Trends in Genetics

Forum

Horizontal Gene Transfer in Vertebrates: A Fishy Tale

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The recent assembly of the herring genome suggests this fish acquired its antifreeze protein gene by horizontal transfer and then passed a copy on to the smelt. The direction of gene transfer is confirmed by some accompanying transposable elements and by the breakage of gene synteny.

There is widespread evidence for **horizontal gene transfer (HGT)** (see Glossary) between single-celled organisms [1] but the discovery a decade ago that a type II **antifreeze protein (AFP)** gene had been horizontally transferred between different fish species was a unique example of direct vertebrate to vertebrate DNA transmission (Figure 1, Key Figure) [2]. Subsequently it was suggested that the gene had been passed from a herring to a smelt [3].

HGT is rampant in prokaryotes, but it has also played a lesser, albeit important role in the acquisition of new traits in eukaryotes [1,4]. Beneficial HGT events from bacteria to single-celled eukaryotes include genes that have facilitated survival in extreme environments with high mercury, arsenic, salt, or temperature, or in icy seas when some diatoms and algae acquired AFP genes from bacteria. Here, any gene incorporated into a chromosome can be transmitted to offspring [1]. Conversely, for most multicellular eukaryotes, the only HGT events that will persist are restricted to the germline, so these most commonly occur between the host and a variety of closely associated organisms, such as bacterial **endosymbiotes**. Consequently, most HGT events to chordates were from single-celled organisms and the majority occurred shortly after this lineage arose [4].

Type II AFP is a homolog of a **calcium-dependent (C-type) lectin** [5] and needs Ca^{2+} to bind to and stop the growth of ice [6]. When AFPs circulate through the body of a fish, they can protect their host from freezing in icy seawater that can be up to 1°C colder than the **unprotected freezing point** of fish blood [7]. Thus, the acquisition of an AFP gene confers a substantial benefit to the recipient in this niche, an advantage that can be naturally

Glossary

Antifreeze protein (AFP): a protein that binds to the surface of ice, thereby preventing its continued growth at subzero temperatures.
Calcium-dependent (C-type) lectin: a carbohydrate-binding protein that requires Ca^{2+} for activity.
Endosymbiote: an organism living within the body or cells of another organism.
Horizontal gene transfer (HGT): (lateral gene transfer) the movement of genetic material between the genomes of organisms by means other than transmission from parent to offspring.
Synteny: conservation of the relative physical positions of two or more genes along the chromosomes of different species.
Transposable elements: DNA sequences that move and duplicate by various methods, spreading to dispersed locations throughout the genome.
Unprotected freezing point: the temperature at which ice can nucleate freezing in a fish lacking AFPs.

Key Figure

Taxonomic Relationships between the Three Lineages That Produce Type II Antifreeze Protein (AFP) (Blue), the Other Fish Examined Herein, and a Variety of Commonly Known Fishes, Adapted from a Time-Calibrated Phylogeny of 1990 Species [13]

