Sculpin Liaisons

The sculpins of **Arne Nolte**, head of a research group at the **Max Planck Institute for Evolutionary Biology** in Plön, near Kiel, are no beauties; yet these unprepossessing fish, first discovered in the Lower Rhine in the 1990s, hold a special fascination for him. After all, these particular sculpins are hybrids, the shared offspring of two species.

TEXT **HARALD RÖSCH**

It was an historic moment as, in 1807, the workers drove their shovels and pickaxes into the muddy ground near Antwerp, Belgium one last time. At Napoleon's behest, they had created a connection between the River Scheldt and a branch of the Rhine, just 35 kilometers away at this point, thus bringing to fruition the emperor's plan to link navigation along the Rhine with the then-French port of Antwerp. The fact that their work was also responsible for the birth of a new species of fish would have gone unnoticed were it not for the fact that, almost 200 years later, ichthyologists discovered a type of fish previously unknown in the Rhine.

The artificial waterway between the Scheldt and the Rhine opened a gateway to the east for the sculpin *Cottus perifretum*. Unfazed by the turmoil and wars in Europe in the 19th and 20th centuries, the small bottom-dwelling fish saw its chance. It let the current carry it out of its habitat in the upper reaches of the Scheldt tributaries,

Photo: Andreas Hartl

Sculpin, bullhead, miller's thumb – the fish of the genus *Cottus* are known by many names. The males mind their offspring with devotion, guarding the eggs on the underside of large rocks from predators, and fanning their large pectoral fins to ensure a constant supply of oxygen.

Photo: Andreas Hartl

swam through the new canal, and ended up in the Rhine estuary.

In fact, *Cottus perifretum* can't survive for long in large rivers, as it doesn't like mud at all. The water there also gets too warm in summer, and there's not enough oxygen. In the Rhine, however, the newcomer from the Scheldt met a close relative, the Rhine sculpin *Cottus rhenanus*. Its name is misleading in that *Cottus rhenanus* doesn't live in the Rhine itself, but in the tributaries of the Middle and Lower Rhine. As with its more westerly counterparts, however, it can happen that individual fish get carried down in the main current from the headwaters of the tributaries. Somewhere in the Lower Rhine region, shortly before the river empties into the North Sea, the two species managed to produce offspring despite the unfavorable conditions.

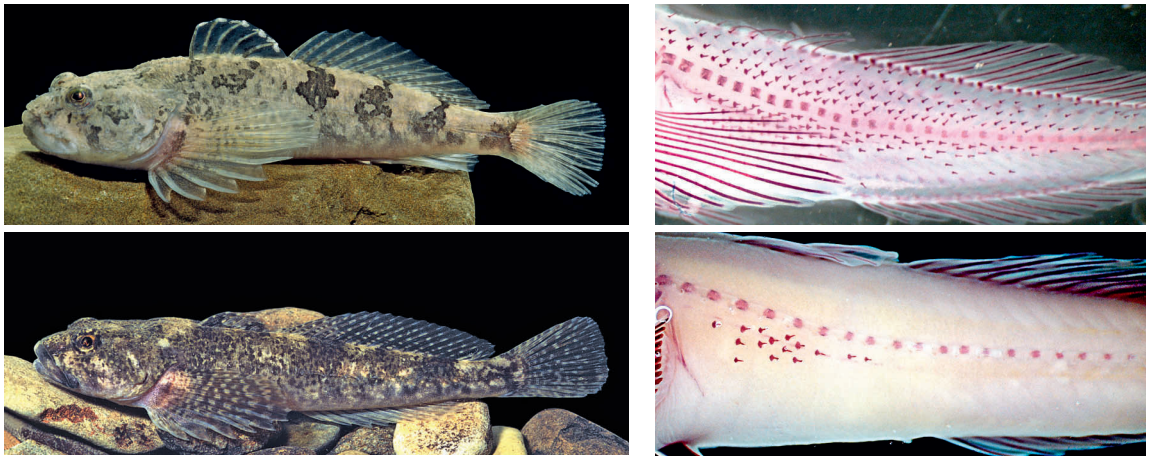
Helped by massive ecological changes in the lower reaches of the Rhine, the offspring of this liaison have been hugely successful since then, conquering almost the entire Rhine. Also known as bullheads, the sculpins have migrated upstream at a rate of five to ten kilometers per year, reaching the Upper Rhine at Karlsruhe and the lower reaches of the Main river.

