n = 12). It is still possible that pericentric inversions occurred but that that they are small and masked by reduced recombination rates near centromeres. We know that other groups of Antarctic fish did experience recent pericentric inversions because, for example, *Pagetopsis macropterus* has a diploid number of 47 or 48, similar to the ancestral state, but an arm number of 58, rather than the ancestral values of 2n = 48 and NF = 52 as in several other icefish, including *Chaenocephalus aceratus*. Although putative paracentric inversions appear in some chromosome arms, without a closer outgroup, it is not possible to determine if the rearrangements happened before or after the chromosome fusions.

Recombination rates

Uneven recombination rates along chromosomes, like the situation discovered here for bullhead chromosomes, occur in other species too. In zebrafish and spotted gar for example, recombination rates are higher at the telomeres in the male map and higher near the centromeres in the female map (Howe *et al.* 2013; Amores *et al.* 2011). In contrast to bullhead notothen, where most chromosomes are similar in size, in zebrafish the female map is more than twice the length of the male map (2.74: 1.0) (Singer *et al.* 2002).

Segregation distortion

Segregation distortion in the male map on Nco1 and in the female map on Nco4 is unlikely to be due to genotyping errors because many adjacent markers show the same distortion; thus, this situation is likely to be biological in origin. Segregation distortion could be explained by interactions between male and female alleles: if one of the two alleles at a locus in the male parent was a partial stage-specific dominant embryonic lethal in the context of the female allele (this would be the $\ll$ 1: 1: 1: 1 case). In other cases, segregation distortion could result from epistatic interactions: if in the male, both alleles at locus "*a*" functioned well in the genomic context of male alleles at locus "*b*" but the male allele at locus "a" was deleterious in the context of heterozygosity for the female allele at locus "*b*." Or if the male were a heterozygote for a paracentric inversion, then after homologous pairing in meiosis, a recombination event within the deletion would result in monocentric and dicentric chromosomes, which would lead to aneuploid gametes, embryo lethality, and segregation distortion. Segregation distortion might also be expected for sex chromosomes, but our results showed distortion on different chromosomes in the female and male, making the sex chromosome explanation less likely. In addition, cytogenetic analyses of bullhead failed to detect any sex chromosomes (Phan *et al.* 1987; Ozouf-Costaz *et al.* 1991; Tomaszkiewicz *et al.* 2011).

Conclusions

The systemic and massive chromosome fusion events that occurred a few million years after the divergence of the *Notothenia* clade from the *Dissostichus* + *Lepidonotothen* + *Trematomus* clade provide a model to explain how teleosts could have evolved fewer chromosomes than their last common ancestor with their Holostean sister group (amia + gars), despite chromosome doubling associated with the TGD. Analyses of conserved syntenies of spotted gar (*L. oculatus*, Loc), chicken (*G. gallus*, Gga), and medaka fish (*O. latipes*, Ola) indicate that chromosome fusions occurred in the teleost lineage before the TGD (Braasch *et al.* 2016). For example, the two orthologous chromosome pairs Loc13/Gga14 and Loc15/Gga27 together show conserved synteny to both Ola19 and Ola8, which are duplicate (paralogous) chromosomes derived from the TGD (Braasch *et al.* 2016). This pattern would appear if, after the divergence of gar and teleost lineages, the teleost orthologs of Loc13 and Loc15 fused together, followed by the duplication of the fused chromosome in the TGD event to produce the homeologous (paralogous) medaka chromosomes Ola19 and Ola8. The organization of the gar genome shows that the fusions envisaged to have preceded the TGD (Nakatani *et al.* 2007) occurred in the few million years after the divergence of gar and teleost lineages but before the TGD. While our comparative genomic analysis of the bullhead map shows that rapid concerted, regular chromosome fusions can and do occur, they leave open several questions, including: what forces would methodically cause chromosomes to fuse regularly one-to-one in a lineage; did the one-to-one fusion of 22 chromosomes to 11 happen all at once or over a protracted period of several million years since the divergence of the *Notothenia* lineage from its sister group; and what caused the remaining two unfused chromosomes in the last common ancestor of *N. rossii* and *N. coriiceps* to fail to fuse to each other but instead join already fused chromosomes? A possible answer might be that some molecular feature of chromosome structure makes it more difficult to fuse a metacentric chromosome with another chromosome than to fuse two acrocentric or telocentric chromosomes.

