Stickleback males show splendid coloration at spawning time – with a red throat and breast and bright blue irises.

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A FISH AT HOME IN ALL WATERS

TEXT: ELKE MAIER

Three-spined stickleback fish live in both salt and fresh water. When the glaciers melted at the end of the last ice age, new lakes were formed, and sticklebacks from the sea found new habitats in those freshwater environments. At the Friedrich Miescher Laboratory on the research campus of the Max Planck Society in Tuebingen, Germany, Felicity Jones and her team are studying how the genome of fish changes as they adapt. 12,000-year-old stickleback bones provide insight into the early phase of this transformation.

The story begins with a chance find. In spring 2018, a team of geologists from the Geological Survey of Norway (NGU) set out on a journey to the northernmost region of Norway. The goal of the expedition was to drill for cores in the sediment of lakes near the coast in order to gain further insight into the sea level fluctuations towards the end of the last ice age. When geologist Anders Romundset filtered his sediment samples through a fine sieve, he found not only algae and other plant residues but also the bony residues of fish that were only a few millimeters in size.

The tiny bones and spines were so well preserved that the scientist knew right away what he was dealing with: the three-spined stickleback *(Gasterosteus aculeatus)*. The four to six-centimeter fish are still found in the sea and lakes of Scandinavia. Using radiocarbon dating, the bones were determined to be around 12,000 years old. They thus date from a time when large parts of northern Europe were still covered by massive ice sheets.

When the glaciers melted towards the end of the Ice Age and the enormous weight of the ice also melted away, the compressed land mass gradually began to rise above sea level. In the process, bays and inlets were cut off from the sea and were subsequently filled with fresh water, thereby creating new lakes. One of those lakes was once home to the stickleback, the bones and spines of which were found by geologist Anders Romundset. During the intervening millennia, the bony relics remained embedded in the sediment at the bottom of the lake. The geologists turned their find over to Andrew Foote, evolutionary ecologist at the Norwegian University of Science and Technology in Trondheim. Foote, an expert in ancient DNA, seized the opportunity. In a special laboratory at the University of Copenhagen, he and his colleague Tom Gilbert began searching the bones for remnants of genetic material. One of the difficulties was that the samples contained not only stickleback DNA, but also fragments of DNA from other organisms (e.g., plants and bacteria) that were also living in that environment at the time. The fragments they were looking for ultimately made up only around 1% of this genetic hodgepodge. Nevertheless, with great effort, Foote managed to 'fish out' and sequence the stickleback DNA.

For Felicity Jones, research group leader at the Friedrich Miescher Laboratory in Tuebingen, Foote's success was a stroke of luck. Together with her team, Jones studies the fundamentals of evolution. The researchers hope to

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find out which molecular mechanisms ensure that organisms can adapt to new habitats or even form new species. Sticklebacks are the ideal model organisms for this purpose. Over the last 10,000 to 20,000 generations, marine sticklebacks have migrated to many different freshwaters (e.g., lakes, rivers, and marshes) and have adapted to the new conditions. These small fish are thus now widespread in the temperate climates of the northern hemisphere.

"The exciting thing for us is that sticklebacks have left the sea to colonize new freshwater habitats and in many cases did so independently," says Jones, who also conducted research in Scotland, New Zealand, and the U.S. before coming to Tuebingen. "This allows us to study the same questions in multiple parallel systems, thereby ruling out the possibility that the genetic adaptations we discover are merely isolated cases." As the fish adapted to their new environment, similar changes in their shape, behavior, and physiology occurred repeatedly at different sites – a process known as parallel evolution. Like in a giant field laboratory, the researchers can use sticklebacks to work out the basic molecular mechanisms that ensure that organisms can adapt to new habitats.

Over 160 years ago, Charles Darwin provided the first plausible explanation of how the diversity of life arose in his book, On the Origin of Species. According to Darwin, all present-day species are descended from common ancestors, the descendants of which have dispersed to different habitats and split into distinct lineages over millions of years. The driving force behind this development is natural selection. Of all the offspring of a living being, those that are best adapted to their environment have the greatest reproductive success. They therefore tend to pass on their hereditary traits to the next generation.

Famous examples of Darwin's theory are the finches of the Galapagos archipelago. Originating from one ancestral species, the birds living on the various islands of the archipelago have evolved quite different beak shapes depending on what types of food they consume. Evolutionary biologists refer to this as adaptive radiation. "Sticklebacks are another example – only much better," says Jones. Unlike the Galapagos finches, the evolutionary split occurred in several places at once. This allows the scientists to repeatedly observe the spectacle and thus verify their findings.

