



Evolution after Darwin

Darwin's pioneering work *On the Origin of Species*, in which he first formulated the principles of his theory of evolution, was published 150 years ago. This theory changed the thinking in biology significantly and influenced almost all areas of biological research. What could be more appropriate, then, than to stage a **Max Planck Symposium on Evolutionary Biology** in the Darwin Year 2009.

TEXT **CHRISTINA BECK**

Evolution goes on all the time." This is the credo of Richard E. Lenski of Michigan State University. The American scientist tracks evolution in the laboratory with his team: some 21 years ago he embarked on a long-term experiment with *E. coli*. Bacteria provide good conditions for such experiments, as a bacterial population grows to billions of cells within a very short time. Studying evolution in action calls for large numbers – it's like playing dice: if you roll the dice a hundred times, you will almost certainly get a six. Lenski hopes to overcome coincidence through the sheer volume of cells and generations alone ("Our lab has produced 45,000 generations of bacteria so far"). His ultimate aim is to identify the events that result in the advent of something new and original in evolution.

Lenski's studies began with twelve identical populations of an *E. coli* strain. His objective was to find out whether random mutations that occurred in the past facilitate the evolution of key innovations.

BILLIONS OF MUTATIONS

"One of the big advantages is that you can freeze bacteria and in this way obtain fossil images," explains the biologist. The bacteria grew on a medium with a limited supply of glucose, which also contained citrate. However, *E. coli* cannot use this citrate as a source of carbon under oxygen conditions. "In the course of 30,000 generations, not a single generation developed the ability to exploit citrates, even though each population had billions of mutations," said Lenski.

It was only after 33,000 generations that a variant (Cit+) capable of exploiting citrate emerged – a fitness advantage that resulted in a clear increase in the size of the population. So was this an extremely rare mutation, which would explain why its arrival was so delayed? Or was it a simple mutation that had to be preceded by other mutations in order for it to be phenotypically effective? "We tested these hypotheses in experiments in which we repeated the evolution from different starting points in the population history," explained Lenski.

Not a single Cit+ mutant occurred among the ten trillion ancestor cells in the repeat experiments, either. All 19 Cit+ mutants obtained by the scientists originated from later clones. A sequence comparison of Cit+ and Cit mutants showed that the necessary citrate transporter was generated only



The research studies presented at the Max Planck Symposium on Evolutionary Biology examined very different animal species including the beach mouse, which is a native of the US, cichlids from east Africa, the European great tit, and primates, including chimpanzees and us humans (l-r).

when the corresponding gene was able to recruit a promoter – the start sequence that made it possible to read it in the first place. And this, in turn, had become possible only as a result of the duplication of another gene. The evolution of this phenotype was thus dependent on the population’s “genetic history.”

HOW MICE ACQUIRE A DIFFERENT COAT COLOR

Genetic research provides the strongest proof of evolutionary theory today. Charles Darwin could not have even guessed at such things. Indeed, the door to a more profound understanding of evolution opened only when James Watson and Francis Crick succeeded in decoding DNA in 1953. “What can genes tell us about evolutionary adaptation processes?” asked

Hopi Hoekstra from Harvard University in her lecture “From Mice to Molecules.” Convergence is a fascinating phenomenon in evolution: unrelated species develop similar characteristics or a similar appearance in response to similar selection pressures. But does this phenotypic convergence imply genetic convergence? In other words: Are the same genes actually responsible for the development of similar forms and patterns? To find out, Hoekstra and her team studied coat color variants in natural mouse populations.

The mouse species *Peromyscus polionotus* populates fallow fields in the southeastern US. However, populations are also found in the bright sand dunes along the Gulf coast and more than 300 kilometers away on the Atlantic coast near Florida. They are known as beach mice and, compared

to their conspecifics inland, they have lighter-colored and significantly reduced coat pigmentation on their faces, flanks and tails. The fact that the lighter-colored coat provides better camouflage in the sand dunes was demonstrated by a simple experiment in which the researchers placed a fake dark mouse coat in the dunes: it was immediately carried off and then later abandoned by a predator (probably an owl or a hawk) when it realized it had been tricked.

“At least three genes – *Mc1r*, *Agouti* and *Corin* – influence coat color,” reported Hoekstra. The Melanocortin-1 receptor, *Mc1r* for short, plays a key role here in that it controls whether the dark pigment eumelanin or the light pigment pheomelanin is produced. The researcher succeeded in demonstrating that the exchange of a single nucleotide (a T instead of a C) in



left | The coffee breaks provided an opportunity for lively conversation and exchange, as seen here between Dieter Ebert from the University of Basle (center) and David G. Heckel from the Max Planck Institute for Chemical Ecology.

right | In her lecture "From Mice to Molecules," Hopi Hoekstra explained the genetic mechanisms that control adaptations in the coat color of beach mice.

the sequence of the receptor gene in the mouse population originating from the Gulf coast altered the effectiveness of the receptor and hence changed the coat color. Arginine is now inserted in position 65 in the amino acid chain instead of cysteine, and this alters both the bonding of ligands and the potential of the receptor for signal transmission. "Such Mc1r mutations have also been observed in mammoths," added Hoekstra.

