Mice Are **Here to Stay**

Wherever people live, there are mice. It would be difficult to find another animal that has adapted to the habitats created by humans as well as the house mouse has. It thus seemed obvious to **Diethard Tautz** at the **Max Planck Institute for Evolutionary Biology** in Plön that the species would make an ideal model system for investigating how evolution works.

TEXT **CORNELIA STOLZE**

The mice at the Max Planck Institute in Plön live in their very own house: they have 16 rooms where they can form their family clans and territories as they see fit. The experiments Tautz and his colleagues carry out to study such facets as the rodents' communication, behavior and partnerships sometimes take months. During this time, the mice are largely left to their own devices. Humans enter their realm only to clean up and provide them with food and water.

The mouse house in Plön meets its inhabitant's requirements fully. After all, house mice also live in large family clans in the wild and maintain extensive physical contact and communication with each other. The squeaking familiar to humans is only a small excerpt of the rodents' vocal and sound repertoire. Most of their communication occurs in the ultrasound range, which is not perceptible to the human ear. And while the sounds can be heard by the mice themselves only within a range of 30 to 50 centimeters, they convey highly complex messages.

Diethard Tautz and his colleagues have discovered that house mice behave naturally only when they live in a familiar environment and interact with other members of their species. When animals living in the wild are captured, they lose their familiar environment: everything smells and tastes different, and they are no longer able to move about freely. And most importantly, they lack contact with the family – a key element in the lives of these extremely social animals.

