What is a lab animal?

Small but dedicated communities are bringing the earliest lineages of the animal kingdom into the lab. Take a look at the ctenophores, the sponges, and the placozoans.

Ellen P. Neff

What does it mean to be an animal? Looking across the kingdom's 35 phyla, it's tempting to think that some are more advanced, more complex, more valuable than others. Aristotle drew such a hierarchy in his *Scala Naturae*. Humans perch on the highest rung of the ladder. Then come primates and the other mammals, followed by fish and reptiles and finally the various invertebrate lineages.

But that's not quite the case. "There are not higher and lower animals," says Casey Dunn, an evolutionary biologist at Yale University. "There are just animals. Period."

"Right now, our conception of what it means to be an animal is very bilaterian-biased," says Scott Nichols, an evolutionary biologist at the University of Denver. Most of the traditional research models—animals like the fly, worm, mouse, and zebrafish—belong to this clade with bilateral symmetry, and people don't tend to encounter the other animals out there that look less like us, he says. "But the reality is, if we want to make these generalizations about what it means to be an animal, we have to take into account these very different animal lineages."

If you look across the tree of animal life, you'll find three phyla about as different from humans as can be: the ctenophores, the sponges, and the placozoans. Maybe you've heard of them?

Working with them can give researchers fresh perspectives about what animals are and how they came to be. "If we look at the genomes of these organisms, they are not very different from the genomes of bilaterian animals. It's their cell biology and their developmental biology and their anatomy that's so different," Nichols says, "I find it fascinating to think that we could take essentially the same genetic toolkit and apply it in different ways to get such different results."

Just don't call them basal, ancestral, or 'living fossils.' "When we talk about integrating these other model systems into this comparative context, it's not that a ctenophore or placozoan or sponge is an old animal. They're not," says William Browne, an evolutionary & developmental biologist at the University of Miami.

Beyond the old hierarchy: an animal is an animal, each with its own unique attributes. Credit: Kelly Cheng Travel Photography / Moment / Getty

Each living animal, since its lineage split from its last shared ancestor hundreds of millions of years ago, has continued to evolve solutions to life on Earth. Some of these solutions are shared across phyla, others are not. "Anything that's different is really cool because it illustrates what's possible," he says.

Simplicity is relative too. When Manu Prakash first laid eyes on a placozoan in a microscope in Leo Buss' lab at Yale, he was hooked and ready to spend the next 50 years with the animal. "I have a fascination for simple things where I can ask a simple question and have a possibility of finding a complete answer," he says. But after a decade of research he's found that working with placozoans is not always so simple.

"When you work with non-model systems, you really have to build all the tools from scratch," he says.

The tools are coming to the lab, as are the animals. So what exactly are they?

Out of the oceans

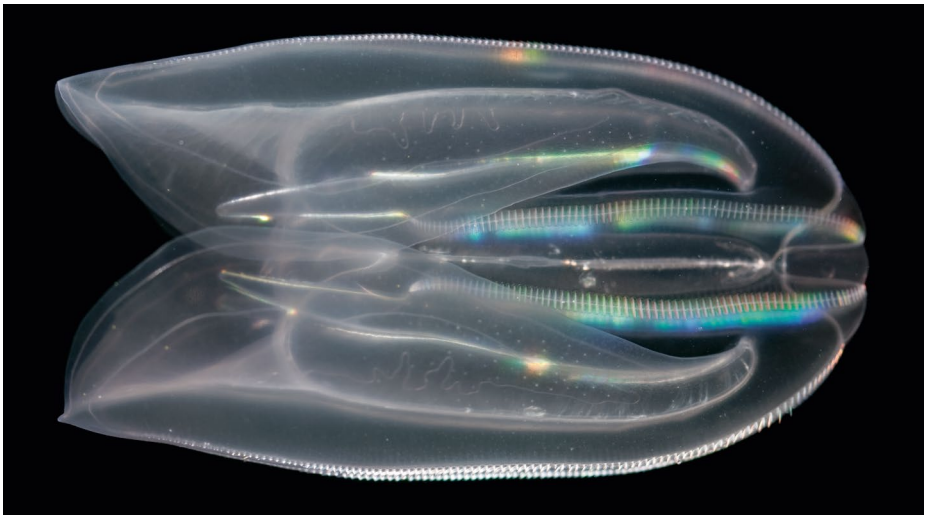
Life evolved in the oceans, and that's where you'll still find placozoans, sponges, and ctenophores.