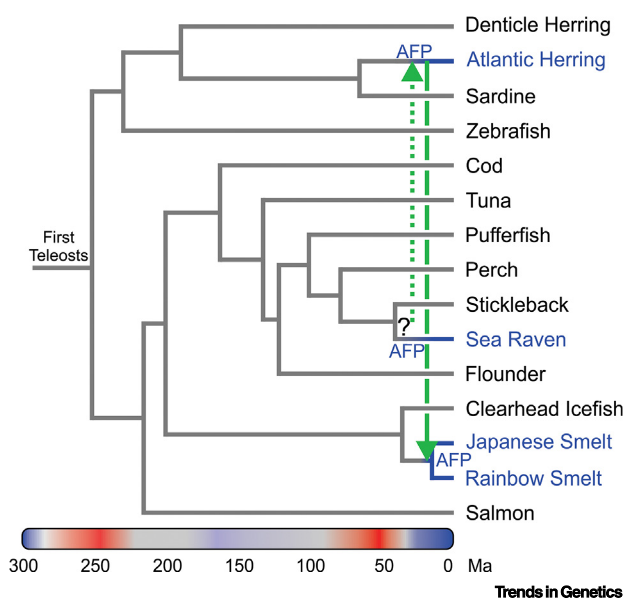

Figure 1. Warm periods, such as the Paleocene–Eocene thermal maximum at ~55 Ma are indicated in the bar at the bottom of the figure in red, whereas cooler periods, such as the late Cenozoic ice age, beginning ~34 Ma, are indicated in blue [14]. The directionality of the second horizontal gene transfer, from herring to smelt (green arrow), has been detailed in this study, whereas the first (green broken arrow), from the sea raven to herring lineages (?), still awaits confirmation.

selected for, as, for example, by amplifying the number of AFP genes [8]. Within fishes, type II AFP is found in three isolated branches of their phylogeny: herrings, cottids (sea raven), and smelts (Figure 1). Given the remarkable sequence conservation (up to 98%) of the introns of the herring and smelt genes over ~250 Ma, HGT is the most logical mechanism for their similarity [2]. The remarkable gain of this advantageous gene was postulated to have occurred via HGT from foreign DNA attaching to sperm during spawning [2], in a manner analogous to the technique of sperm-mediated gene transfer employed in laboratories to transfer genes to organisms, including fish [9].

The recently deposited Atlantic herring (*Clupea harengus*) genome sequence (GenBank Assembly Accession GCA_ 900700415.1) [10] has enabled examination of the AFP loci within this species and provides conclusive evidence for HGT. There are two loci containing AFP genes in the herring: one on chromosome 15 contains three AFP genes in tandem (Figure 2A); the other on chromosome 26 has seven AFP genes in tandem, the third and fourth of which appear to be pseudogenes (Figure 2B). Neither of these loci contains a progenitor gene (lectin) that could have given rise to the AFP gene through duplication and divergence [11] and all lectins elsewhere in the herring genome are quite dissimilar (<40% sequence identity). Moreover, the herring AFP genes arrived recently in their present loci because both sets interrupt a string of genes (**synteny**) that are intact in other Clupeiformes, including the denticle herring (*Denticeps clupeoides*) and the closely related sardine [*Sardina pilchardus*, same subfamily (Clupeinae)] (Figure 2A,B). Thus, the gene must have arrived in the Atlantic herring lineage after the recipient and the sardine lineages diverged.