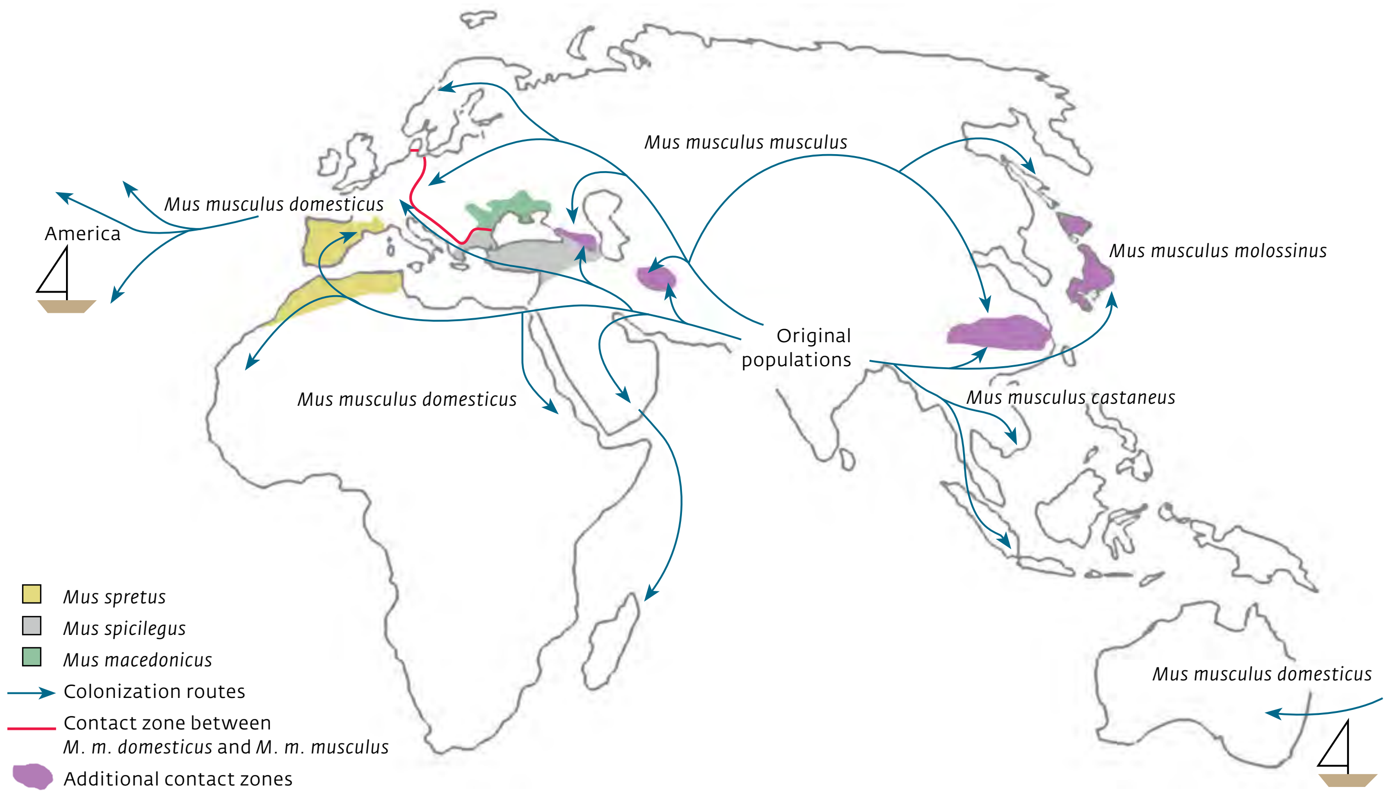
A MODEL FOR EVOLUTION

The mice in the mouse house, in contrast, can indulge in their entire repertoire of behaviors. This is the only way the latest evolutionary development of the rodents can be studied, as the results of research carried out in the past showed that differences in behavior can be a key factor in the emergence of new species. For Tautz, the house mouse is a model for the processes of evolution: it would be difficult to find another animal species that lends itself so well to the study of the genetic mechanisms of evolution.
"Not only is this species extremely adaptable, as demonstrated by its dispersal all over the globe, but we also know its genome better than that of almost any other species," says Tautz. After all, scientists have been breeding and studying mice in the laboratory for more than a hundred years, observing their behavior and decoding their genome. Today's laboratory mice are descendants of the house mouse, but they are far less genetically diverse than their wild relatives.

The house mouse's first ancestors lived around 500,000 years ago in the region that is now Iran, where they split into subspecies. When humans settled there around 14,000 years ago and started farming, a new age began

While the eyes of the house mouse (*Mus musculus*) tend to be underdeveloped, the rodent's nose and ears play a very important role in the social life of its species: their vocalizations are so complex that the animals can even develop "dialects." Such regional differences influence the mice's choice of reproductive partner.

Photo: MPI for Evolutionary Biology



Graphic: TRENDS in Genetics, vol.19, no.1, January 2003

for the mice: they couldn't resist the corn fields and storerooms of the first farmers. It's hard to imagine a more reliable and convenient source of food.

From then on, the house mouse conquered the world in the wake of humans. As part of this process, it followed different dispersal routes: the eastern European house mouse (*Mus musculus musculus*) populated northern Asia and eastern Europe and adapted to the continental climate there. The western European house mouse (*Mus musculus domesticus*) reached western Europe, with its Atlantic climate, on Phoenician merchant ships crossing the Mediterranean. Then, with the first European seafarers, they made it as far as America, Australia, Taiwan and even remote archipelagoes such as the Faroe Islands.

With the first European whalers, the mouse also penetrated almost as far as Antarctica, populating regions where it quickly adapted to average temperatures of just under 5 degrees Celsius. To research this diversity, there are mice from all over the world living at the Max Planck Institute in Plön: in addition to mice from Germany and France, there are conspecifics from the Faroe Islands, Spain, Austria, Kazakhstan, Iran and even Taiwan.

FROM VEGETARIAN TO CARNIVORE

When necessary, the house mouse even adapted its originally vegetarian eating habits. The descendants of the former sea-faring mice, for instance, feed on insects, worms and larvae. On the island of Helgoland, they even became accustomed to eating dead sea birds. They are so flexible that, as long as they have a good food supply, mice don't even need direct access to water.