Placozoans are tiny—these asymmetrical "flat animals" are a few millimeters in size and consist of just six known cell types. They have two epithelial layers with cilia to propel them around in search of algae, and fibers in between that allow the animals to change shape[1]. They turn up in temperate and subtropical waters all over the world.

Long considered the representative placozoan species, the "sticky hairy plate" *Trichoplax adhaerens* was found clinging to the glass of an aquarium tank in Graz in the late 19th century; its genome was sequenced in 2008[2]. Most placozoan studies use clones of this species, though researchers like Bernd Schierwater, who's worked with the animal since the 80s, and his colleagues say there is much greater diversity waiting to be documented.

It's been 135 years, but a second placzoan species has just been named and sequenced: *Hoilungia hongkongensis*[3]. The two placozoans may be morphologically identical, but have considerable genomic differences according to the authors.

Sponges, the "pore-bearers" of Phyla Porifera, are a bit more diverse—over 8500 species have been described so far[4]. These sessile filter feeders inhabit both shallow seas and the deep ocean, along with one lineage that's overcome the osmotic challenges and made the move



Bioluminescent beauty: An adult ctenophore, *Mnemiopsis leidyi*, with its characteristic, colorful comb rows. Credit: W. Browne

into freshwater. They have endodermal and ectodermal epithelia, lined with cilia that help filter in the animal's planktonic meals. Sandwiched in between their epithelial layers is a gelatinous mesohyl. Spicules, small structures made of silica or calcium depending on the class of sponge, help give the animals shape.

Like placozoans, sponges lack a nervous system and muscle cells. They might not look like they move much, but they do contract and expand in response to stimuli, a behavior professor Sally Leys at the University of Alberta likens to a slow sneeze.

"Comb jellies", the ctenophores, are not to be mistaken for jellyfish. The distinct phylum contains about 200 known species throughout the world's oceans[4]. Most freely swim throughout various depths of the water column, but a few attach themselves to substrates at the bottom. The animals are characterized by eight ciliated rows, the combs that give them their colloquial name and propel them about, and by their bioluminescent abilities. Like sponges, they have two body layers separate by jelly-like mesoglea. They are the earliest example of an animal with a simple nervous system and musculature, and most are aided in their predatory ways by tentacles covered in sticky cells called colloblasts.

While ctenophores can be abundant in the wild, the gelatinous animals can be quite fragile. "If you handle them too roughly, they will literally vanish before your eyes," says Browne.

Debate rages about the phylogenetic placement of these three phyla relative to the rest of the animals. Cnidarians seem to have settled as the closet sister group to the bilaterians, but placozoans, sponges, and ctenophores have all had their moment as the earliest branching animal lineage. "It's become one of the best characterized, difficult phylogenetic problems," says Dunn. The relationships are sensitive to how they are analyzed, he says, "which means we don't have conclusive evidence one way or the other, even though it seems every year or two a paper comes out that states that we've conclusively settled the debate."

Let the phylogenetic chips ultimately fall where they may—there's still a lot each of these animals can tell us.

Filling in gaps

These days April Hill works with sponges in her lab at Bates College in Maine, but they weren't always her animal of choice. She started her career with people, working on the Human Genome project, and then moved on to study the *PAX6* gene network and development in mice. It was her husband, a marine ecologist, who sparked the first curiosity to look outside mammals. "It was actually him who said to me, I wonder if those genes that you are studying in mice are in sponge?" she recalls.

"At the time I knew much less about sponges than I do now, and so you know I was a bit naïve. I thought they couldn't really do anything but hang out at the bottom of the water. But it was a really fascinating question." When people think of Pax genes, they often think of eyes, she says, "and of course sponges don't have eyes." But they do have genes in the network[5]. In sponges, these are involved in the epithelial lining of their canal system. "If you look deeply in humans and mice, that's another role that's played," she says.

The last common ancestor of all animals no longer exists, but searching for similarities across the living clades can help fill in gaps about the basics of biology and how animals evolved.

Take the nervous system. Ctenophores have one, sponges and placozoans don't (Box 1). If ctenophores are truly the sister group to the rest of the animals, it means that either the nervous system evolved twice, in ctenophores and again in cnidarians, or just once, only to be lost in the sponges and placozoans.

Both are fascinating scenarios to Pawel Burkhardt, whose lab at the Sars Centre

Box 1 | Nerves? Who needs them?

A lack of nerves isn't holding sponges or placozoans back. They've each done just fine without a formal nervous system for millions of years, *thank you very much*. But to coordinate movement and behavior, there still has to be some kind of intercellular signaling involved.

