C. ALBERTSON

BIOLOGY'S NEXT TOP MODEL?

From Antarctic icefish to Galapagos finches, there are some interesting characters at the fringes of developmental biology. **Brendan Maher** explores a world of alternative model organisms.

Debate wears on as to how, exactly, it happened. Some say it could have been a global cooling event brought on by falling greenhouse-gas levels. Others argue that it was the drifting continents, which opened up Drake Passage and kick-started frigid, circumpolar currents. Whatever the reason, to the fish that lived on the Antarctic shelf about 34 million years ago, all that mattered was that it was getting cold, very cold.

As the water temperatures plunged by about 5 °C to below zero over the next few million years, most fish became extinct or moved on to warmer climes. But one group, the Notothenioidei, remained. Thanks to some extraordinary evolutionary innovations, these bottom-dwellers radiated, speciated and ultimately dominated. Crucial proteins shifted shape so they could work at cold temperatures, and a digestive enzyme fragment took on a new role as anti-freeze. Because colder waters hold more dissolved oxygen, red blood cells became dispensable, and the 16 species of the Channichthyidae family no longer make them at all. These are the icefish of the Antarctic: clear blooded, up to almost a metre long and with eerie, crocodilian features.

To John Postlethwait, though, the icefish are "beautiful". It's not just their haunting looks that captivated Postlethwait, a developmental biologist at the University of Oregon, Eugene. It's what they might do for the study of osteoporosis. A quirk of their evolution, he says, may make icefish a valuable model animal for discovering genetic controls on bone density.

For decades, developmental biology has been dominated by an established A-list of models including the mouse (*Mus musculus*), fruitfly (*Drosophila melanogaster*), nematode (*Caenorhabditis elegans*), zebrafish (*Danio rerio*), African clawed frog (*Xenopus laevis*), chicken (*Gallus gallus*) and mustard weed (*Arabidopsis thaliana*). It has been rare for scientists' fancies to stray beyond these supermodels. The ease with which the animals can be bred, their small size, fast generation times and the slew of laboratory tools with which to manipulate them make them irresistibly appealing. According to the Thompson Reuters Web of Science, more than 50,000 publications in 2008 mentioned mice, and around 6,000 featured *Drosophila*.

"You end up having to start from square one."
— Marianne Bronner-Fraser

Icefish, on the other hand, appeared in about 20. Yet just as in advertising — where hand and foot models ply their modest trades on the basis of singular features rather than overall glamour — there is a place in science for models that have a specialist role. And the demand for them could grow as new tools make them easier to use. In November 2008, 23 alternative models, including fruit bats, comb jellies, wandering spiders and blind Mexican cave fish were featured in the first volume of *Emerging Model Organisms*, a laboratory manual from Cold Spring Harbor Laboratory Press in New York. (Icefish, whose larva is pictured above, haven't yet made the cut.)

Alternative models present considerable obstacles: they are often difficult to collect and maintain, and genetic and genomic tools have to be custom-built. "You end up having to start from square one," says Marianne Bronner-Fraser, a biologist at California Institute of Technology in Pasadena and current president of the Society for Developmental Biology based in Bethesda, Maryland. On the other hand, barriers to working with these organisms are less than they were, thanks to rapid, cheap genome sequencing and advances in other techniques for genetic manipulation. "The demarcation of what makes a good model is beginning to be blurred," says Craig Albertson from Syracuse University in New York who studies craniofacial development in African cichlids and, with Postlethwait, has begun work on icefish. As the field of evolutionary development or 'evo-devo' continues

to captivate biologists, ever stranger critters may find themselves the subject of attention.

Icefish and their ilk are what Albertson and his colleagues call an "evolutionary mutant model" — one in which evolutionary processes have produced characteristics that imitate human disease[1] (see 'The making of a model', overleaf). Researchers can compare the genomes of closely related populations or species to find out how they changed as their bones demineralized, their eyesight deteriorated or another peculiarity arose. By doing so they may reveal the genes or genetic elements that are involved in parallel human processes, such as osteoporosis, blindness or even obesity. Russell Turner, who directs the bone-research laboratory at Oregon State University in Corvallis, says that "evolutionary mutant models have enormous potential". But, he adds, this kind of work is "going to tell us more about how we got to where we are than help us find something that we can make use of for therapy, at least over the short run".