NO PLACE FOR SCULPINS

Arne Nolte has been tracking the spread of the new sculpins, which were first sighted some 20 years ago in the lower reaches of the Rhine. At that time, fish experts were at a loss, insofar as sculpins had never been observed in a similar habitat, let alone one that had been so significantly altered by human activity. "It was like finding brown trout in the Rhine, or carp in a mountain stream," says Nolte; but that wasn't the only surprise. Using genetic analysis, Nolte verified in 2005 that the newcomers were the result of crossbreeding, or hybridization, as biologists call it. Although they look more like the Scheldt parental species on the outside, genetically they are a mixture of *Cottus perifretum* and *Cottus rhenanus*.

The fact that these crossbreeds can apparently conquer a new habitat with more success than their parent species goes against the conventional wisdom that has long prevailed among evolutionary biologists. "Hybrids used to be seen as slip-ups that weren't really intended in nature. They were thought of as low-value rejects that couldn't reproduce even if they arose in the first place," explains Nolte. A prime example of this is the mule, a sterile creature arising from the hybridization of a horse and a donkey.

The reason for this attitude lies in the definition of a biological species as set out by biologist Ernst Mayr in the 1940s: a community of individuals that reproduce among themselves. All individuals that can come together to pro-



left: *Cottus perifretum* (top) and *Cottus rhenanus* (bottom) look quite similar to the layperson. Their markings and pigmentation don't aid identification, since they also vary from habitat to habitat within a species. Hybrids of both species resemble *Cottus perifretum*.

right: Ichthyologists distinguish between the two sculpin species by the degree to which spinelike scales cover the body (made visible here by staining). In *Cottus perifretum*, this prickling covers the sides and caudal peduncle (top), while in *Cottus rhenanus*, it occurs only behind the pectoral fins (bottom), if at all.

duce viable offspring would then belong to the same species. However, it has since become apparent that Mayr's definition is too restrictive and disregards many clearly different species.

Botanists were quick to realize that different plant species often interbreed and yet still generate viable offspring. Zoologists weren't so quick off the mark, but the new sculpins in the Rhine are just one example of the fact that animal species, too, regularly intermingle in nature. Not only that, but Nolte is convinced that hybrids may play a central role in the emergence of new species. It is estimated that approximately one out of every ten fish and bird species hybridizes with another species. This would suggest that hybridization constantly provides for new genetic variants, especially between closely related species. "Hybrids are more than just accidents of nature; on the contrary, they may have made a major contribution to biodiversity on Earth. Were it not for that intermingling, we would have fewer species," says Nolte.

A BIG SMORGASBORD

Nolte hopes the sculpins will show him how hybrids generate new species, but he began by focusing on the diversity of European fish fauna. This had received only cursory attention until the 21st century, the first comprehensive and systematic compilation of European fish being published in 2007. Little wonder, then, that the nature of the relationships between sculpin populations had attracted little attention. Ichthyologists had often lumped the sculpins of Central Europe together in one basket, referred to as the *Cottus gobio* complex. Working at the time with Maurice Kottelat and Jörg Freyhof at the Alexander Koenig Research Museum in Bonn, Nolte identified three different species out of that conglomeration, all of which occur in the Lower Rhine region and in directly neighboring rivers.

HOW IS A SPECIES BORN?

For a new species to develop, the gene flow within a species must be prevented or at least restricted. This is the case when individuals are spatially isolated from the population, by a river or mountain range for example (**allopatric speciation**). The two groups then evolve differently so that, given sufficient time, two different species emerge. Scientists have often observed this form of speciation in birds.

