

## **A potential role for antifreeze protein gene dosage in wolffish speciation**

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Atlantic wolffish (*Anarhichas lupus* – AW) is a marine fish found in the ice-laden shallow waters of the North Atlantic Ocean. It resists freezing in this environment by producing high levels of type III antifreeze protein (AFP) that provide a plasma freezing point depression of ~1.85 °C. In comparison, the deeper-dwelling spotted wolffish from the same genus (*A. minor* – SW) produces only a fraction of this amount of AFP, which is insufficient to prevent its plasma from freezing in ice-laden seawater.

The main reasons for the large difference in winter AFP levels between these two species are gene dosage and mRNA levels. Southern blotting shows that the AW has approximately three times as many AFP gene copies than the SW, with two thirds arrayed in tandem repeats that appear absent in SW. AFP mRNA levels in SW liver are more than three-times lower than those in AW liver, suggesting decreased rates of AFP gene transcription in this tissue. Interestingly, levels in some other tissues appear elevated for SW.

A difference in AFP gene dosage may have led to, or accelerated, speciation from a common ancestor of AW and SW, whereby individuals with high AFP expression levels were able to colonize ice-laden shallow seawater habitats during glacial episodes. They may then have become reproductively isolated from populations with lower AFP levels confined to ice-free waters below 50 m, where the temperature is always above the freezing point of seawater.

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