For osteoporosis, the gold standard preclinical model is an ovariectomized rat, which has mammalian physiology and bone loss analogous to that of postmenopausal women. But although the rat is very useful, the story of how it got to be that way is not. The icefish, on the other hand, has a tale to tell. As competitors in the freezing Antarctic waters disappeared millions of years ago, some icefish began to explore niches above the sea floor, something for which they needed buoyancy. They had long ago lost their swim bladders, the air sacs that perform this function in many fish — and structures rarely re-evolve. Instead, their dense skeletons began to demineralize and soften, even to the point that one can see the outlines of their brains through their translucent skulls. Thus, several species of icefish, in addition to living happily with extreme anaemia, have essentially acquired adaptive osteoporosis.

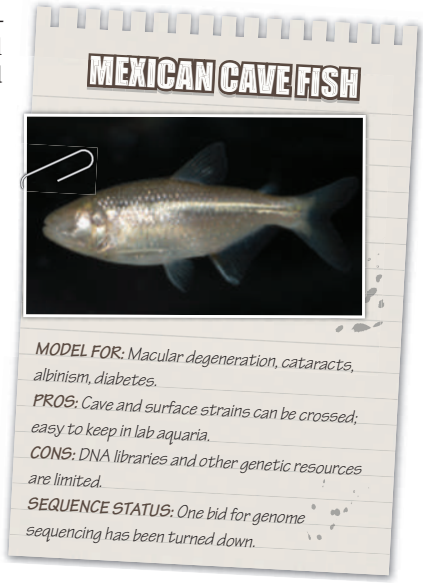
Blind cave fish, top, can be bred with sighted surface fish to explore forms of blindness.

Y. YAMAMOTO

Cold introductions

Postlethwait was introduced to icefish during a talk in 2004 by William Detrich, a marine biologist at Northeastern University in Boston, Massachusetts. Detrich studies their unusual blood development in part to understand human blood diseases such as anaemia. He has been collecting icefish for more than 25 years now, and has fished out a bevy of genes necessary for red-blood-cell development.

R. BOROWSKY



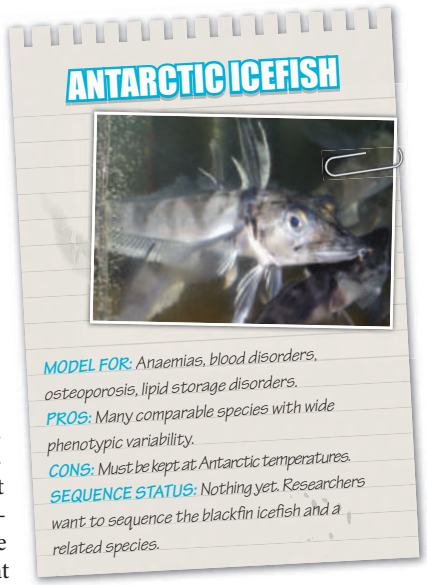
When the two met again in February 2007, at the 2nd Strategic Conference of Zebrafish Investigators in Asilomar, California, they, Albertson and Pamela Yelick, who studies tooth development at Tufts University in Boston, laid out a plan to investigate the underlying cause of bone loss in icefish. It would be a physical as well as an intellectual challenge: they would have to acquire their specimens during Antarctica's winter when the fish are gravid. But the team suspected that the naturally occurring mutations that have led to adaptive bone loss over the past 34 million years, could teach them something important about osteopenia and osteoporosis. Their US$2.48-million proposal to the National Institute on Aging was accepted on the first try. John Williams, the programme officer on the grant, says that there has been ongoing discussion about bringing new models into the field of ageing, and this seemed to fit the bill. "Postlethwait's work gets neatly into that and with a very well designed study," he says.

Postlethwait and the other investigators reason that human and icefish bone loss may have evolved in similar ways. Although strong teeth and bones are necessary early in human life, the pressure to maintain these amenities wanes in later years. For the icefish expanding into niches above the sea bed, the need for strong, heavy bones also lessened. Thus conserved programs involved in bone formation and maintenance may shut off through similar mechanisms, but earlier in the development of the fish than in humans. "You can use these slow evolutionary changes to study changes that might occur in a human in a lifetime," says Yelick.