LITERATURE CITED

Albertson, R. C., Y. L. Yan, T. A. Titus, E. Pisano, M. Vacchi *et al.*, 2010 Molecular pedomorphism underlies craniofacial skeletal evolution in Antarctic notothenioid fishes. BMC Evol. Biol. 10: 4.

Allendorf, F. W., and G. H. Thorgaard, 1984 Tetraploidy and the evolution of salmonid fishes, pp. 1–53 in *Evolutionary Genetics of Fishes*, edited by Turner, B. J.. Plenum Press, New York, NY.

Amores, A., and J. H. Postlethwait, 1999 Banded chromosomes and the zebrafish karyotype. Methods Cell Biol. 60: 323–338.

Amores, A., A. Force, Y. L. Yan, L. Joly, C. Amemiya *et al.*, 1998 Zebrafish hox clusters and vertebrate genome evolution. Science 282: 1711–1714.

Amores, A., J. Catchen, A. Ferrara, Q. Fontenot, and J. H. Postlethwait, 2011 Genome evolution and meiotic maps by massively parallel DNA sequencing: spotted gar, an outgroup for the teleost genome duplication. Genetics 188: 799–808.

Amores, A., J. Catchen, I. Nanda, W. Warren, R. Walter *et al.*, 2014 A RAD-tag genetic map for the platyfish (*Xiphophorus maculatus*) reveals mechanisms of karyotype evolution among teleost fish. Genetics 197: 625–641.

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver *et al.*, 2008 Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3: e3376.

Braasch, I., A. R. Gehrke, J. J. Smith, K. Kawasaki, T. Manousaki *et al.*, 2016 The spotted gar genome illuminates vertebrate evolution and facilitates human-teleost comparisons. Nat. Genet. 48: 427–437.

Buckley, B. A., and G. N. Somero, 2009 cDNA microarray analysis reveals the capacity of the cold-adapted Antarctic fish Trematomus bernacchii to alter gene expression in response to heat stress. Polar Biol. 32: 403–415.

Buckley, B. A., S. P. Place, and G. E. Hofmann, 2004 Regulation of heat shock genes in isolated hepatocytes from an Antarctic fish, *Trematomus bernacchii*. J. Exp. Biol. 207: 3649–3656.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko, and J. H. Postlethwait, 2011 Stacks: building and genotyping Loci de novo from short-read sequences. G3 (Bethesda) 1: 171–182.

Chakravarti, A., L. K. Lasher, and J. E. Reefer, 1991 A maximum likelihood method for estimating genome length using genetic linkage data. Genetics 128: 175–182.

Chen, L., A. L. DeVries, and C. H. Cheng, 1997 Evolution of antifreeze glycoprotein gene from a trypsinogen gene in Antarctic notothenioid fish. Proc. Natl. Acad. Sci. USA 94: 3811–3816.

Cheng, C. H., and L. Chen, 1999 Evolution of an antifreeze glycoprotein. Nature 401: 443–444.

Cheng, C. H., and H. W. Detrich, III, 2007 Molecular ecophysiology of Antarctic notothenioid fishes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 362: 2215–2232.

Detrich, H. W., III, B. Buckley, D. Doolittle, C. D. Jones, and S. Lockhart, 2012 Sub-Antarctic and high Antarctic notothenioid fishes: ecology and adaptational biology revealed by the ICEFISH 2004 cruise of the RVIB Nathaniel B. Palmer. Oceanography (Wash. D.C.) 25: 184–187.

Dettai, A., M. Berkani, A. C. Lautredou, A. Couloux, G. Lecointre, C. Ozouf-Costaz, and C. Gallut, 2012 Tracking the elusive monophyly of nototheniid fishes (Teleostei) with multiple mitochondrial and nuclear markers. Mar Genomics: 8: 49–58.

DeVries, A., 1988 The role of antifreeze glycopeptides and peptides in the freezing avoidance of Antarctic fishes. Comp. Biochem. Physiol. 90B: 611–621.