SUMMARY

Sticklebacks live in both salt and fresh water. They have colonized new freshwater habitats from the sea multiple times.

These small fish are ideal model organisms for studying how living organisms can adapt to changing environments and even form new species.

Max Planck researchers have analyzed DNA from 12,000-year-old stickleback bones found in ice-age lake sediment in Norway. The data provide insight into the evolutionary history of Scandinavian sticklebacks.

How the sticklebacks change in the course of adaptation is superbly demonstrated by the formation of bone plates on the sides of their bodies. Marine stickleback have armor plates covering the entire sides of their body, while freshwater sticklebacks have only a few plates towards the front of their body.

The reasons for this are still being investigated. Scientists hypothesize that the armor plates on individuals in the open sea protect them from predators that freshwater sticklebacks do not encounter. An additional explanation is that limited amounts of calcium in freshwater habitats make growth of bony plates costly. Amazingly, when new freshwater habitats are colonized by high plated marine sticklebacks, the population rapidly evolves to a low-plated form within less than one hundred generations.

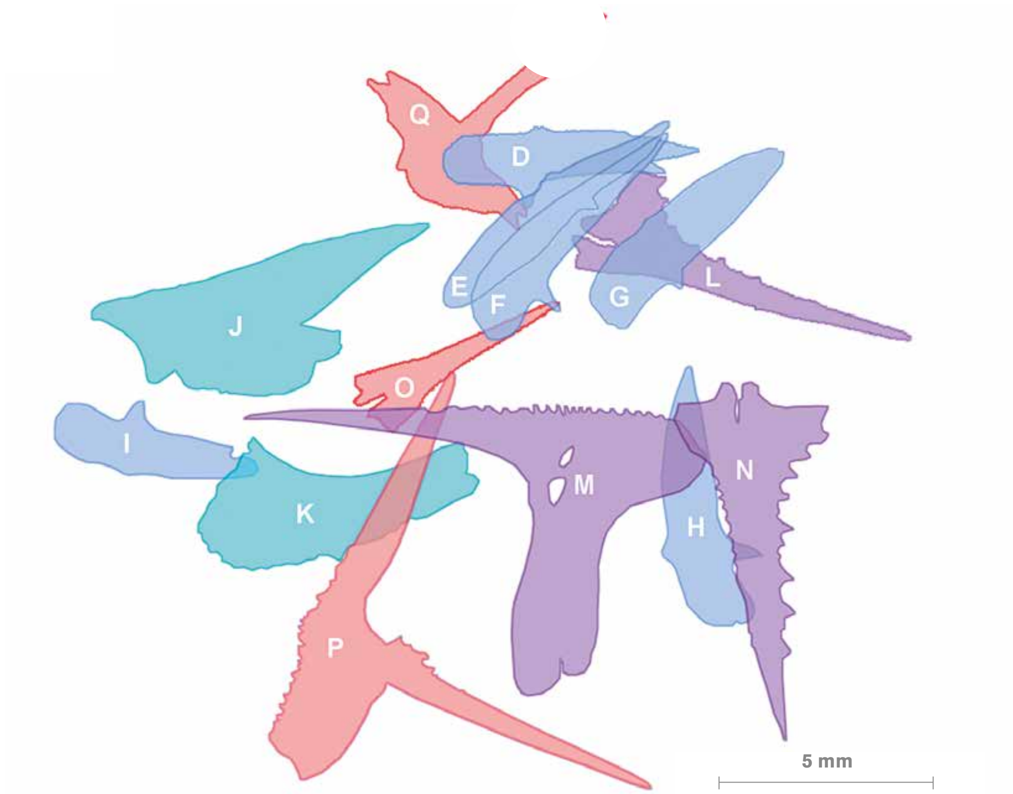
Evolutionary biology has made tremendous progress since Darwin. Thanks to modern analytical methods, researchers can now search the genome for the characteristic traces that evolution has left behind. "Most of our knowledge about how the genome works comes from model organisms specially bred in the laboratory. In contrast, we still know relatively little about how naturally occurring genetic differences influence the evolution of wild populations," says Jones.