The collaborators have begun by comparing patterns of gene expression between developing icefish embryos and those of a closely related but dense-boned notothenioid species. They have already found delays in the expression of genes involved in bone mineralization. Postlethwait suspects that further work will uncover mutations that regulate the timing at which these bone genes act. As many of the genes are crucial for development, he says "you can't destroy the gene. But you can destroy the regulatory elements that cause a gene to be expressed in a specific tissue at a specific time." If they do identify potentially important genetic elements, the group will turn to model organisms that are easier to work with to test their function by amplifying or knocking them out. Three-spined sticklebacks (*Gasterosteus aculeatus*), for example, are closely related to the icefish but they have a fully sequenced genome and tools available for genetic tinkering. Alas, recent research has suggested that regulatory

sequences are less likely than the genes they control to be conserved across evolutionary time, potentially challenging the researchers' ability to translate the results to humans. "We need to do the work to see," says Postlethwait, "with the constant honest realization that an icefish does not ride a bicycle."

Neither does the blind Mexican cave fish (*Astyanax mexicanus*) — but that hasn't stopped a small group of researchers from looking at them for hints about human conditions. These cave fish, deprived for more than a million years of light and the trophic abundances it brings, have evolved a slew of 'troglomorphic' traits: they are pale, eyeless and have a keen sense of smell and a slow, efficient metabolism. The 29 populations in limestone caves scattered around Mexico can interbreed both with each other and with sighted *Astyanax* populations from the surface. This sets up opportunities to understand the nature of the cave fish's peculiar qualities because researchers can cross together different cave and surface fish in order to track down the genes inherited alongside its oddities.



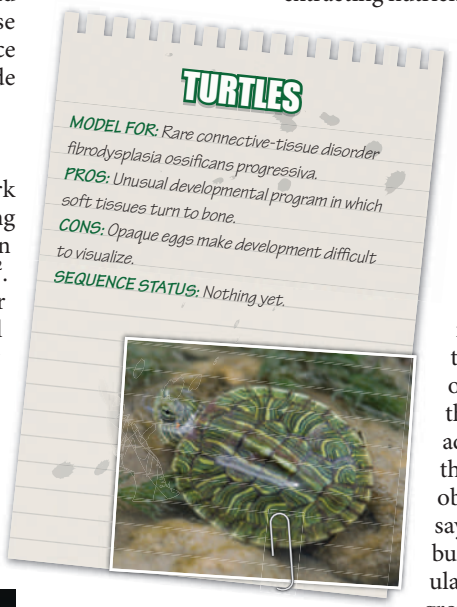
Sight to the blind

Last year, Richard Borowsky, a biologist at New York University, and his collaborators showed that breeding together blind cave fish from two different populations can produce a brood in which about 40% of offspring can see[2]. The results suggest that the parental fish have lost their eyesight through mutations in different developmental pathways, and that two sets of incomplete eye-making instructions can, when combined, make up enough of a readable manual to build a working eye. William Jeffery, an *Astyanax* researcher at the University of Maryland in College Park, says that cave fish may help provide clues about human forms of blindness such as macular degeneration and cataracts. "The cave-fish lens is one of the first things to decay in the embryo, leading to the loss of the eye," says Jeffery. One of the genes implicated is *αA crystallin*, a factor in the lens that prevents apoptosis. It has limited expression during cave-fish development and causes cataracts when mutated in zebrafish[3].

W. DETRICH

Why cave fish lost their eyesight isn't clear. Some have hypothesized that the energetic cost of eye development and maintenance is so high that selection pressure in the dark environment quickly acts against it. Alternatively, eye loss could simply have arisen through neutral changes in each population that became fixed by genetic drift. Or it could be an inadvertent result of another adaptive change. Still, several populations have converged on this phenotype. Contrast that to albinism, which has happened in almost the same way in various cave-fish population, via a mutation to the *OCA2* gene[4]. This gene makes a protein crucial to development of pigmented 'melanocytes' and happens to be the most commonly mutated gene in human albinism. "It may be simply that this gene is a frequent target of mutation in all organisms," Jeffery says.