A few years ago, Diethard Tautz discovered a possible key to the evolutionary success of the house mouse: new, functional genes can form from parts of the genome within a short period of time. These genome sections had previously baffled researchers, as they don't contain any information that can be translated into functional proteins. For this reason, up to 90 percent of the mouse genome was classified as junk DNA. However, the Max Planck working group discovered that these originally functionless DNA sequences could give rise to new genes. "They really are complete reinventions – something that previously would have been considered impossible," explains the evolutionary biologist.

By genetically analyzing several mouse species, Tautz and his colleagues discovered that only a small percentage of the DNA is translated into proteins. Nevertheless, almost every DNA section is transcribed into an RNA molecule and can thus be a candidate for a new gene. Sections of DNA that are read but not translated into proteins

Left page Origin and range of the house mouse: Two million years ago, their closest relatives *M. spretus* (yellow), *M. spicilegus* (gray) and *M. macedonicus* (green) separated; today their ranges overlap in different regions of Europe. Half a million years ago, subspecies of the house mouse then emerged in the area that is now India and Iran. The rodents gradually spread across the globe in the wake of the first farmers: the eastern European house mouse (*M. m. musculus*) migrated to northern Asia and eastern Europe, the southeastern Asian house mouse (*M. m. castaneus*) to eastern Asia, and the western European house mouse (*M. m. domesticus*) to western Europe. From there, the western European house mouse spread to all the other continents by ship. The different subspecies encountered each other in different regions of the world and cross-bred with each other (purple). One example of this is a narrow hybrid zone in central Europe (red line). A new hybrid subspecies, *M. m. molossinus*, emerged in Japan as a result of the contact between the eastern and Asian house mice.

Right Diethard Tautz at the demonstration enclosure in the visitors room at the Max Planck Institute for Evolutionary Biology. The mice live here in a highly diverse environment under nearly natural conditions. The actual experiment rooms are fitted with the same structural elements.

are thus called precursor genes or protogenes. Whether they become genes or not depends on the environment: if an RNA molecule assumes an important function, the coding section of the DNA remains active and is retained. If the RNA molecule fails to find a useful task, the section becomes non-coding DNA again.

But new genes also arise through a second mechanism: when a gene for a protein arises from an originally non-coding section due to a change in the DNA reading frame. A reading frame comprises three consecutive letters of the genetic alphabet. Each of these triplets stands for an amino acid into which the genetic code is translated. If this reading frame shifts, new triplets arise and the sequence of letters can be translated into amino acids. The scientists in Plön identified several genes that were overwritten due to such a change in the reading frame. One example is the Hoxa9 gene

Photo: Tom Pingel

Diethard Tautz and his colleague Christine Pfeifle have completed all the preparations. The inhabitants will now be left undisturbed for months; their behavior is recorded by cameras. The rooms are equipped with wood shavings for burrowing and digging, and with feeding dishes and water bottles; the red "huts" with access tubes and removable lids serve as nests. The mice live in differently equipped environments depending on the nature of the experiment: in this relatively unstructured experiment room, the researchers are studying the social behavior and the territory formation of *Mus spicilegus*, a particularly social species. Working together, the animals built themselves a nest in a corner under a pile of bedding (back) and assembled all the food supplies in the middle of the room.

– a gene that controls embryonic development. In rodents and primates, this gene uses an additional, alternative reading frame of this kind.

The researchers suspect that genes frequently form anew. According to their findings, only around 60 percent of the mouse genes originate from the early phase of evolution. Over time, apparently every section of the DNA is, at some point, read and tested to see whether it is suitable as a gene. "The non-coding sections of the genome are therefore a reservoir for new genes that can thus help the animals rapidly adapt to new habitats. This harbors enormous potential for evolution, and we previously knew nothing about it," says Tautz.