"It's just fascinating that there's an animal out there that doesn't have what we would think of as a nervous system," says Adriano Senatore, a researcher at the University of Toronto-Mississauga who studies the evolution of voltage-gated calcium channels and calcium signaling. "And yet it's able to conduct behavior that you normally think you'd need a nervous system for. I think *Trichoplax* helps blur the lines," he says.

With collaborators Thomas Reese and Caroline Smith at the NIH, Senatore has observed neuropeptides that can influence *Trichoplax* behavior, arresting their movement as if they've discovered food. They are currently following up to figure out whether genes that are involved in neural signaling in other animals are at work in *Trichoplax* too. He's also studying the molecular properties of the calcium channels found in *Trichoplax*—it has all three types found in humans.

And those sneezing sponges? Leys studies nonelectrical signaling and how it can give rise to such movements in a muscleless, nerveless animals. She's found evidence that the amino acids and neurotransmitters glutamate and GABA are behind the behavior in the freshwater species *Ephydatia muelleri*, chemical messengers that are also common in other animals[11].

at the University of Bergen studies the evolution of synapses. He works with a trio of organisms: the ctenophore *Mnemiopsis leidyi* and the calcareous sponge *Sycon ciliatum*, both locally available in Norwegian waters, as well as choanoflagellates, the unicellular sister group to animals. Genomes and transcriptomes of all three are available, which lets him compare genetic similarities and differences between the morphologically different organisms, two that are animals and one that isn't.

"You have to study exactly these organisms because they are at the right phylogenetic spot," Burkhardt says. The genetic machinery to make synaptic proteins exists in all three groups, he says, even though the physical structures only turn up in ctenophores.

There are advantages in the animals' simplicity, relatively speaking. *Trichoplax* for example has a little over 11,500 protein-coding genes, many of which overlap with other animals[2]. But because the number of genes per family is smaller, there are fewer possible interactions to tease apart to understand a more complicated network, says Schierwater.

Take, for instance, uncontrolled cell growth—aka cancer. *Trichoplax* has a homologous gene for human p53, a cancer suppressor, and its ligase, Mdm2, a protein found in mammals but lost in model invertebrates like *C. elegans* and *Drosophila*. Chemically inhibiting the proteins in *Trichoplax* increases programmed cell death and eventually kills the animals, suggesting a conserved role in an animal that's genetically simpler than a human[6].

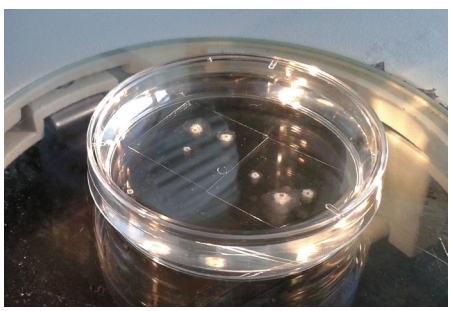
From the field: The sponge *Ephydatia muelleri* in its natural freshwater habitat. Credit: J. Mitchell

In Miami, Browne is interested in a family of genes called Krüppel-like factors (KLF) and the role these transcription factors play in stem cell maintenance. The family predates the rise of animals and turns up in humans too. The complicated part is, humans have 17 KLF genes, Browne explains. Ctenophores have just three. Every time the animals snag a meal, the colloblasts at the end of their tentacles are damaged and must be replaced before they can hunt again. Determining how the KLF genes contribute to that replenishment in ctenophores might in turn shed new light on how the gene family, despite its duplications over evolutionary time, functions in humans as well.

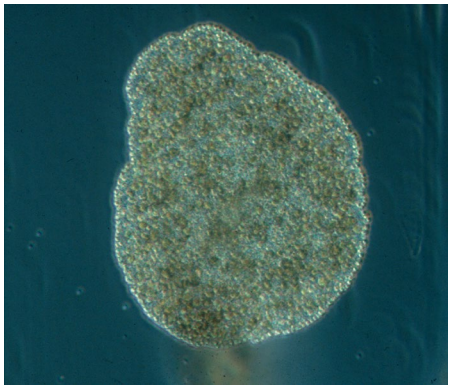
Differences matter too. When your worldview is limited to vertebrates, says Browne, "you miss what could be different, and what can be different can be the most important means for addressing how to get around a problem."

