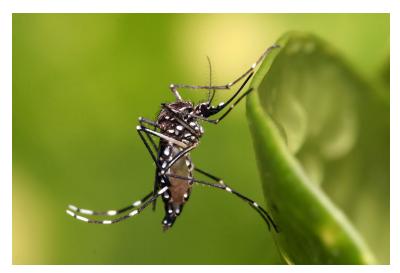# Revolutionising comparative physiology through genome editing

Aedes aegypti in Dar es Salaam, Tanzania. Photo credit: Muhammad Mahdi Karim (GFDL 1.2 http://www.gnu.org/licenses/old-licenses/fdl-1.2.html)

Enthralled by the diversity of lifeforms and the ecological challenges that they encounter, comparative and ecological physiologists have been investigating the planet's most weird and wonderful species to reveal their mysteries for more than a century. With the advent of recombinant DNA and cloning technologies in the late 1970s and 1980s, a super league of model organisms – from yeast and mice to nematode worms, fruit flies and zebra fish – associated with a barrage of molecular tools have become the mainstay of many modern laboratories addressing fundamental questions regarding the nature of life. However, the development of novel genome editing technologies – such as CRISPR/Cas9, zinc finger nucleases, transcription activator-like effector nuclease and gene silencing morpholinos – over the past decade have allowed scientists to begin unravelling the molecular origins of biological questions in species that until recently were genetically intractable. With the revolution in its early stages, Michael Dickinson (The California Institute of Technology, USA), Julian Dow (University of Glasgow, UK) and Leslie Vosshall (The Rockefeller University,

USA) have invited researchers from diverse fields to review and discuss the fascinating biological problems that are beginning to reveal their mysteries thanks to the opportunities presented by genome editing.

Genome editing – from mosquitoes and fruit flies to lampreys and songbirds

Beginning with a practical introduction to the field, Benjamin Matthews from the University of British Columbia, Canada, and Leslie Vosshall outline the factors that researchers need to consider when developing a new model organism (jeb218198). Based on their experiences with the mosquito disease vector *Aedes aegypti*, the pair describe the practical challenges that researchers must consider, from rearing animals in the lab to the availability of a well-documented genome to the technologies – such as CRISPR/Cas9 genome editing – to knock out genes and generate precisely targeted mutations. Describing the decade-long 10-stage process during which they harnessed the genetics of the mosquito, the duo hopes that their example will 'inspire scientists to pursue mechanistic

genetics-driven studies in new and exciting species'.

Returning to the tried and tested model organism *Drosophila melanogaster*, Yoshinori Tomoyasu (Miami University, OH, USA) and Marc Halfon (State University of New York at Buffalo, USA) discuss the powerful lessons that have been learned by investigating the role of enhancers in the fly (jeb212241). Enhancers, also known as *cis*-regulatory elements or CREs, are DNA sequences that determine when, where and how much a gene will be transcribed. After outlining the techniques for enhancer sequence identification in standard model organisms, Tomoyasu and Halfon review the emerging methods, including computational and functional genomics approaches, that allow researchers to identify potential enhancer sequences in non-model organisms. Once these sequences have been isolated, their activity can be tested directly, either using CRISPR/Cas9 or by conventional reporter techniques.

In a review that will inspire researchers to address novel questions about insect olfaction in non-model insects, Claude Desplan (New York University, USA) and an international team of collaborators discuss our current understanding of the development of the *Drosophila* olfactory system (jeb208215). In addition, Adrian Brückner and Joseph Parker from the California Institute of Technology, USA, then branch out to discuss what is currently known about the evolution of biosynthetic pathways for chemical secretion in animal glands (jeb211938). The duo is optimistic that novel gene editing technologies in non-model organisms, as well as advances in single-cell genomics, will permit researchers to reveal 'how gland cells are assembled and functionally configured at a molecular level'.

To answer fundamental questions about the evolution of developmental mechanisms in vertebrates, Joshua York

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Inside JEB highlights the key developments in Journal of Experimental Biology. Written by science journalists, each short report gives the inside view of the science in JEB.