However, new species can also develop without spatial separation. In a given population, individuals with an extreme characteristic may be ideally adapted to the environment, such as when small individuals specialize in one food type and large individuals in another. In this kind of **sympatric speciation**, the gene flow is inhibited if the two groups prefer their own type and no longer reproduce with the other. This process has been well documented particularly for fish species and insects.

Genetic exchange between populations can also be prevented if, for example, they specialize in different habitats or develop different courtship behavior, mate at different times, or if the hybrids are inferior to both original populations.

Photos: Jörg Freyhof – Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin (left, 2), Jie Cheng – MPI for Evolutionary Biology (right, 2)

» Without hybrids, there would be fewer species on Earth.

Arne Nolte has had an interest in fish since his childhood years. Cichlids, loach, scalar, killifishes – his aquariums were a veritable "Who's Who" of popular pet fish. His inventory comprised up to 40 different tanks at its height; even the vegetables had to be moved out of his parents' greenhouse to make room for the fish. It was already clear to the budding biologist that he wanted to work with fish. Since his university in Oldenburg offered no such opportunity, he traveled through Europe on his own and collected sculpins in order to identify them. As a result, practically no one knows these fish as well as Nolte.

His analyses soon showed that morphology alone would no longer be sufficient for species identification. Genome analysis became an increasingly important tool for identifying taxonomic relationships. In Diethard Tautz's laboratory at the University of Cologne, Nolte appropriated the molecular biology equipment that he would use for sculpin analysis. In 2008, Tautz, by then Director at the Max Planck Institute in Plön, called on him to join his team.

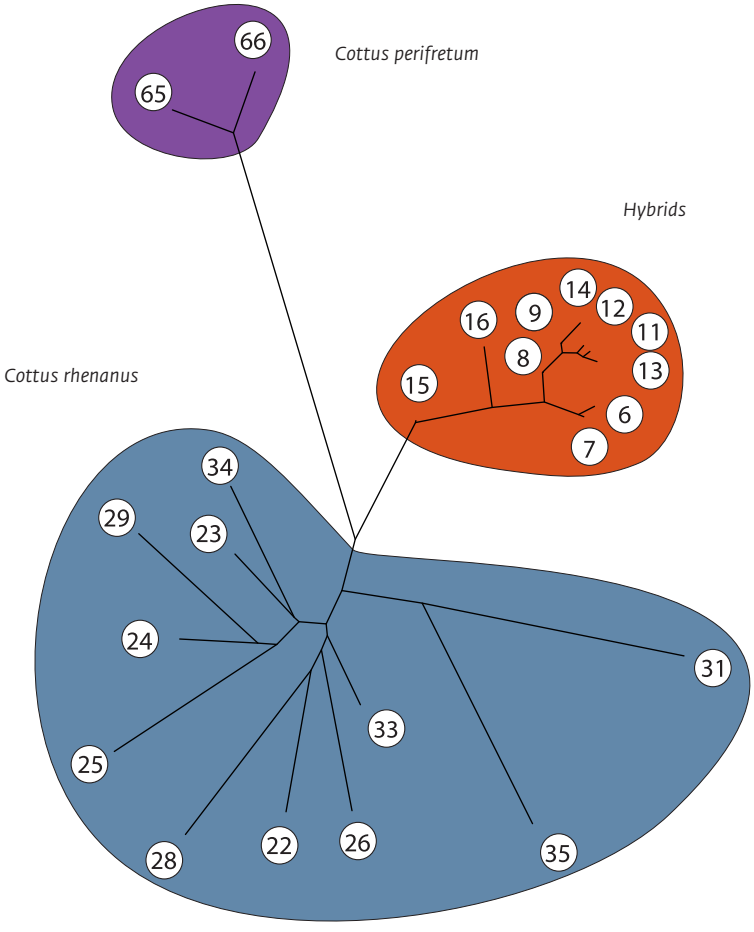
A look at the sculpin genome uncovered what wasn't visible to the naked eye: the sculpins that turned up in the Rhine are genetic hybrids. Since that discovery, genetic analysis has shaped Nolte's day-to-day research. With its help, he hopes to find out how the hybrid sculpins evolve into a new species in the new habitat, and what makes them so successful.

