his team created fish with a range of lifespans. They then compared the genomes and longevities of parent and second-generation progeny, and identified a few chromosomal regions, each with hundreds of genes that might influence ageing. Although these did not directly reveal genes involved in longevity, they suggested possible candidates. From this study, the scientists estimated that about 32% of variation in lifespan among turquoise killifish results from genetics, a figure comparable to the 20–35% estimated genetic contribution in mice[6].

From then on, the killifish's transformation into a valid research model accelerated. Anne Brunet, a geneticist studying ageing at Stanford University in California, had longed for a short-lived vertebrate and was delighted to hear about killifish when Valenzano visited Stanford for a summer course. She recruited him to her lab for a postdoc, and in 2006, Valenzano brought the killifish to California. There, he copied and modified protocols for zebrafish to transfer in foreign genes, starting with the green-fluorescent-protein gene from jellyfish[7]. In 2015, Brunet and her colleagues reported the successful use of CRISPR–Cas9 gene editing in killifish, generating fish with mutations in 13 genes involved in key ageing events such as telomere shortening and mitochondrial dysfunction[1].

As enthusiasm for *Notho* grew, two groups tackled its genome sequence: Brunet's lab at Stanford, and Cellerino and collaborators at the Leibniz Institute on Aging–Fritz Lipmann Institute in Jena, where Cellerino worked for a time and still maintains a cooperative group. Both groups published genome sequences[2,3] in December 2015. "The two papers are complementary," says molecular geneticist Matthias Platzer at the Leibniz Institute, who collaborates with Cellerino. Researchers from the teams now plan to make a consensus sequence.

Beyond the genome, scientists are exploring which genes are transcribed into RNA and used for protein production during different stages in the life cycle. Platzer and his colleagues are interrogating messenger RNA molecules — the killifish transcriptome — to find out. To put together a transcript catalogue[8], they sequenced RNA from killifish whole body, brain and skin, taken at a range of ages, from the embryonic period to 39 weeks old.

Cellerino's team used similar techniques to track what happens in tissues from the same killifish as it develops. By taking small fin clips, they let the fish live long enough to be sampled again. They found that the transcriptomes of short- and long-lived killifish differ when those fish are only ten weeks old, and identified a protein that is a key controller of lifespan[9].

Because the killifish is not a mammal, linking fish genes to human ones will require a leap. Fish genes often have a human counterpart, but these can be difficult to find. This is in part because the killifish's ancestor underwent a whole-genome duplication: where human DNA has one copy of a gene, the killifish often has two. But at the Jena meeting, geneticist John Postlethwait of the University of Oregon in Eugene offered a potential solution. The trick, he explains, is to use an intermediate genome from another fish: the spotted gar (*Lepisosteus oculatus*). The gar's ancestors diverged from the killifish's before the duplication event, so its genome is in some ways more similar to that of a mammal. Scientists may be able to find a killifish gene's counterpart in the gar, and from there, find a match in people[10].

"The killifish work clearly is very innovative and potentially could be a really valuable model," says Matt Kaeberlein, a molecular biologist at the University of Washington in Seattle who studies ageing. But he is unsure how popular the fish could become, noting that its adoption will depend on how difficult it is to work with and whether killifish scientists can obtain sufficient funding. Ron Kohanski, programme officer at the US National Institute on Aging in Bethesda, Maryland, says that the agency is not funding killifish research, but is interested in the fish: "The killifish constitutes a good model for ageing on several levels," he says.

> ***"You need to have a 'blue thumb'. You need one person who is 100% of the time taking care of these fish."***

BLUE THUMBS

Yet the African fish has its disadvantages. For one, it's not as easy to keep in a lab as other fish, such as zebrafish. "You need to have a 'blue thumb'," says Cellerino. "You need at least one person who is 100% of the time taking care of these fish." They also need more space than zebrafish, which thrive in crowded conditions; killifish males sometimes fight and might interfere with each other's growth. Because killifish develop so quickly, they eat a lot — and so produce a lot of waste, leading to water-quality challenges. "One of the things we joke about is we don't keep fish, we maintain biofilters," says Mickie Powell, a comparative physiologist at the University of Alabama at Birmingham.

Killifish spawn readily; a couple can produce 20–40 eggs a day. But then things get tricky, because the eggs need to develop in a fairly dry place. Scientists often transfer the eggs to peat for a couple of weeks, but the eggs don't hatch at the same time, so require a watchful eye.

Many researchers feed their killifish bloodworms, but the quality of that foodstuff varies with season and by supplier. Food matters, points out Powell, who is working on a standardized killifish food; for example, diet affects epigenetic markers that in turn influence longevity. She thinks that food choice might explain why some labs report different killifish lifespans.

Researchers also need a better understanding of how to keep lab collections healthy. Brunet's lab was blindsided in 2008 when several fish started to act weirdly, rolling awkwardly instead of swimming straight. A veterinary surgeon diagnosed the parasite *Glugea*, which the scientists suspect came in with other species of killifish that they bought from a fish store. "That was the lowest point," Brunet says. "We had to bleach everything and start from scratch."

Scientists still hanker after tools that are easily obtainable for other model systems. Valenzano and Brunet wish for antibodies to study fish proteins, and Valenzano also dreams of more strains and a stock centre to provide them. These will probably come as the community of killifish researchers grows.

That community is growing beyond those who study ageing, Platzer says. Developmental biologists are interested in the suspended animation, or diapause, that the eggs undergo, and evolutionary geneticists are intrigued by the killifish's use of XY chromosome sex selection. Many other fish use mechanisms such as population density, ambient temperature or ZW chromosomes, in which the egg, not sperm, determines offspring gender. The Jena meeting attracted scientists interested in using killifish to study epigenetics during blood formation, toxicology and shift-worker biology, says co-organizer Christoph Englert of the Leibniz Institute.

Valenzano says that discussions among *Notho* researchers have shifted from tool development to biology. For example, in a study posted on the preprint server bioRxiv[11], Cellerino and his colleagues describe how a microRNA involved in controlling excessive iron levels is upregulated in ageing killifish to protect the brain from iron accumulation. The human version of this microRNA is associated with Alzheimer's, a condition in which high iron levels have been implicated, he adds.

"The fun part is just about to start," says Valenzano. ■

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CORRECTION

In the Toolbox article 'The visualizations transforming biology' (*Nature* **535,** 187–188; 2016), the CellPACK image was labelled as an HIV-1 particle, rather than a *Mycoplasma mycoides* cell. Also, the text implied that Nico Scherf is leader of a cell-biology research group, whereas he is a postdoc studying bioinformatics.