Doussau de Bazignan, M., and C. Ozouf-Costaz, 1985 Une technique rapide d'analyse chromosomique appliquee a sept speces de poissons antarctiques. Cybium 9: 57–74.

Eastman, J. T., 1993 *Antarctic Fish Biology: Evolution in a Unique Environment*. Academic Press, San Diego.

Eastman, J. T., 2005 The nature of the diversity of Antarctic fishes. Polar Biol. 28: 93–107.

Eastman, J. T., and A. R. McCune, 2000 Fishes on the Antarctic continental shelf: evolution of a marine species flock? J. Fish Biol. 57(Suppl. A): 84–102.

Egginton, S., C. Skilbeck, L. Hoofd, J. Calvo, and I. A. Johnston, 2002 Peripheral oxygen transport in skeletal muscle of Antarctic and sub-Antarctic notothenioid fish. J. Exp. Biol. 205: 769–779.

Fishman, L., A. Kelly, E. Morgan, and J. Willis, 2001 A genetic map in the Mimulus guttatus species complex reveals transmission ratio distortion due to heterospecific interactions. Genetics 159: 1701–1716.

Friedrich, C., and W. Hagen, 1994 Lipid contents of five species of notothenioid fish from high-Antarctic waters and ecological implications. Polar Biol. 14: 359–369.

Ghigliotti, L., F. Mazzei, C. Ozouf-Costaz, C. Bonillo, R. Williams *et al.*, 2007 The two giant sister species of the Southern Ocean, *Dissostichus eleginoides* and *Dissostichus mawsoni*, differ in karyotype and chromosomal pattern of ribosomal RNA genes. Polar Biol. 30: 625–634.

Ghigliotti, L., S. E. Fevolden, C. H. Cheng, I. Babiak, A. Dettai *et al.*, 2012 Karyotyping and cytogenetic mapping of Atlantic cod (Gadus morhua Linnaeus, 1758). Anim. Genet. 43: 746–752.

Ghigliotti, L., C. C. Cheng, C. Ozouf-Costaz, M. Vacchi, and E. Pisano, 2015 Cytogenetic diversity of notothenioid fish from the Ross sea: historical overview and updates. Hydrobiologia 761: 373–396.

Grutzner, F., G. Lutjens, C. Rovira, D. W. Barnes, H. H. Ropers *et al.*, 1999 Classical and molecular cytogenetics of the pufferfish *Tetraodon nigroviridis*. Chromosome Res. 7: 655–662.

Hagen, W., G. Kattner, and C. Friedrich, 2000 The lipid compositions of high-Antarctic notothenioid fish species with different life strategies. Polar Biol. 23: 785–791.

Hemmingsen, E. A., 1991 Respiratory and cardiovascular adaptations in hemoglobin-free fish: resolved and unresolved problems, pp. 191–203 in *Biology of Antarctic Fish*, edited by di Prisco, G., B. Maresca, and B. Tota. Springer Verlag, Berlin, Heidelberg.

Hoegg, S., H. Brinkmann, J. S. Taylor, and A. Meyer, 2004 Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. J. Mol. Evol. 59: 190–203.

Hofmann, G. E., B. A. Buckley, S. Airaksinen, J. E. Keen, and G. N. Somero, 2000 Heat shock protein expression is absent in the Antarctic fish, *Trematomus bernacchii* (Family Nototheniidae). J. Exp. Biol. 203: 2331–2339.

Howe, K., M. D. Clark, C. F. Torroja, J. Torrance, C. Berthelot *et al.*, 2013 The zebrafish reference genome sequence and its relationship to the human genome. Nature 496: 498–503.

Hurley, I. A., R. L. Mueller, K. A. Dunn, E. J. Schmidt, M. Friedman *et al.*, 2007 A new time-scale for ray-finned fish evolution. Proc. Biol. Sci. 274: 489–498.

Huth, T. J., and S. P. Place, 2013 De novo assembly and characterization of tissue specific transcriptomes in the emerald notothen, *Trematomus bernacchii*. BMC Genomics 14: 805.

Jaillon, O., J. M. Aury, F. Brunet, J. L. Petit, N. Stange-Thomann *et al.*, 2004 Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. Nature 431: 946–957.