Nolte seldom goes out on fishing trips now, because his analyses require that the fish be held under controlled conditions and that they interbreed in a specific way. Consequently, his preferred subjects swim in the fish room of Plön's Max Planck Institute, in neat rows of aquariums supplied with fresh water from Schöhsee, a nearby lake.

It's a stroke of luck for scientists to be able to observe the birth of a species at such an early stage. In many cases, biologists study populations that have already been diverging for thousands or even millions of years. "Our hybrid lineage is probably no more than 200 years old. That's quite unique," says Arne Nolte.

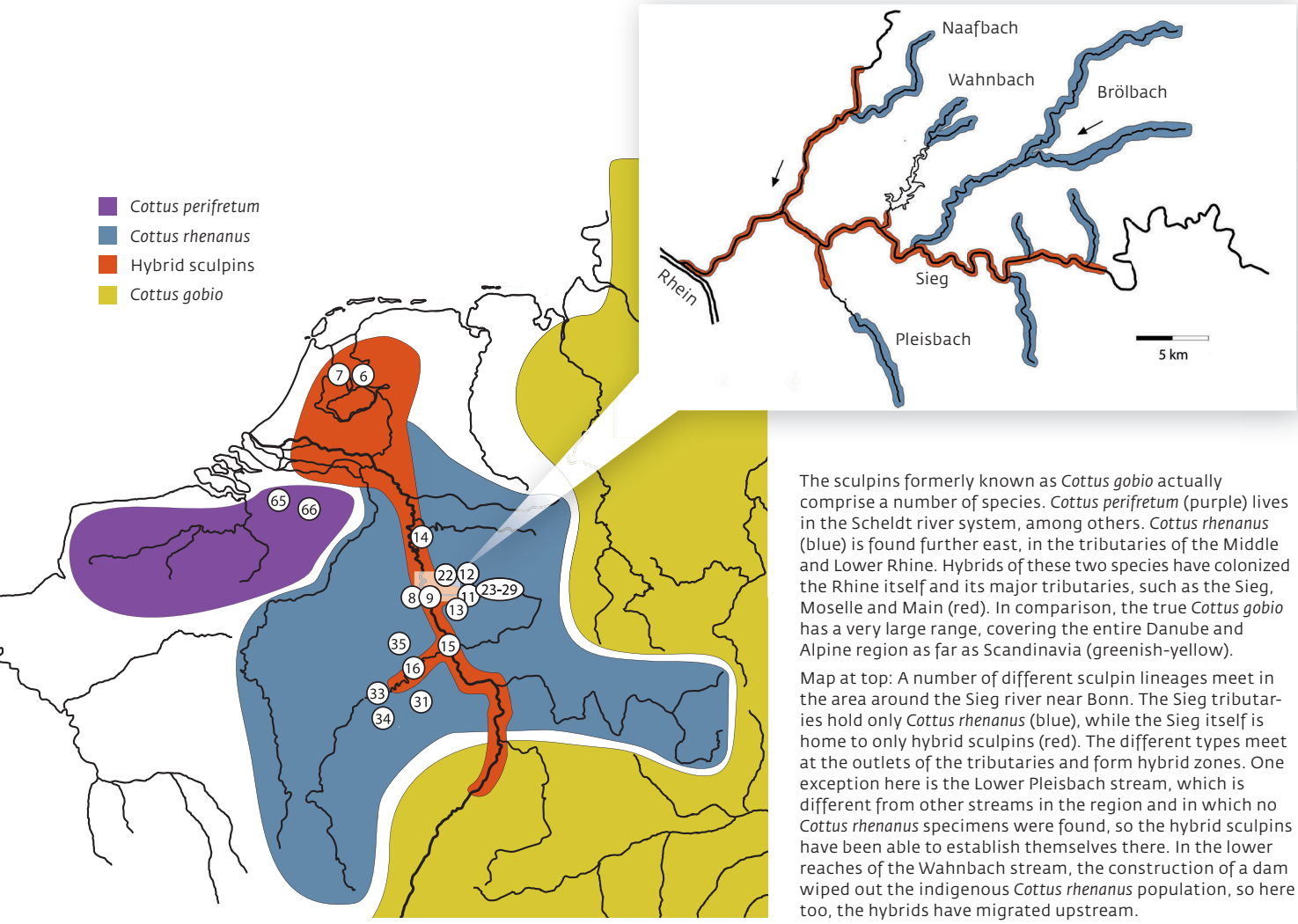
HYBRID FITNESS

First, the researchers wanted to clarify whether the offspring of the hybrid sculpins inherit a genetic handicap. When hybrids reproduce, parts of the genotypes from both parent species are mixed together, and specific mutations that affect the viability of the hybrids can occur. Sometimes, for example, selfish genetic elements called transposons are activated. These are jumping DNA segments that are integrated at some point in the genome and interrupt genes. In many animal species, male hybrids are less viable because they have only a single copy of the X



Sculpin family tree: A comparison of six especially variable regions in the genome shows that the hybrid sculpins (red) form a genetically distinct group from both parent species. They differ to practically the same degree from both *Cottus perifretum* (purple) and *Cottus rhenanus* (blue). Moreover, the hybrids are genetically more similar to one another than the parent species, which are subdivided into individual lines. (The numbers correspond to the find sites on the map on page 60.)

Graphic: designergold, based on original material from the MPI for Evolutionary Biology



The sculpins formerly known as *Cottus gobio* actually comprise a number of species. *Cottus perifretum* (purple) lives in the Scheldt river system, among others. *Cottus rhenanus* (blue) is found further east, in the tributaries of the Middle and Lower Rhine. Hybrids of these two species have colonized the Rhine itself and its major tributaries, such as the Sieg, Moselle and Main (red). In comparison, the true *Cottus gobio* has a very large range, covering the entire Danube and Alpine region as far as Scandinavia (greenish-yellow).