WHY DARWIN'S FINCHES APPEAR IN EVERY TEXTBOOK

Interestingly, the lighter-colored coat in the mice from the Atlantic coast could not be explained by the same allele – the latter did not occur in them at all, nor could the researchers find any new mutations in the Mc1r gene that would have influenced the activity of the receptor. "Our findings show that a pigmentation pattern that arose convergently in different mice populations that actually developed under very similar conditions is clearly the result of completely different genetic mechanisms," said Hoekstra. So there are different molecular solutions for attaining the same phenotype under comparable environmental conditions. The combination of different alleles of the aforementioned genes Mc1r, Agouti and Corin may well play a role here.

Using the Galapagos finches, Darwin had already clearly demonstrated the fact that patterns, colors and forms can change as a result of evolutionary adaptation processes. His drawings on this topic can still be found in every school science textbook today, and show how the finches developed a thick beak for biting seeds, a longer and sharper one for flowers, and a short, pointed beak to access tiny insects in rock crevices. As the famous naturalist wrote in his travel report of 1839: "Seeing this gradation and diversity of structure in one small, intimately related group of birds, one might really fancy that from an original paucity of birds in this archipelago, one species had been taken and modified for different ends."

Rosemary and Peter Grant from Princeton succeeded in demonstrating the effect of natural selection directly. They studied the beaks of ground finches on the island of Daphne Major and, at the same time, recorded their food sources. In years of extreme drought, a large part of the population died and only the finches with larger beaks survived because they could crack thicker and harder seeds when the seeds they usually fed on became scarce. The beak shape is not only inherited, as the Grants discovered. It also becomes established as an adaptation far faster than had

been thought. Natural selection, that apparently endlessly cumbersome and slow mechanism of evolution, can clearly create new conditions within a single generation.

In another exciting finding, the researcher couple discovered that a newly migrated larger species of bird could make better use of the thicker seeds than the long-established birds. The established residents responded to the arrival of the new competition through adaptation – in precisely the opposite direction as before, in that they now developed smaller beaks. This meant they could access food sources in rock crevices, for example, which their thick-beaked rivals could not reach. This adaptation, too, was quantifiable in one generation of the birds. The researchers call this phenomenon microevolution.

HOW GREAT TITS FOLLOW CLIMATE CHANGE

Other mechanisms also exist that could be the result of adaptation processes. Based on a long-term study carried out in the UK, Ben Sheldon from the University of Oxford demonstrated that great tits are so plastic, in other words adaptable, in their behavior that the population as a whole is successfully adapting to the rapidly emerging changes in the climate. The researchers observed the breeding be-

behavior of the great tits in Wytham, a 375-hectare wooded area near Oxford, for five decades. No other bird population had been observed on an ongoing basis in a single location for such an extensive period.

Between 1947 and 2009, the time of egg deposition shifted forward by around 14 days. "All of the data would indicate a close link between the average time of egg deposition in the population and the temperature that prevails in the period leading up to it," explained Sheldon. Temperatures in spring – that is, before egg deposition – have increased markedly since the mid-1970s.

Over the same period, the time window in which the majority of winter-moth grubs hatch has also moved forward. "The correlation between temperature and mating time is the same," says Sheldon. The grubs are a key resource for the great tits, and the success of their breeding is largely dependent on the presence of these insects. Correct timing is important for breeding and the selection pressure on the timing of egg deposition is high. The synchronization between egg deposition and the peak in the hatching of the grubs is maintained through the process's close linkage with spring temperatures. "And this would suggest that this adaptation is achieved solely through individual phenotypic plas-



ticity," stressed Sheldon. The British scientist also referred to "phenodynamics" in this context.

Russell D. Fernald of Stanford University proposed a bridge between genes and behavior in his lecture. Although genes do not directly specify the behavior of an organism, they code for the molecular products that

develop and control brain function, and therefore make behavioral reactions possible in the first place. There are growing indications that information generated in the social context can alter gene expression in the brain, and therefore also behavior. In order to find out how social information is translated into cellular and molecular



changes, the researchers looked for a corresponding model system. A species of cichlid from Lake Tanganyika in east Africa proved a lucky find: Fernald and his colleagues were able to use it to demonstrate the influence of social interaction on the brain.

SOCIAL CLIMBING GOES STRAIGHT TO THEIR HEADS

There are two different types of adult male cichlids: those that have a territory (T) and those with no territory (NT). Territorial males defend their territory and are extremely aggressive toward neighboring males. They scare off non-territorial males and court the females, with whom they eventually mate. In contrast, between 70 and 90 percent of NT males do not reproduce at all. Their gonads are thus small in comparison with those of the T males. They also lack the dark stripe on their heads and dots on the anal fin. These dots look like eggs to the females. In their attempts to gather them up, they collect the seminal fluid of the T male, thus ensuring fertilization.