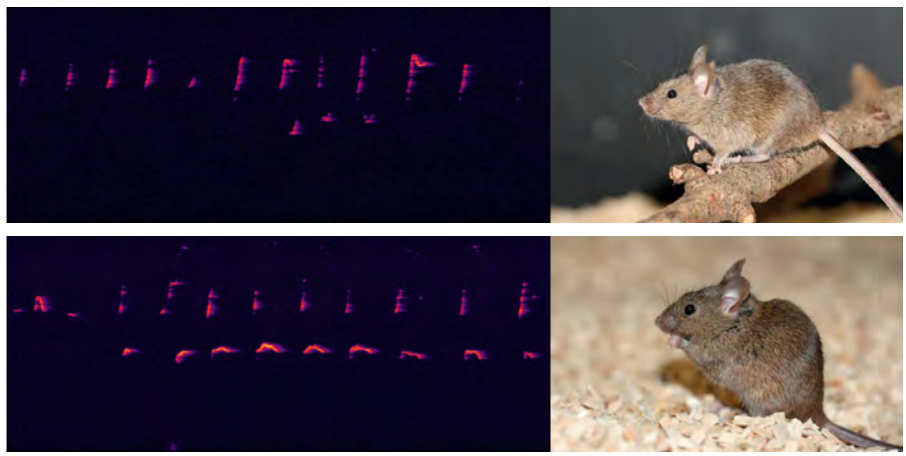
The studies carried out at the Max Planck Institute in Plön also showed that the emergence of new genes can increase significantly under certain circumstances. A lot of new genes arise after major ecological changes: for instance, a particularly high number of new genes emerged during the transition to the Cenozoic era, when the dinosaurs became extinct, and in the most recent lineage leading to mice.

In addition, whenever the house mouse encountered a new habitat over the course of evolution, new populations formed with different characteristics and occupied different ecological niches – a phenomenon known as adaptive radiation. "It was long believed that this kind of adaptation to new living conditions was a very slow process," explains Tautz. "Recent studies have shown, however, that animals sometimes adapt after just a few dozen generations – so, for mice, within just a few years."

ISLAND MICE GO THEIR OWN WAY

Tautz and his colleagues are able to study the consequences of adaptive radiation and the very rapid adaptation of animals to new conditions using the house mouse in different locations in Germany, including the island of Helgoland. Located 50 kilometers from the mainland, house mice arrived on this

Photo: Tom Pingel



Comparison of some ultrasounds of animals from the same subspecies, *M. m. domesticus*, from France (top) and Germany (bottom). The animals communicate using complex syllables that can differ from one region to the next. The females communicate a lot with each other – especially when they are alone together. The females make fewer and different sounds when in contact with males.

North Sea island on board ships only a few hundred years ago. "This is a mere blink of an eye in terms of evolution. Nevertheless, the animals on the island already differ considerably from their conspecifics living on the mainland," says the Max Planck researcher.

Evolutionary biologists attribute this to, among other things, differences in the food supply: while house mice on the mainland have a mostly plant-based diet, their relatives on Helgoland were forced to become accustomed to eating meat, as there is no farming on the island. They now presumably eat dead sea birds instead of plants, and their chewing apparatus has developed toward that of a predominantly meat-eating species.

The Helgoland mice hardly mix with their relatives that are constantly arriving from the mainland, so it's only a matter of time before the Helgoland animals become a separate species: when two populations no longer exchange genes, different mutations arise in the two groups and they develop in different directions. This kind of geographical isolation, combined with new adaptations, is considered to be one of the mechanisms for the emergence of new species.

This species formation, which is known as allopatric speciation, is already further advanced in the eastern and western subspecies *M. m. musculus* and *M. m. domesticus*. The two still belong to one species. However, they are clearly distinguishable both genetically and in terms of their external appearance, and the two groups' ability to reproduce with each other is now limited. This can be observed particularly well in the range boundary of the two subspecies along the climate divide between the Atlantic and continental climates: their ranges overlap in a 40-kilometer-wide strip – known as a hybrid zone – along this divide. The animals come into contact with each other here and reproduce regularly.