We surmised that the subsequent transfer went from herring to smelt (*Osmerus*

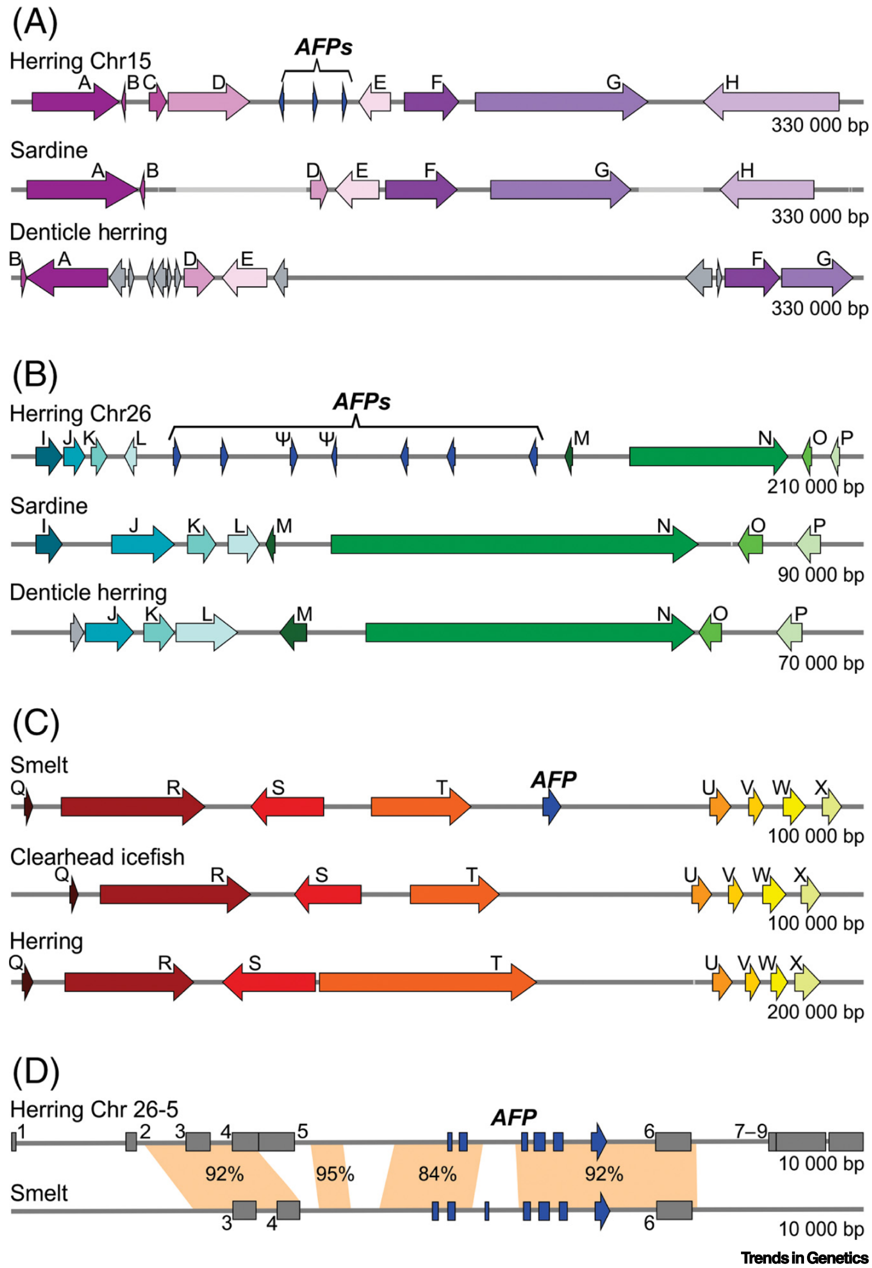

Figure 2. Comparison of the Antifreeze Protein (*AFP*)-Containing Regions of the Herring and Smelt Genomes to Those of Closely Related Fish. The AFP genes are colored blue and the syntenic flanking genes are lettered sequentially and colored in graduated shades, with arrows indicating their orientation. Nonsyntenic genes are colored gray. Gaps in the sequence are only indicated if in intergenic regions, by a lighter gray on the line. (A) Region of herring from base 4 210 211 to 4 540 210 of NC_045166.1, sardine from base 1 200 001 to 1 530 000 of UIGZ01000042.1, and denticle herring from base 1 371 001 to 1 701 000 of NC_041720.1. (B) Region of herring from base 8 065 000 to 7 855 001 of NC_045177, sardine from base 256 000 to 166 001 of UIGZ01000477.1, and denticle herring from base 23 430 001 to 23 500 000 of NC_041709.1. (C) Region of smelt from base 15 001 to 115 000 of JQ514278.1, icefish from base 11 075 001 to 11 175 000 of JAAIVE010000069.1, and herring from base 16 600 000 to 16 400 001 of NC_045152.1. (D) Detailed comparison of the smelt AFP gene with the rightmost herring AFP gene on chromosome 26. Individual exons are shown in blue and transposable elements are presented as gray rectangles. Pale orange shading shows matching regions with percent identity indicated.

mordax) [3] because the herring has a larger number of AFP gene copies within its genome (eight versus one). A phylogenetic analysis led Sorhannus to conclude the opposite [12]. However, the direction of transfer is confirmed here to be from herring to smelt because the matching segments of the AFP gene in the smelt, which share 84 to 95% identity with the herring AFP gene, contain three putative **transposable elements** (Figure 2D). Each transposable element is of a different type and each is found hundreds of times in the herring genome, but two of the three (3 and 6) are absent from the genomes of the other fish examined herein, including that of the closely related sardine. This fortuitous 'tagging' of the gene in the smelt with transposable elements, two of which are peculiar to the herring, substantiates both horizontal transfer and the direction from herring to smelt.