A diablito frog (*Oophaga sylvatica*) with tadpoles. Photo credit: Lauren O'Connell.

and David McCauley from the University of Oklahoma, USA, have turned to one of the earliest surviving vertebrate ancestors, the lamprey (jeb206433). Initially, they used morpholinos to knock out genes in developing lamprey embryos. However, practical hurdles surrounding the technology led them to switch to CRISPR/Cas9 to target and disable genes with a role in neural crest development. The technology also allowed the duo to identify novel cells in the developing nervous system. Hoping that CRISPR/Cas9 genome editing can be applied to learn about the role of gene regulatory networks in evolution, York and McCauley suggest that transgenic changes could be used to control invasive lamprey populations in the Great Lakes.

Switching to consider the genetic underpinnings of behaviour, Jason Gallant from Michigan State University, USA, and Lauren O'Connell from Stanford University, USA, review the power of molecular techniques to address questions about the link between behaviour and the underlying genes (jeb213447). They focus on weakly electric fish and families of poison dart frogs, investigating the selection of voltage-gated sodium channels in electric discharge organs of the fish, and toxin tolerance in the amphibians. Gallant and O'Connell are excited by the opportunities offered by new gene knockout technologies in non-model species; however, they warn that interpreting the outcomes of single gene knockouts may be challenging given the complex genetic networks that mediate behaviour.

Another animal set to divulge the molecular origins of behaviour is the

blind cavefish, *Astyanax mexicanus*, with sighted and blind populations living in close proximity. Listing the novel behaviours developed by the blind subterranean populations – including improved hunting strategies in the dark, altered body clocks and reduced sleep – Johanna Kowalko from Florida Atlantic University, USA, describes how crossbreeding closely related populations has allowed researchers to identify regions of DNA where genes that alter the fish's physiology and behaviour are clustered (jeb208835). Kowalko reviews how altering gene expression with morpholinos and CRISPR/Cas9 has allowed researchers to begin understanding how cave-bound populations have lost pigment and how the fish's sensory systems and behaviours have evolved to compensate for the lack of light.

Precisely coordinating reproduction with the time of year is essential for the survival of many species, so Asano Ishikawa and Jun Kitano from the National Institute of Genetics and the Graduate University for Advanced Studies, Japan, have focused on the hormonal mechanisms that allow three-spined stickleback populations across the northern hemisphere to coordinate breeding with the seasons in their local habitat (jeb208975). Explaining how the expression patterns of the hormones that regulate breeding are affected by day length at different latitudes, the duo is keen to identify the genes that control when sticklebacks breed using omics approaches and genome editing.

The turquoise killifish (*Nothobranchius furzeri*), which lives in the transient water holes that appear during the 3 month rainy season in Central and East Africa, has also fine-tuned its physiology to the seasons of the year. Yet, despite the brevity of its lifespan, the species experiences many of the symptoms of ageing, including pigment loss, declines in vision and fertility, and shortening of the telomeres at the ends of chromosomes. In addition, the cellular powerhouses – mitochondria – also undergo age-related dysfunction. Dario Valenzano and Micheal Poeschla from the Max Planck Institute for Biology of Ageing, Germany, review how new genome editing technologies are allowing scientists to investigate how knocking out a key component of the enzyme that

An adult (left) and old (right) turquoise killifish (*Nothobranchius furzeri*). Photo credit: Dario Riccardo Valenzano.

maintains telomere length affects how fish age (jeb209296) to begin unravelling the molecular mechanisms.

Finally, Sarah London from the University of Chicago, USA, discusses the lessons that we have learned about the neurobiology of learning using male zebra finches (jeb206516). Defining the three forms of learning that underpin how juvenile males pick up songs, London reviews the key brain regions involved in the process. She then focuses on a cascade of genes that are initiated in learning and memory in zebra finches and other species – known as the mTOR cascade (the mechanistic target of rapamycin) – which, alongside the genes that they activate, are potential targets for understanding the molecular regulation of song learning. After discussing the techniques available to create transgenic birds and monitor the impact of gene knockouts, London says, 'Songbirds thus represent great potential for application of genetic manipulations to questions of genome–brain–behaviour relationships across species and lifespan'.