Consequently, the genome of the resulting hybrids consists of those of the western and eastern subspecies. However, the two species' genomes are evidently now compatible only to a limited extent, as the hybrid offspring suffer from a weak immune system and are more vulnerable to parasite infestations. This also has an impact on the rodents' intestinal flora. John Baines, a visiting professor at the Max Planck Institute in Plön, carried out a genetic analysis of the biodiversity of these intestinal bacteria. He found that the hybrids' intestinal flora includes fewer bacterial species, and that the different species also occur with different levels of frequency. For example, a hybrid mouse has considerably more helicobacter bacteria than its purebred parents. In humans, these microbes can cause intestinal ulcers.

IMMUNE SYSTEM ALTERS INTESTINAL FLORA

The scientists explain this as follows: Different variants of genes for the immune system result in the formation of different immune cells. The hybrid mice thus have different T cells. These immune cells also occur in intestinal tissue and evidently have an adverse ef-

Photos: MPI for Evolutionary Biology

fect on the bacteria there. This doesn't appear to benefit the mice, as the hybrids are more prone to intestinal tissue infections than their parents.

In addition, sperm maturation is severely impaired in the hybrids, causing them to produce fewer offspring. Leslie Turner and Bettina Harr, two former colleagues of Tautz, discovered a complex network of interactions between different gene regions that can prevent reproduction between the hybrids over the course of evolution. In this way, the differences between the forms of mouse constantly reinforce each other until the two subspecies eventually give rise to completely separate species. Crossbreeds of the two subspecies can't survive in nature in the long term. "If the offspring of a hybrid are less fit than their parents, the populations inevitably drift apart," concludes Diethard Tautz.

But a species split isn't triggered solely by physical differences; differences in behavior are often the starting point for separation. In this way, a highly diverse group in which all males and females can theoretically produce offspring together can lead to populations that cease to operate harmoniously – be it because the animals no longer find each other attractive, because one group builds different nests, or because the females are fertile at a different time. Such changes in behavior are the main reason for the incipient isolation of one or more groups within a population. Researchers call this sympatric speciation.

GENES FOR PARTNER SELECTION

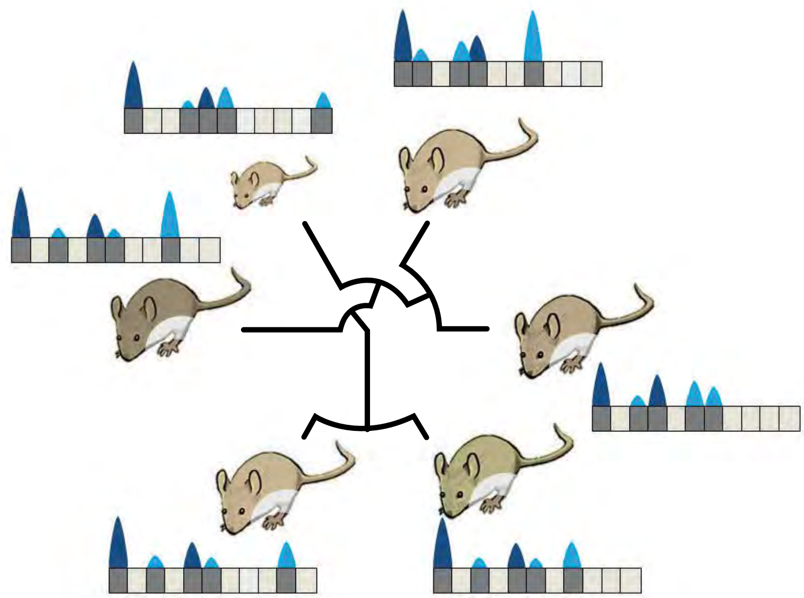
Tautz and his team recently encountered a complex in the mouse genome that is made up of several genes and that crucially influences the animals' partner selection. This PWS region, as it is known, controls several behaviors and ensures that different "personalities" form between individual populations. The researchers discovered, for example, that western house mice from western Germany and the south of France differ in their mating behavior.