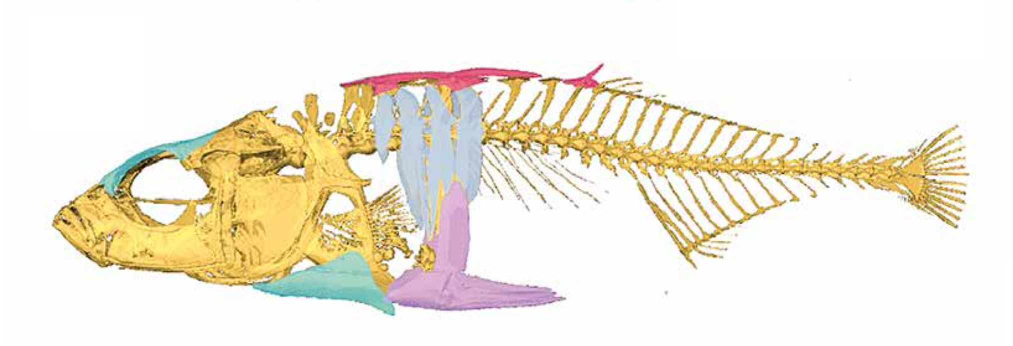
A window into the past

The discovery of prehistoric bones opened up completely new possibilities for the Tuebingen team and their research partners. For the first time, they had an ancestor of the freshwater stickleback in front of them. This fish, which lived about 12,000 years ago, also revealed genetic information. "To our knowledge, these are the oldest fish bones from which genomic data have ever been obtained," says Jones. "They open a window into the past, allowing us to trace which genetic variation the fish brought with them as they adapted to their new habitats."

The bony relics were found in a sedimentary layer marking the transition from salt to fresh water. As individual coves and inlets gradually became cut off from the sea toward the end of the Ice Age, sticklebacks were left behind in the isolated waters. They adapted to the new conditions of brackish water and reproduced. Over time, numerous new types emerged in different waters; these sticklebacks are quite distinct from their marine relatives.

IMAGE: ANDY FOOTE, MELANIE KIRCH, ANDERS ROMUNDSET





Relics of the ice age: in order to identify them, the researchers compared the skeletal elements they found (top) with X-ray images of a present-day stickleback (below). This allowed them to match the ancient bones to a single individual.

The fish varied in body size and pigmentation, in the length of their dorsal spines, and in the size and number of their bony plates.

In order to get an idea of what had changed at the genetic level, the researchers compared the genetic makeup of the Ice Age stickleback with that of its descendants. To do so, they analyzed sticklebacks from two near-shore lakes south of the Norwegian city of Hammerfest. The bones that the geologists discovered and which contributed their prehistoric DNA also came from one of these lakes. The researchers also sequenced the genomes of marine sticklebacks from the same area.

The comparison of the genetic material revealed that the ice age fish was genetically quite similar to its present-day marine relatives. “The bones contained mostly those gene variants that are advantageous for living in salt water,” explains Melanie Kirch, a doctoral student in Jones’s research group, who analyzed much of the genomic data. There were also variants that showed an adaptation to freshwater.

Such gene variants are also found in isolated cases among today’s marine sticklebacks. The researchers assume

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Fish in color: the researchers stained a stickleback skeleton to make individual bone fragments visible. Freshwater and saltwater sticklebacks differ in the length of their spines or the size and number of bone plates they grow on their bodies to protect themselves from predators.

that the latter occasionally mate with conspecifics from freshwater habitats (e.g., in the estuaries of rivers). As a result, freshwater genetic variants keep entering the marine population. For marine sticklebacks, these variants are useless or even disadvantageous and therefore do not spread. In contrast, they prove to be wild cards when colonizing new freshwater habitats. If evolution can access such ready-made building blocks, adaptation is possible in a relatively short time (in the case of the stickleback, within only a few decades). If, on the other hand, the appropriate gene variants first have to be created by chance through mutation, millions of years can sometimes pass.

The genome comparison provided even more insight into the evolution of freshwater sticklebacks. It revealed that the fish from the two lakes were genetically less diverse than their marine ancestors. This was partly because the isolated individuals that recolonized the lakes at that time brought with them only a small fraction of the gene variants that were present among marine sticklebacks. On the other hand, some variants within the newly established populations randomly disappeared from the gene pool over time – a process known as genetic drift. “That is how even those variants that would be beneficial for freshwater life have been lost – by chance alone,” says Jones. Such severe genetic impoverishment – typical of small founder populations – is often referred to as a “genetic bottleneck”.

For freshwater sticklebacks, this bottleneck was momentous. Genetic variation is the material from which evolution produces new adaptations. If many different gene variants are present, it can draw from the full range. In contrast, variation was greatly reduced in fish from the two lakes. “We suspect they are not as well adapted to their habitat as they could be,” says Jones.

Jones and Foote are excited about the new possibilities that ice age DNA is offering. They would like to study the evolutionary history of Scandinavian sticklebacks in greater detail. One of their goals is to genetically evaluate even more prehistoric stickleback bones from younger sedimentary layers. “If we had bones not just from a single fish but rather from multiple individuals that lived hundreds of years apart, we could directly track how the genome changed over time after the sticklebacks arrived in their new habitat,” says Jones.