Map at top: A number of different sculpin lineages meet in the area around the Sieg river near Bonn. The Sieg tributaries hold only *Cottus rhenanus* (blue), while the Sieg itself is home to only hybrid sculpins (red). The different types meet at the outlets of the tributaries and form hybrid zones. One exception here is the Lower Pleisbach stream, which is different from other streams in the region and in which no *Cottus rhenanus* specimens were found, so the hybrid sculpins have been able to establish themselves there. In the lower reaches of the Wahnbach stream, the construction of a dam wiped out the indigenous *Cottus rhenanus* population, so here too, the hybrids have migrated upstream.

and Y sex chromosomes, so genetic defects have an immediate impact on male animals. However, the researchers in Plön haven't detected any harmful genetic effects in the hybrid sculpins.

This means that the first hybrids weren't at a genetic disadvantage after *Cottus perifretum* and *Cottus rhenanus* met in the Rhine. However, being no worse than the parent species isn't enough. After all, the parent species hadn't managed to survive long-term in the Rhine.

Consequently, the crossbreeds must have mastered something that was beyond the abilities of their predecessors. Nolte and his colleagues have yet to identify exactly what that is. "We assume that the hybrids are better able to adapt their metabolism to higher temperatures. They tolerate warmer water and grow faster in it," he says.

CLUES IN THE GENOME

Once again, the genome contains important clues. Some 500 genes are active to different degrees between the parent species and the hybrids, and these genes hold the secret to the hybrids' success. Moreover, today's hybrid sculpins are no longer genetically identical to the original hybrids. A comparison between wild hybrids and recent laboratory crossbreeds yielded marked differences in their genomes. This means that the sculpins currently living in the Rhine have already evolved further and adapted to the prevalent environmental conditions.

Arne Nolte wants to delve still deeper, and the European Research Council (ERC) has furnished him with almost 1.4 million euros to do just that. Over the next five years, he plans to conduct extensive genetic analyses to find out which genetic mutations enable the hybrids to survive in large rivers, and what ecological advantages those mutations confer.