Jones, F. C., M. G. Grabherr, Y. F. Chan, P. Russell, E. Mauceli *et al.*, 2012 The genomic basis of adaptive evolution in threespine sticklebacks. Nature 484: 55–61.

Kasahara, M., K. Naruse, S. Sasaki, Y. Nakatani, W. Qu *et al.*, 2007 The medaka draft genome and insights into vertebrate genome evolution. Nature 447: 714–719.

Kennett, J. P., 1977 Cenozoic evolution of Antarctic glaciation, the circum-Antarctic Ocean, and their impact on global paleooceanography. J. Geophys. Res. 82: 3843–3860.

Koch, K.-H., 2005 Antarctic icefishes (Channichthyidae): a unique family of fishes. A review, part I. Polar Biol. 28: 862–895.

Mazzei, F., L. Ghigliotti, G. Lecointre, C. Ozouf-Costaz, J.-P. Coutanceau *et al.*, 2006 Karyotypes of basal lineages in notothenioid fishes: the genus *Bovichtus*. Polar Biol. 29: 1071–1076.

Mazzei, F., L. Ghigliotti, J.-P. Coutanceau, H. W. Detrich, III, V. Prirodina *et al.*, 2008 Chromosomal characteristics of the temperate notothenioid fish *Eleginops maclovinus* (Cuvier). Polar Biol. 31: 629–634.

McCluskey, B. M., and J. H. Postlethwait, 2015 Phylogeny of zebrafish, a "model species," within danio, a "model genus". Mol. Biol. Evol. 32: 635–652.

Miller, M. R., T. S. Atwood, B. F. Eames, J. K. Eberhart, Y. L. Yan *et al.*, 2007a RAD marker microarrays enable rapid mapping of zebrafish mutations. Genome Biol. 8: R105.

Miller, M. R., J. P. Dunham, A. Amores, W. A. Cresko, and E. A. Johnson, 2007b Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. Genome Res. 17: 240–248.

Miyaki, K., O. Tabeta, and H. Kayano, 1995 Karyotypes in six species of pufferfishes, genus *Takifugu* (Tetraodontidae, Tetraodontiformes). Fish. Sci. 61: 594–598.

Morescalchi, A., J. C. Hureau, E. Olmo, C. Ozouf-Costaz, E. Pisano *et al.*, 1992a A multiple sex-chromosome system in Antarctic ice-fishes. Polar Biol. 11: 655–661.

Morescalchi, A., E. Pisano, R. Stanyon, and M. A. Morescalchi, 1992b Cytotaxonomy of Antarctic teleosts of the Pagothenia/Trematomus complex (Nototheniidae, Perciformes). Polar Biol. 12: 553–558.

Morescalchi, A., A. M. Morescalchi, G. Odierna, V. Stingo, and T. Capriglione, 1996 Karyotype and genome size of Zoarcids and Notothenioids (Teleostei, Perciformes) from the Ross Sea: cytotaxonomic implications. Polar Biol. 16: 559–564.

Nakatani, Y., H. Takeda, Y. Kohara, and S. Morishita, 2007 Reconstruction of the vertebrate ancestral genome reveals dynamic genome reorganization in early vertebrates. Genome Res. 17: 1254–1265.

Naruse, K., M. Tanaka, K. Mita, A. Shima, J. Postlethwait *et al.*, 2004 A medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. Genome Res. 14: 820–828.

Near, T. J., and C. H. Cheng, 2008 Phylogenetics of notothenioid fishes (Teleostei: Acanthomorpha): inferences from mitochondrial and nuclear gene sequences. Mol. Phylogenet. Evol. 47: 832–840.

Near, T. J., J. J. Pesavento, and C.-H. C. Cheng, 2004 Phylogenetic investigations of Antarctic notothenioid fishes (Perciformes: Notothenioidei) using complete gene sequences of the mitochondrial encoded 16S rRNAq. Mol. Phylogenet. Evol. 32: 881–891.

Near, T. J., A. Dornburg, K. L. Kuhn, J. T. Eastman, J. N. Pennington *et al.*, 2012a Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proc. Natl. Acad. Sci. USA 109: 3434–3439.