“What is exciting about this,” said Fernald, “is that this phenotypic state can be completely reversed.” When the researchers placed NT males in a pool with smaller conspecifics, they became T males. Conversely, T males in a community with bigger territorial males became NT males. “The chang-

es in behavior – that is, whether the animals were aggressive or not – arose within a matter of minutes,” reported the American scientist.

The changes in the brain were particularly impressive: the gonadotropin-releasing hormone controls the release of gonadotropins and thus the growth of the gonads. In T males, the GnRH-releasing neurons are eight times larger than they are in NT males. When a male was elevated from NT status to T status, the neuron size changed within a day and had reached the typical size of the GnRH-releasing neurons of T males in less than a week. The researchers also recorded an increase in the GnRH messenger RNA and the corresponding protein. Conversely, the volume of links between these neurons declined with loss of status – in other words, when a T male was transformed into an NT male. “The social behavior actually alters the brain in real time,” explained Fernald in summary.

David Haig threw light on a completely different link between genes and behavior. The researcher from Harvard University compared, first, the reproductive history of two individuals: a young woman who gave birth to her first child in her early 20s, bore additional children at intervals of two years, and eventually died at the ripe old age of 80, and the female chimpanzee Fifi from Gombe National

Park, who had her first offspring at the age of 13 and gave birth to subsequent offspring at intervals of four to five years and died at the young age of 45.

EARLY WEANING OFFERS ADVANTAGES

A slide presented by Haig highlighted the central observation deduced from this: early weaning is a feature that is specific to the human lifecycle. Human children are weaned at two to three years of age, while chimpanzee young remain at their mothers’ breast for an average of five years, and even as long as seven to eight years in the case of the Orangutan. This change in the course of the evolution of human children is based on reliable access to “supplementary” nutrition. “This results in childhood in humans including an extended juvenile phase in which the offspring are weaned but still dependent on their parents for their nutrition,” said Haig. Early weaning is advantageous to the mother as she is required to invest less of her own resources (in the form of mother’s milk) in rearing the young.

Haig expects to gain a deeper insight into this mother-child relationship through the study of genomic imprinting and its effects. The following phenomenon lies behind this: two copies of each gene are found in the fertilized ovum – one originating



- 1 | Hopi Hoekstra, Harvard University
- 2 | Richard E. Lenski, Michigan State University
- 3 | Ben Sheldon, Oxford University
- 4 | Russel D. Fernald, Stanford University
- 5 | David Haig, Harvard University

from the mother and the other from the father. Imprinted genes are genes in which different effects arise depending on whether the maternal or paternal allele is read. And it may well be that there is a parental conflict at work here: the paternal genes are generally less related than the maternal genes to the fetuses arising from subsequent pregnancies – since, unlike the mother, the father is not always the same. Therefore, according to Haig’s hypothesis, the paternal genes try to mobilize more of the mother’s resources for the current fetus. The researchers thus presume that imprinting developed at the genetic loci at which either the paternal or maternal inclusive fitness is maximized through the extent of genetic expression.

MISSING GENE COPY AFFECTS APPETITE

With a view to testing these ideas, David Haig examined the genetic condition Prader-Willi Syndrome in detail. Subjects with this disorder lack the paternal copy of a gene that is imprinted, or switched off, on the maternal side, with interesting effects: in the immediate aftermath of their birth, babies with PWS have little appetite and little inclination to nurse. Within the first two years of their lives, however, they develop an insatiable appetite for food. As a result, in the course

of their further development, the children become obese, while simultaneously suffering from delayed growth.

WHAT LIES BEHIND GENOMIC IMPRINTING?

This phenotype confirms the theory according to which the expression of the paternal gene should strengthen the appetite of the offspring in the phase in which they are fed exclusively on breast milk. However, the question that arises here is whether the theory can also explain the change in the appetite in the course of the further development of PWS patients. The expression of paternal genes should obviously inhibit the child’s appetite for replacement nutrition. Haig speculates as to the reasons for this: milk could be the more nutritious or immunologically more valuable nutrition. Moreover, a longer nursing phase may well delay the birth of a younger sibling. In both cases, the child that is already born benefits. “The study of imprinted genes in humans can thus shed light on the relational interactions in our evolutionary past,” stressed Haig in conclusion.

As the famous geneticist and evolutionary biologist Theodor Dobzhansky said: “Nothing in biology makes sense except in the light of evolution.” This was impressively confirmed in the 14 lectures presented at this symposium. ◀

GLOSSARY

Mutant

A bacterial cell in which a genetic modification has occurred.

Clone

A population of bacterial cells whose phenotype is identical.

Phenotype

The overall appearance of an organism, the sum of its characteristics.

Ligand

A substance that can bind to a target protein, such as a receptor.

Allele

Different expressions of a gene generated through minor variations in the base sequence.

Inclusive fitness

The optimization of the passing on of an organism’s own genes.