The impact of evolution on the hybrids in the Rhine can be observed in natural hybrid zones, areas where the hybrids living in the large rivers encounter the native sculpins in the tributaries. Nolte and his team have examined several of these hybrid zones along

Graphics: Proceedings of the Royal Society

top: Arne Nolte loves to busy himself with fish on his own time, too. Here he poses with a freshly caught brook trout (*Salvelinus fontinalis*) from the Sainte-Marguerite River in Quebec, Canada.

bottom left: Jörg Freyhof (left) and Arne Nolte scour the stream for sculpins in the Pleisbach, a tributary of the Sieg in North Rhine-Westphalia. Sculpins live on the beds of streams and hide under stones and branches. They are poor swimmers, having no swimbladder, so they use jerky zigzag movements to flee from would-be catchers.

bottom right: The fish room at the Max Planck Institute in Plön houses dozens of aquariums with sculpins from different populations. Scientists can observe the lives of the fish and carry out crossbreeding experiments in these shallow tanks, which have been furnished with river gravel and shards of pottery as hiding places. The temperature and day length in the room can be regulated precisely, because the sculpins need cool water and seasonal fluctuations in order to survive and reproduce.

Photos: MPI for Evolutionary Biology (3)

Showy, but not assertive: In general, cultivated forms such as these aquarium bettas are no longer able to hold their ground in their original habitat, and also don't interbreed with wild members of the same species. During mating, the male betta encircles the female, who lies on her back, and catches the eggs in his mouth. He then spits them into a nest of air bubbles that he has created on the surface.

the Sieg river, which flows into the Rhine at Bonn. The headwaters of the Sieg tributaries contain only *Cottus rhenanus*, while the Sieg itself yields the hybrids with *Cottus perifretum*. At the river mouths, the two lineages meet and propagate, and backcrossing occurs, giving rise to secondary hybrids.

CAUGHT AT RIVER MOUTHS

Parent species, hybrids, secondary hybrids – the taxonomic relationships could now become impossibly complex, but the secondary hybrids seem to have a problem. They colonize only the river mouths in the long term – areas where hardly any other sculpins occur. They seem unable to disperse from there, because while the sculpins in the Sieg have spread at a rate of approximately four kilometers a year, the hybrid zones have remained about two kilometers wide for years. The conclusion is clear to Nolte: "Tributary outlets constitute ecological boundaries, and thus barriers to dispersal. The secondary hybrids are inferior to both the original Rhine sculpins in the headwaters and the Rhine-Scheldt hybrids in the main rivers."

Genetic analysis of the fish from hybrid zones provides a further pointer that natural selection determines the dispersal of secondary hybrids. It has shown that the secondary hybrids are both viable and able to reproduce. They breed with one another and, at the edges of the hybrid zones, with the parental lines, resulting in a true hybrid swarm. This means that the expansion of hybrid zones isn't limited by genetic causes alone. The only possible explanation is that hybrids in the transition zones are less adapted to the habitats of their parents. They can establish themselves only at river mouths, where conditions change abruptly.

The findings from hybrid zones along the Sieg are important to Nolte in another regard, as well: "They are an example of the fact that hybrids are inferior to the parental lines in their original habitat when in direct competition with them. This is especially true when the habitats are intact." This news will reassure conservationists, who warn of the risk of bastardization of fauna through the release of cultivated lines. It confirms observations of pike, char and trout, which revealed that cultivated fish that escaped have left practically no genetic traces in wild populations.

A similar case is that of *Betta splendens*, a fighting fish that is popular with aquarium enthusiasts. In its native Thailand, breeders cultivate colorful variants with eye-catching fins for the aquarium market; but for centuries now, they have also produced aggressive, short-finned variants for fighting contests. It's safe to assume that cultivated forms have repeatedly escaped over the years and mixed with wild variants. Some ichthyologists have even gone so far as to state that the original *Betta* is extinct – an unlikely scenario, since cultivated forms and their hybrids don't stand a chance against their indigenous relatives in the wild.

HYBRIDS FILL NICHES

The concept that hybrids are less adapted to the environment than their parent species is valid only in relation to the original habitat. They are often better at occupying new habitats, as revealed by the sculpins in the Rhine. "Hybrids are better at conquering new territory, and that's why they play such an important role in speciation. With their genomes from different lines, they deliver new templates for evolution to play with," says Arne Nolte.