Near, T. J., R. I. Eytan, A. Dornburg, K. L. Kuhn, J. A. Moore *et al.*, 2012b Resolution of ray-finned fish phylogeny and timing of diversification. Proc. Natl. Acad. Sci. USA 109: 13698–13703.

Near, T. J., A. Dornburg, R. C. Harrington, C. Oliveira, T. W. Pietsch *et al.*, 2015 Identification of the notothenioid sister lineage illuminates the biogeographic history of an Antarctic adaptive radiation. BMC Evol. Biol. 15: 109.

O'Brien, K. M., H. J. Xue, and B. D. Sidell, 2000 Quantification of diffusion distance within the spongy myocardium of hearts from antarctic fishes. Resp. Phys. 122: 71–80.

Ocalewicz, K., 2004 Cytogenetic analysis of platyfish (Xiphophorus maculatus) shows location of major and minor rDNA on chromosomes. Hereditas 141: 333–337.

Ohno, S., J. Muramoto, L. Christian, and N. B. Atkin, 1967 Diploid-tetraploid relationship among old-world members of the fish family Cyprinidae. Chromosoma 23: 1–9.

Ozouf-Costaz, C., and M. Doussau de Bazignan, 1987 Chromosome relationship among 15 species of Nototheniidae, pp. 413–419 in *Proc. Vth Congr. Europ. Ichtyol., Stockholm, Sweden*, edited by Kullander, S. O., and B. Fernholm, Swedish Museum of Natural History, Stockholm.

Ozouf-Costaz, C., J. C. Hureau, and M. Beaunier, 1991 Chromosome studies on fish of the suborder Notothenioidei collected in the Weddell Sea during EPOS 3 cruise. Cybium 15: 271–289.

Papetti, C., H. S. Windisch, M. La Mesa, M. Lucassen, C. Marshall *et al.*, 2016 Non-Antarctic notothenioids: past phylogenetic history and contemporary phylogeographic implications in the face of environmental changes. Mar. Genomics 25: 1–9.

Phan, N. V., V. Gomes, H. Suzuki, and M. J. A. C. R. Passos, 1987 Karyotypes of two Antarctic fishes, *Notothenia gibberifrons* and *Notothenia coriiceps neglecta*. Jpn. J. Ichthyol. 33: 384–387.

Pisano, E., C. Ozouf-Costaz, J.-C. Hureau, and R. Williams, 1995 Chromosome differentiation in the subantarctic Bovichtidae species *Cottoperca gobio* (Gunther, 1861) and *Pseudaphritis urvillii* (Valenciennes, 1832) (Pisces, Perciformes). Antarct. Sci. 7: 381–386.

Pisano, E., F. Mazzei, N. Derome, C. Ozouf-Costaz, J.-C. Hureau *et al.*, 2001 Cytogenetics of the bathydraconid fish *Gymnodraco acuticeps* (Perciformes, Notothenioidei) From Terra Nova Bay, Ross Sea. Polar Biol. 24: 846–852.

Pisano, E., E. Cocca, F. Mazzei, L. Ghigliotti, G. di Prisco *et al.*, 2003a Mapping of alpha- and beta-globin genes on Antarctic fish chromosomes by fluorescence in-situ hybridization. Chromosome Res. 11: 633–640.

Pisano, E., L. Ghigliotti, F. Mazzei, and C. Ozouf-Costaz, 2003b Cytogenetic features of Notothenia angustata Hutton, 1875, an Antarctic fish living in non-Antarctic water, pp. 117–120 in Antarctic Biology in a Global

G3·Genes|Genomes|Genetics

Context, edited by Huiskes, A. H. L., W. W. C. Gieskes, J. Rozema, R. M. L. Schorno, and S. M. van der Vies et al. Backhuys Publishers, Leiden, The Netherlands.

Place, S. P., and G. Hofmann, 2005   Constitutive expression of a stress-inducible heat shock protein gene, hsp70, in phylogenetically distant Antarctic fish. Polar Biol. 28: 261–267.

Postlethwait, J. H., Y.-L. Yan, M. Gates, S. Horne, A. Amores et al., 1998   Vertebrate genome evolution and the zebrafish gene map. Nat. Genet. 18: 345–349.