The location of the single AFP gene in rainbow smelt is known [3] and the synteny of the surrounding genes has been well conserved over the 250 million years since the smelt and herring diverged (Figure 2C). The conspicuous absence of an AFP gene at this location in the herring (Figure 2C), as well as in the clearhead icefish (*Protosalanx chinensis*), which is a close relative of the smelt, again confirms that these HGT events are evolutionarily recent. An earlier HGT, between the herrings and the group from which the sea raven emerged, is also postulated (Figure 1) [2], but a detailed analysis is not possible because key genomic sequences have yet to be determined.

The sequential transfer of an advantageous gene between fishes leads us to suggest that these events, while extremely rare, might happen as consequence of external fertilization in a medium containing the shed DNA of all the ecosystem's inhabitants. It will be worth examining fish for other examples of HGT.

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Declaration of Interests

The authors declare that they have no competing interests.

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References

1. Van Etten, J. and Bhattacharya, D. (2020) Horizontal gene transfer in eukaryotes: not if, but how much? *Trends Genet.* 36, 915–925
2. Graham, L.A. *et al.* (2008) Lateral transfer of a lectin-like antifreeze protein gene in fishes. *PLoS One* 3, e2616
3. Graham, L.A. *et al.* (2012) Smelt was the likely beneficiary of an antifreeze gene laterally transferred between fishes. *BMC Evol. Biol.* 12, 190
4. Crisp, A. *et al.* (2015) Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. *Genome Biol.* 16, 50
5. Ewart, K.V. and Fletcher, G.L. (1993) Herring antifreeze protein: primary structure and evidence for a C-type lectin evolutionary origin. *Mol. Mar. Biol. Biotechnol.* 2, 20–27
6. Ewart, K.V. *et al.* (1996) Ca2+-dependent antifreeze proteins. Modulation of conformation and activity by divalent metal ions. *J. Biol. Chem.* 271, 16627–16632
7. DeVries, A.L. (1971) Glycoproteins as biological antifreeze agents in Antarctic fishes. *Science* 172, 1152–1155
8. Hew, C.L. *et al.* (1988) Multiple genes provide the basis for antifreeze protein diversity and dosage in the ocean pout, *Macrozoarces americanus*. *J. Biol. Chem.* 263, 12049–12055
9. Lavitrano, M. *et al.* (2013) Methods for sperm-mediated gene transfer. *Methods Mol. Biol.* 927, 519–529
10. Pettersson, M.E. *et al.* (2019) A chromosome-level assembly of the Atlantic herring genome-detection of a supergene and other signals of selection. *Genome Res.* 29, 1919–1928
11. Deng, C. *et al.* (2010) Evolution of an antifreeze protein by neofunctionalization under escape from adaptive conflict. *Proc. Natl. Acad. Sci. U. S. A.* 107, 21593–21598
12. Sorhannus, U. (2012) Evolution of type II antifreeze protein genes in teleost fish: a complex scenario involving lateral gene transfers and episodic directional selection. *Evol. Bioinform. Online* 8, 535–544
13. Betancur, R.R. *et al.* (2017) Phylogenetic classification of bony fishes. *BMC Evol. Biol.* 17, 162
14. Hansen, J. *et al.* (2013) Climate sensitivity, sea level and atmospheric carbon dioxide. *Philos. Trans. A Math Phys. Eng. Sci.* 371, 20120294