Applications of novel genetic techniques that benefit humankind
In a quartet of reviews, scientists based in Europe and the USA discuss applications of genome editing technologies to address challenges in human health and agriculture. One group of animals that is already being targeted is disease-causing nematodes. 'Infections with parasitic nematodes are some of the oldest known diseases of humans', say Elissa Hallem (University of California, Los Angeles, USA) and colleagues, who wish to learn about nematode reproduction, development and behaviour in the hope of developing novel drugs to combat infection (jeb206482). The team outlines a wide range of molecular techniques to

identify key points in nematode development that could be targeted and components of the animals' nervous systems that are essential for the parasites to locate victims. Using a CRISPR/Cas9 targeted mutagenesis technique developed in her lab, Hallem and her colleagues have identified two critical genes: *tax-4*, which allows *Strongyloides stercoralis* to move toward warmth and a second gene that is necessary for movement that allows the animals to locate hosts.

An alternative strategy for combating disease that is becoming more feasible with the development of genome editing technologies is the creation of populations of disease carriers that have been engineered with a genetic insertion, known as a gene drive, that should spread through the population, either to disrupt disease transmission or extinguish the population. Omar Akbari and Robyn Raban from the University of California, San Diego, USA, with John Marshall from the University of California, Berkeley, USA, discuss the strengths and weaknesses of the current technologies for creating stable transgenic populations (jeb208181). Currently, the resilience of gene drives has only been tested in the laboratory over a few generations and the scientists emphasise that longer-term studies of gene drive stability are essential if the strategy is to alleviate the spread of global killers such as dengue fever and malaria.

Agriculture is another sector that could benefit from the application of genome editing technology. Thomas Bishop and Alison Van Eenennaam, from the University of California Davis, USA,

Nellore cattle in Brazil. Photo credit: Alison Van Eenennaam.

discuss the techniques that could allow the targeted introduction of new genetic traits to improve the health and welfare of animals (jeb207159). The pair discuss methods for introducing gene editing components into tissue cells or early-stage embryos, before moving on to review how sperm-generating stem cells from genetically elite males could be transplanted into the testes of infertile males to produce surrogate sires. However, regulation currently hinders the application of gene editing in agriculture, despite the considerable potential.

Another limitation to the application of genome editing technologies is the difficulty in identifying genes that underpin specific traits. Recognising the challenge, Virginie Courtier-Orgogozo from the Institut Jacques Monod, France, and Arnaud Martin from The George Washington University, USA, have compiled a database, GePheBase, of more than 2000 mutations spanning more than 700 genes in all plants, animals and yeasts, which have been associated either

with natural evolutionary change or domestication (jeb208934). Analysing the database, they discovered that the number of genes involved in toxin tolerance and colouration variation is much broader than for other traits, such as drought adaptation. In addition, Courtier-Orgogozo points out that 'the large-effect mutations contributing to natural evolution tend to disrupt genes less than the ones selected by breeders'.

Concluding the collection, Martin, Nora Wolcott and O'Connell review how they are teaching genome editing techniques through course-based undergraduate research experiences to the next generation of bench scientists (jeb208793). Listing the factors that teachers should consider when selecting a study organism, they also describe bringing hands-on experience of CRISPR genome editing into the classroom. Martin and O'Connell then outline their course structures, which include lectures and practical sessions targeting the pigmentation pathway in *Xenopus laevis* tadpoles and the wing patterning genes of painted lady butterflies. They also explore the ethics of genome editing through presentations and written assignments. 'Importantly, undergraduate students [are] successfully performing CRISPR-based tests of gene function', they say, adding, 'Our basic structure lays out a clear path for designing course-based undergraduate research experiences where students […] actively participate in the creation of scientific knowledge'.

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Kathryn Knight
kathryn.knight@biologists.com