In addition to living without light, cave fish have evolved to live without much of the bounty that sunlight produces, extracting nutrients from waterborne organisms that slip through the cracks into their limestone caves, or living off the organic matter deposited by overhead bats. Both Borowsky and Jeffery have been studying how the cave-fish metabolism has adapted to the extremely low nutrient level. As it turns out, says Jeffery, they're fatter and more resistant to starvation than their surface-dwelling populations, and Jeffery thinks there could be some parallels to humans. "We're trying to study it genetically. It would seem that this could be a very good model for obesity," Jeffery says. According to some theories, humans are also evolutionarily adapted to survival with little nutrition and this could explain why they easily become obese when food is abundant. Borowsky says his studies, which include an effort to build gene maps for various blind fish populations, have uncovered a variant for the growth hormone GH1 and he is currently sequencing the gene in more populations. Borowsky says of his cave-fish research. "It's certainly not going to cure blindness tomorrow or diabetes, but it's really relevant." Although Borowsky and other cave-fish researchers have attempted to drum up the interest of a high powered sequencing laboratory to sequence *Astyanax* populations, they've so far been unsuccessful.



R. NUSSBAUMER/NATUREPL.COM

Arkhat Abzhanov, at Harvard University's department of organismic and evolutionary biology, is another researcher whose move to obscure models has forced him to build his own genetic resources. Abzhanov had worked with fruitflies and chickens in his studies of *HOX* genes, which are involved in establishing the animal body plan. But to understand how these genes work in an evolutionary context, he turned to Darwin's finches. The vast phenotypic diversity of the Galapagos finch populations that Charles Darwin marvelled at includes a variety of beak shapes that seem

J. BURTON/NATUREPL.COM

Swordtails reproducibly develop melanoma in hybrid crosses.

The making of a model

Some features can make an outlandish organism more suitable as a model for human disease:

Rapid evolution
Organisms that have undergone rapid adaptive radiation are likely to have a lot of related species that researchers can cross together to find the genes responsible for unusual traits.

Many differences
A wide divergence of physical characteristics (phenotypes) offers greater opportunity to study 'quantitative traits', characteristics that vary in degree. For example, if every species has a different number of stripes, it will be easier to discern the genetic controls for stripe number.

A history
Organisms with a well understood evolutionary backstory will more easily offer clues as to how traits developed over time.

Convergence
Different species or populations in which the same trait — such as blindness — has evolved independently essentially count as replicates of the original natural experiment.

A good pedigree
Rapid development to sexual maturity, small adult size, easy availability and the ability to alter and track genes, all make organisms better for experimentation. **B.M.**

custom built for the food source where a given finch lives: thick heavy bills for cracking seeds, or more elongate bills for probing cactus fruit.

By building microarrays for the different species of finch and comparing their gene expression, Abzhanov has been able to get a picture of the genes that are differently regulated in their embryos. He found, for instance, that higher expression of calmodulin, a protein that mediates calcium signalling, is associated with longer beaks. He went back to chickens and used established experimental methods to boost calmodulin signalling in the beaks of embryos. The manipulated chickens recapitulated the finches' elongated beaks[5].

Better safe than sorry?

Abzhanov is also working with African seedcrackers, *Pyrenestes ostrinus*, another bird with an assortment of beak shapes. He is currently applying to the National Institute of Dental and Craniofacial Research in Bethesda, Maryland, for a grant to explore whether comparisons of these birds can identify genes and regulatory pathways that sometimes go awry in human craniofacial development, resulting in conditions such as cleft palette. "They're interested because this is a story of naturally occurring variants which genetically change the integration of craniofacial components," says a hopeful Abzhanov.

Not everyone finds funding agencies receptive though. Bronner-Fraser says that some of her best postdocs are interested in alternative models, but "a lot of times they come and realize that it might be difficult to get jobs and money and they might branch out to more traditional models".

Working with alternative models also requires passion and patience. Abzhanov can't order the animals he needs online. He must go on lengthy field trips to the Galapagos Islands, taking great care not to disrupt their breeding and placing mock eggs for every egg he takes from a nest. Postlethwait and Detrich arguably had it harder, spending weeks in Antarctica in the middle of its sunless winter, trawling for notothenioids. Postlethwait was able to bring back developing embryos, but he found that maintaining the fish in captivity is extremely difficult. "You can have a freezer at minus 20 and you can have a fridge at plus 4. But the temperatures in between, especially temps at around 0 are very hard to maintain," Postlethwait says. Many of the embryos hatched after about six or seven months, he says, when they should take nine.

