Model organisms: beyond the inner circle

Vivien Marx

Progress in genomics offers researchers many new reasons to expand the universe of organisms they study.

The scientific limelight comes naturally to model organisms such as baker's yeast, the fruit fly or the house mouse. The promise of genetics to shed light on heredity, development or behavior attracted many researchers to focus on these organisms. Now, powerful genetic tools and data can be applied within and outside the cabal of established models, swinging lab doors open to biodiversity. But not every sequenced organism will have an easy ascent to stardom.

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Genomic buzz

The mosquito whizzes toward the model organism circle in neurobiologist Leslie Vosshall's lab at Rockefeller University. She and her colleagues previously determined the genetic basis for why men's sweat smells floral to some women and offensive to others[1]. Expanding this olfactory system research to a public health challenge, she studies why humans smell desirable to blood-feeding mosquitoes carrying malaria or yellow fever.

"For the first time, we can produce strains of mosquitoes lacking a given candidate gene and can ask in what way their ability to hunt a human host is impaired," says Vosshall. Researchers now use gene editing on nonmodel insects such as mosquitoes, crickets and silk moths[2]. Although RNA interference is considered easier than gene editing, this method does not lead to heritable changes or completely abolish gene activity, and it cannot produce the numbers of animals needed for large-scale behavioral genetics, she says.

Vosshall began her mosquito project in 2009 with zinc-finger nucleases (ZFNs), which she calls "the only system ready for prime time." More recently, she has added approaches such as transcription activator–like effector nucleases (TALENs) and noncoding RNA with clustered, regularly interspaced, short palindromic repeats (CRISPRs). In her view, these approaches offer researchers more control than ZFNs in targeting gene sections, and they carry "a fraction" of the production price.

The genomic package is ready now that the mosquito genome is in hand along with tools such as ZFNs, TALENs and CRISPRs. "Our hope is that we can fast-track what took the *Drosophila* community a century to achieve by using these powerful tools to make targeted mutations in important mosquito genes," says Vosshall. Other research teams harbor fast-track hopes, too.

Behold the pig

Pigs are potential transplant donors for human patients. Patients who receive organs from human donors need immunosuppressant drugs, and organs of porcine heritage may cause an even heftier immune response. Scientists at Indiana University (IU) School of Medicine have edited the pig genome using ZFNs to produce piglets whose cell surfaces lack the antigens *N*-glycolylneuraminic acid and galactose a-1,3-galactose[3]. According to the authors, these pigs' cells are less likely to trigger the acute antigenicity that hinders pig-to-human transplantation.

The IU team members acknowledge that ZFN-based editing can lead to random integration of plasmids into the genome. They note that whole-genome sequencing to compare modified and unmodified pig genomes will help avoid such effects if, for example, ZFN-engineered pigs are being considered as sources of tissue for clinical use.

This study is one of four publications in the last 2 years in which research teams applied Sigma-Aldrich's ZFNs to genetically modify pigs, says Shawn Shafer, who directs the company's functional genomics technologies. Because the IU findings address complex genetic challenges with high efficiency,

Pigs might be the right model organisms for some biological questions, as new genetic tools become increasingly available.

they encourage researchers to explore "previously untouchable" challenges, he says.

In response to scientists seeking to expand the use of ZFNs, his company set up a yeast-based validation strategy. Sigma wants to remove from biology the "shackles of specific gene targets in a subset of species," he says, thereby opening up any genome to modification. Gene editing could become a "commodity experimental strategy."

An edited genome was part of the artificial parenting that led to Pig 26, the recently announced piglet born at the University of Edinburgh's Roslin Institute, where Dolly the sheep was cloned. Scott Fahrenkrug, a geneticist at the University of Minnesota and the CEO and cofounder of a start-up called Recombinetics, is a collaborator on the Pig 26 project. He is focusing separately on using gene editing to create pigs as preclinical models of cardiovascular disease, diabetes and cancer. He does not wish to disclose his customer base beyond saying that his clients develop therapeutics.

Gene-editing approaches might be more readily accepted in pigs than in dogs, says Scott Fahrenkrug.

The Ossabaw Island hog is a breed descended from feral pigs on an island near Savannah, Georgia, USA. Similarly to humans, these pigs can develop metabolic syndrome, which brings on elevated blood sugar, weight gain, high blood pressure and a heightened risk of diabetes, heart disease and stroke. "You give them a high-caloric diet, and they pack it on quick and they get type 2 diabetes," Fahrenkrug says.

He and his team believe that the availability of many pig-breed genomes, a wealth of gene-editing tools and information about human patients with diabetes can inch researchers closer to replicating such diseases in pigs[4]. A few spots in the pig genome are not easily edited with TALENs, so Fahrenkrug is exploring the CRISPR system. "A bad carpenter's committed to one tool," he says. "A good one has many and knows which, when and how to use them all."

Pigs are already used as human disease models, alongside dogs. Gene-editing approaches might be more readily accepted in pigs than in dogs, says Fahrenkrug. Pigs

might even replace human patients in early-stage clinical trials. "We've got a long way to go from here to there. We'd like to think that we can help accelerate translation in that way," he says. Pigs will not be right in every case, but these new tools help scientists select the best model for their biological questions.