Not only do hybrids colonize new habitats more easily, but they are also at an advantage if the original environment changes. In such situations, new adaptations are required and the hybrids can outflank the parent species.

Photo: ZooFari/wikipedia

» With climate change, more species will probably originate from hybrid lines in the future.

One such changing habitat is the Rhine. Straightened by humans and corseted by dams and shoreline stabilization, the extent of change has accelerated over the last 20 years. Since water protection in Germany was enhanced after a series of chemical spills in the 1980s, water quality in the Rhine has seen continual improvements, and the river is once again home to a number of species that had vanished. At the same time, however, the amount of living organisms in the Rhine has fallen, since there are now fewer nutrients available.

In addition, a number of animal and plant species have been introduced by humans or have migrated there in the course of climate change. In fact, the Rhine has experienced several waves of migration within just a few years, and non-indigenous Asian clams, amphipods and bristle worms now dominate large parts of its ecosystem. "This type of habitat upheaval gives hybrids the opportunity to fill new niches, and it is probable that climate change will further accelerate the emergence of new species from hybrids," says Nolte.

In other words, hybrids aren't just an undesired byproduct in the interplay between species, but are ubiquitous in the natural world as an indispensable driver of evolution. Hybridization even played its part in the evolution of modern humans. *Homo sapiens*, Neanderthals and perhaps other human types interbred during the course of their history, so humans also benefited from foreign genetic material as they spread across the globe.

Consequently, the advance of the sculpin hybrids in the Rhine doesn't worry Nolte; he considers it a completely natural process. In fact, he is far more concerned about other invaders. In recent years, black spotted gobies, bighead gobies, racer gobies and tubenose gobies have been migrating upstream from the Danube estuary to the Black Sea. They entered the Rhine via the Rhine-Main-Danube Canal, among other channels, and are spreading like wildfire.

GOBIES WITH A MIGRATION BACKGROUND

Gobies are in direct competition with Nolte's sculpins. These are especially large and powerful fish that avail of a broad spectrum of food sources and are capable of winning through against other species. Bit by bit, the newcomers are displacing indigenous fish species. In some places, the invasive gobies now comprise over 70 percent of catches from the river bed.

Large populations of hybrid sculpins are still found in the main tributaries of the Rhine, but there is a risk that the new expansion will put an end to the furtive spread of the hybrids, less than 200 years after it began. The specimens in Nolte's fish room would then be the last of their kind, survivors of an initially successful, but ultimately failed experiment of nature. ◂

TO THE POINT

- **In nature, different species regularly breed with each other. Under certain conditions, these hybrids can give rise to new species.**
- **The combination of the genomes of both parent species can give hybrids new characteristics and abilities, enabling them to colonize new habitats more easily.**
- **The parent species generally predominate in the original habitat, and hybrids can't prevail there. However, this applies only in the case of undisturbed habitats. Shifts such as climate change increase the hybrids' chances of survival.**

GLOSSARY

Biological species: Classification by species is an attempt by scientists to describe the diversity of nature. However, living creatures differ to varying degrees, so the dividing lines researchers draw between species are necessarily arbitrary. Today, there are more than 20 different definitions of species. They divide organisms on the basis of external characteristics, genes, behavior, physiology, or their ability to reproduce. According to one prevalent concept, all individuals that are capable of breeding together and producing fertile offspring belong to one species. Be that as it may, no definition has yet emerged that categorizes individuals consistently in every case.

Hybrids: In the strict sense, hybrids are the offspring resulting from the cross-breeding of individuals that differ from each other in one or more characteristics. In general, though, the term refers to reproduction between different species. The colloquial expression "half-breed" is a bit of a misnomer, since hybrids may be more like one parent. Some hybrids even surpass their parental types, or display completely new characteristics.

Hybrid swarm: A hybrid swarm occurs when hybrids continue to breed with the parental types they originally evolved from. Their offspring interbreed with each other, with their hybrid parents and with the parental types, giving rise to many different genetic lines.