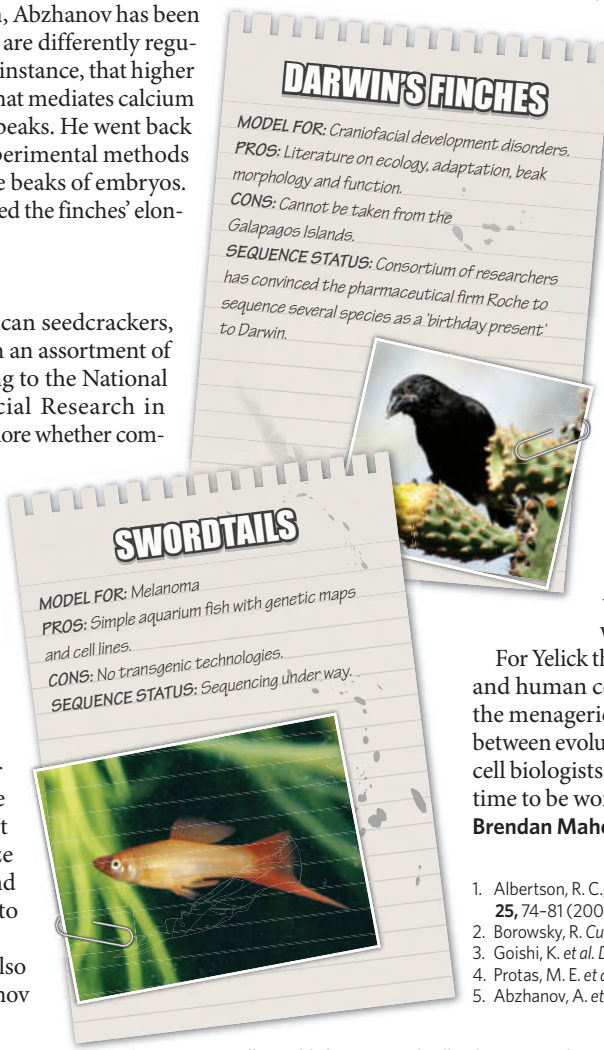
For many researchers, the adventure is part of the attraction. "I think that the people who work on the non-traditional models are often people who want to get out of the lab and into the real world," says developmental biologist Scott Gilbert at Swarthmore College, Pennsylvania. Gilbert, who works on shell development in red-eared terrapins, says they're a "horrible system to use if one wants reproducibility or to have research material at any given day". But he persists because turtles naturally turn soft tissues into bone and so hold broader lessons for these types of transition from one cell type to another.

Gilbert says he is taking cues from human biology to inform his studies on turtle development. Research on fibrodysplasia ossificans progressiva (FOP) — a rare and devastating human genetic disorder in which normal muscle tissue turns into bone — has revealed some of the key proteins involved in the turtle shell development program that he studies. Frederick Kaplan, an orthopaedic surgeon at the University of Pennsylvania, Philadelphia, had discovered mutations in patients with FOP affecting the expression of bone morphogenetic protein. Similar programs seem to direct extension of bony plates from turtle ribs in forming the shell. "I think we're benefiting in a way from his work more than he is from ours," says Gilbert.

When it comes to the Antarctic icefish, it is unclear who, if anyone, will benefit: the researchers trying to lift them out of frozen obscurity, or the people with osteoporosis who could share some part of their biology with these fish.

For Yelick though, who has worked with mouse, zebrafish and human cells prior to her work on icefish, expanding the menagerie is invigorating. "Getting these interactions between evolutionary biologists, molecular biologists, stem-cell biologists and tissue engineers, it's just a really exciting time to be working in this field." ■

Brendan Maher is *Nature*'s Research Highlights editor.



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A. ABZHANOV

1. Albertson, R. C., Cresko, W., Detrich, H. W. & Postlethwait, J. H. *Trends Genet.* **25,** 74–81 (2008).
2. Borowsky, R. *Curr. Biol.* **18,** R23–R24 (2008).
3. Goishi, K. *et al. Development* **133,** 2585–2593 (2006).
4. Protas, M. E. *et al. Nature Genet.* **38,** 107–111 (2006).
5. Abzhanov, A. *et al. Nature* **442,** 563–567 (2006).