Cost and husbandry are barriers to seeing pigs as just big lab mice. Fahrenkrug's plan is to offer scientists the animals on demand so that they need not aspire to become master pig handlers. His team has broken ground on a pig genetic innovation facility to be completed next year, with more attention to animal welfare given there than they receive in industrial agriculture.

His company is making all its gene-editing components available through the plasmid repository Addgene. "It's not a drug model; it's a software model," Fahrenkrug says of his approach, which is to widen the circle of tool users who pay royalties only if they create a commercial product. His company has a global exclusive agreement with the gene-editing company Cellectis, which licensed TALENs from the University of Minnesota.

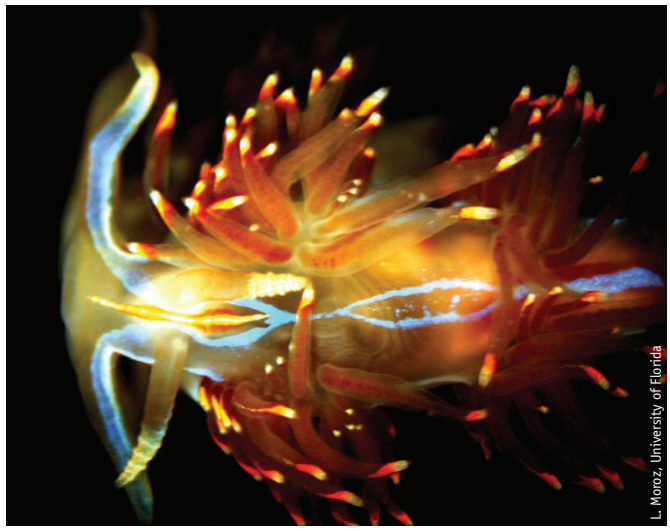
A learned slug

When the sea slug feels threatened, it squirts sticky purple ink that coats its predator's antennae, rendering the pursuer unable to sense its potential lunch. Scientists have chosen to work with the sea slug because its behaviors can be correlated with events in neurons that are so large they can be seen with the naked eye. Now genomic analysis brings this model organism to the fore in new ways.

This animal, first described by Roman natural philosopher Pliny the Elder, figures prominently in Nobel laureate Eric Kandel's work. Much of what is known about memory circuits has come from sea slugs, particularly Kandel's work, says neurobiologist Leonid Moroz from the University of Florida. But despite this track record in biology, scientists working with sea slugs have difficulty landing grants, he says—for example, because of doubts about the animal's translational value for biomedicine.

The molecular events involved in human memory—whether in learning the alphabet, recalling a first kiss or forgetting a decade of one's life—are poorly understood. Moroz says that work in nontraditional models and approaches such as single-cell genomics help researchers study such events in the complex human brain. His group is developing genomic methods and software to integrate, in a parallelized fashion, data captured across many neurons.

He and his team study neuroplasticity in sea slug neurons on a molecular level and look at single-cell genomic changes[5]. "Technically and conceptually it is only possible to do this in invertebrate organisms," Moroz says. As the sea slug learns and remembers, the team's protocols allow researchers to track and capture its gene expression through RNA-seq and to look at DNA methylation dynamics. "Within 2 hours of memory formation, half of the genome is chemically reorganized," he says.



Genomics helps sea slugs, such as this opalescent nudibranch, re-emerge as a model organism. Studying such slugs, Leonid Moroz combines single-cell genomics and behavior to paint a molecular portrait of neurons and reconstruct the evolution of neural circuits.

The work will soon be able to draw on more genomic information. Moroz initiated the sequencing of the sea slug genome, a collaboration with the Broad Institute that is nearing completion. His lab is now expanding this approach to study hippocampal neurons, which make up a much larger memory circuit, in mice. The cells differ between species, but the principles stay the same, he says.

He believes that the US government's new Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative rightfully draws attention to the brain. "Currently, neurological disorders are only a one-way ticket," he says, which underscores the need to reverse or slow down these diseases. Tackling neurological disorders calls for understanding how the genome shapes dynamic events in specific cells in real time, he says.

The decoded genome fills scientists' molecular toolbox to the brim with letters: "We have the alphabet, but we have no idea of grammar," Moroz says. He believes that by integrating genomic analysis, physiology and behavior, researchers can begin to work out the needed grammar. This research can help scientists explore the genomic basis of neuronal identity, wiring and plasticity as well as topics such as the epigenomics of memory persistence and cellular activity in heterogeneous tissue surrounded by an influential microenvironment.

Translational scientists build the bridge from fundamental discovery to medicine, but for this task they need a wealth of basic research to translate from, Moroz says. Nature and evolution have delivered a biodiversity of experiments waiting for scientific discovery, all of which will help address human disorders, he says. But right when genomic methods let researchers expand the small circle of traditional model organisms, budget belts are being tightened. Cuts will hit the smaller basic-research–oriented groups harder than applications-oriented scientists, he says. Yet he hopes that the new genomic tools and the value of nontraditional models for neuroscience, cancer and systems biology will all factor into funding decisions.

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