"If we put animals from the area around Cologne, far west of the hybrid zone, together with members of the same species from the French Massif Central in our mouse house, they all mate with each other initially," says Tautz. "However, something surprising happens with their offspring: the mice with one German and one French parent later show a preference for partners of the same origin as their father."

It is still unclear precisely what causes such preferences. What is known is that, in mice, a partner's attractiveness is conveyed through odorants and sounds in the ultrasound range of 50 to 70 kilohertz. "We originally suspected that the males, in particular, convey mating signals through the sounds they make, just like the chirping of songbirds. But then we observed that females communicate far more with each other, particularly when they are alone together," says Tautz.

French and German mice differ in the sounds they make, and thus speak, as it were, different languages – despite belonging to the same subspecies. This contributes to the separation of the populations, but the search for the cause of the paternal preference continues. The German and French mouse populations clearly were physically separated from each other long enough

Photo: Tom Pingel



Left page Diethard Tautz (standing), Johana Fajardo, Derek Caetano-Anolles, Chen Xie, Kristian Ullrich and Wenyu Zhang (clockwise) discuss their projects.

Right DNA sections with varying degrees of activity in the genomes of different mouse species (black lines indicate the relationships between them): dark gray boxes represent regions that are translated into RNA molecules and the height of the triangles above the boxes indicates the volume of RNA produced. While 10 percent of the DNA in all the animals is used identically (box on the left), non-coding sections are read at varying levels of intensity. The resulting RNA molecules are retained only if they offer an evolutionary advantage. New genes can then form from these sections.

for the initial indications of separate development to emerge within the subspecies.

In addition, another aspect of sexual behavior accelerates speciation: although mice have many different sexual partners, the researchers also regularly observed cases of fidelity and inbreeding. Large families in which fathers produce offspring with daughters and mothers with sons often arise in the behavioral rooms. This promotes the formation of genetically uniform groups and thus strengthens the speciation process. This natural inbreeding is also the reason why mice are such a good genetic and biomedical model system: the requisite genetically uniform inbred strains are particularly easy to establish with mice.

The research by Diethard Tautz and his colleagues shows that mice are much more than pests and carriers of disease. On the contrary, their proliferation across the globe is an object lesson in how organisms can adapt to new habitats. The different house mouse populations on earth constitute evolutionary experiments from which new species could one day emerge. ◂

Graphic: MPI for Evolutionary Biology

TO THE POINT

- **The modification of existing genes isn't the only thing that gives rise to new characteristics in evolution: completely new genes can also be created from previously non-coding sections of DNA. This happens especially when major ecological changes occur or when species populate new habitats.**
- **As hemerophiles – animal and plant species that thrive in habitats altered by humans – house mouse populations and subspecies have spread throughout the world and repeatedly adapted to new environmental conditions. Accordingly, they constitute a natural experiment on the part of evolution – one that enables scientists to study the emergence of new species.**
- **Differences in behavior, for example in partner selection, and in the formation of social systems are the first steps in one species splitting into two separate species.**

GLOSSARY

Allopatric speciation: Individuals of one species are separated by external factors such as mountains or estuaries. Over the long term, this spatial isolation results in the accumulation of different mutations in the subpopulations and, consequently, the formation of genetic differences. The animals can no longer reproduce successfully with each other, and two new species form. When species split in the absence of spatial separation, it is referred to as sympatric speciation.

Radiation: Diversification of a group of organisms or a species into a larger number of new species, which adapt to new ecological niches and thus form new characteristics. This can cause an abrupt increase in the number of new species, for example when a new habitat is populated. In contrast, the fragmentation of an originally contiguous habitat can also trigger the emergence of new species, but this is a continuous process that unfolds over a long period of time. New species can also take over the vacated ecological niches when competitors disappear, as was the case with mammals when the dinosaurs went extinct, for example. A successful evolutionary innovation can also result in the emergence of many new species.