Postlethwait, J. H., Y. L. Yan, T. Desvignes, C. Allard, T. Titus et al., 2016   Embryogenesis and early skeletogenesis in the antarctic bullhead notothen, Notothenia coriiceps. Dev. Dyn. 245: 1066–1080.

Prirodina, V. P., 1997   The direction of the karyotype specialization in the suborder Notothenioidei (Pisces, Perciformes). Cybium 21: 393–398.

Prirodina, V. P., and A. V. Neyelov, 1984   Karyotypes in two species of the genus Notothenia s.str. (Fam. Nototheniidae) from the West Antarctica. Proc. Zool. Inst. 127: 32–37.

Robertson, W. R. B., 1916   Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae. V-shaped chromosomes and their significance in Acrididae, Locustidae and Gryllidae: chromosome and variation. J. Morphol. 27: 179–331.

Santini, F., L. J. Harmon, G. Carnevale, and M. E. Alfaro, 2009   Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. BMC Evol. Biol. 9: 194.

Schartl, M., R. B. Walter, Y. Shen, T. Garcia, J. Catchen et al., 2013   The genome of the platyfish, Xiphophorus maculatus, provides insights into evolutionary adaptation and several complex traits. Nat. Genet. 45: 567–572.

Scherthan, H., 2012   Chromosome numbers in mammals. eLS. DOI: 10.1002/9780470015902.a0005799.pub3.

Shin, S. C., H. Ahn do, S. J. Kim, C. W. Pyo, H. Lee et al., 2014   The genome sequence of the Antarctic bullhead notothen reveals evolutionary adaptations to a cold environment. Genome Biol. 15: 468.

Sidell, B. D., M. E. Vayda, D. J. Small, T. J. Moylan, R. L. Londraville et al., 1997   Variable expression of myoglobin among the hemoglobinless Antarctic icefishes. Proc. Natl. Acad. Sci. USA 94: 3420–3424.

Singer, A., H. Perlman, Y.-L. Yan, C. Walker, G. Corley-Smith et al., 2002   Sex-specific recombination rates in zebrafish (Danio rerio). Genetics 160: 649–657.

Taylor, J., I. Braasch, T. Frickey, A. Meyer, and Y. Van De Peer, 2003   Genome duplication, a trait shared by 22,000 species of ray-finned fish. Genome Res. 13: 382–390.

The International Brachypodium Initiative, 2010   Genome sequencing and analysis of the model grass Brachypodium distachyon. Nature 463: 763–768.

Tomaszkiewicz, M., M. Hautecoeur, J.-P. Coutnceau, C. Bonillo, A. Dettai et al., 2011   Comparative cytogenetic studies of the Nototheniidae (Teleostei: Acanthomorpha) from the Indian (Kerguelen-Heard Plateau) and Atlantic (South Georgia, South Sandwich, Falkland/Malvinas, Bouvet Islands) sectors of the Southern Ocean, pp. 109–121 in The Kerguelen Plateau: Marine Ecosystem and Fisheries, edited by G. D. a. D. Welsford. Societe Francaise d'Ichtyologie, Paris.

Urton, J. R., S. R. McCann, and C. L. Peichel, 2011   Karyotype differentiation between two stickleback species (Gasterosteidae). Cytogenet. Genome Res. 135: 150–159.

Van, N. P., V. Gomes, H. Suzuki, and M. J. D. Passos, 1987   Cytogenetical studies of Antarctic fishes. 2. Karyotypes of 2 Antarctic fishes, Notothenia-Gibberifrons and Notothenia-Coriiceps-Neglecta. Jpn. J. Ichthyol. 33: 384–387.

Van Ooijen, J. W., 2006   JoinMap 4, Software for the Calculation of Genetic Linkage Maps in Experimental Populations., Kyazma B.V., Wageningen, The Netherlands.

Wible, J. R., G. W. Rougier, M. J. Novacek, and R. J. Asher, 2007   Cretaceous eutherians and Laurasian origin for placental mammals near the K/T boundary. Nature 447: 1003–1006.

Communicating editor: J. Ross-Ibarra