Nichols studies the epithelium of sponges in an evolutionary context, as a barrier to the outside world and as a means for multicellular organisms to partition their bodies into physiologically unique environments. He's found cell-adhesion molecules like cadherins in sponges, as well as conserved genetic machinery for things like collagen, integrins, and laminins. In some cases the proteins identified in the sponge function in the same ways as they do in other animals, but not always, he says. That can make interpretation a bit challenging, given limited technical options to genetically perturb the animals, at least at the moment.

In addition to novel functions, he's also intrigued by what the sponge has that traditional models lack. Take binding proteins in the Vinculin family. Two are well known from studies with traditional models, but there's actually a third, Nichols says. It's just been lost in worm, fly, and vertebrates. His lab is starting to study this third protein, which he suspects is involved in actin binding and may play a critical, butyetunacknowledged, role in cell adhesion.



A sponge is born: *Ephydatia muelleri* clones growing from gemmules in the lab. Credit: S. Leys



More than meets the eye: *Trichoplax adhaerens* in all its cellular simplicity. Image from Eitel, M. et al. *PLoS One* **8**(4): e57131 (2013).

The makings of a "model"?

Mark Martindale has been interested in ctenophores since "before anybody knew what a ctenophore was," he laughs. For his dissertation, he did some dicing. A ctenophore embryo, cut in half, will grow up into two half adults—literally. But when he took some those half animals and cut them in half again, some regenerated into whole animals, despite never having ever been "whole"[7].

An adult regenerative program exists, and it's a story he's ready to return to three decades later. "We have functional genomics, we can raise them in the lab," he says. "We can do all the kinds of things that you used to only be able to do in flies and mice and nematodes."

But relying on individuals sampled from wild populations means having to cope with population-level differences that can complicate attempts at genetic manipulation, Martindale says, "We can't always know what every base pair in the genome of the animal that we're working on today actually is."

Getting animals into the lab can be the first step; then what?

Taking care of *Trichoplax*. It's not too hard to collect placozoans: simply leave a few glass slides in shallow coastal waters for a few days or weeks, and eventually some animals will turn up, says Schierwater. Back in the lab, they're happy in a small dish with some algae and a biweekly seawater change, says Andreas Heyland, a researcher who spent his post-doc working with the animals, describing their behavior and developing assays to study them[8]. "It's literally five minute to ten minutes a week to maintain them in relatively large colonies," he says.

People think fruit flies are inexpensive compared to an animal like a mouse,

Big stretch: A juvenile *Mnemiopsis* with tentacles extended. Credit: W. Browne

Heyland laughs. "That's not even on the same level as something like *Trichoplax*." Though he no longer actively works with them, he still keeps a few cultures around his lab at the University of Guelph for interested students.

In the lab, *Trichoplax* is clonal and reproduces by binary fission. Sexual reproduction is unconfirmed, as is the location of the animal's stem cells. No genetic model has been developed yet as a result, but the animals are amenable to more classical approaches, says Senatore: identifying cells and where they localize to in *Trichoplax*, making comparison between those in placozoans and those in other animals, and isolating placozoan genes to express in human cell cultures as a way to probe their molecular properties and potential phenotypes.

But they can still require some customization. After Prakash decided that he wanted to image every cell in a freely moving *Trichoplax*, it took him and his lab at Stanford three years to build the necessary set-up. In the dorsal epithelium of *Trichoplax*, they recorded the quickest epithelial cellular contractions in any known organism—they hypothesize that the principle of "active cohesion" needed to accomplish the feat could yield insight outside biology in materials engineering[9].

Suitable sponges. If you know where to look, sponge embryos can be easy enough to find during spawning season, but most species need to be collected from the field. No sponge to date has been found where both eggs and sperm predictably spawn to allow fertilization in culture and development into new sponges, says Leys. Those that do reproduce in the lab, like *Tethya Wilhelma*, "bud" asexually, or "brood," like *Amphimedon queenslandica*. Many researchers rely on animals that are locally available as a result.

There are pharmacological means of manipulating protein expression in sponges, but no genetic model yet, notes Hill. Hill also studies the microbial symbionts that can make their way inside the cells of sponges; she suspects that tapping that relationship could be a way to sneak a tool like CRISPR/Cas9 into the animals in the future. Viral transduction might be possible too. Sponges have a conserved homolog of the receptor for adeno-associated virus, says Nichols, the viral vector that is used to manipulate genes in other animals. It's an approach he sees as potentially promising in *Ephydatia muelleri*, the freshwater species that could be a leading contender in the search for a "model" sponge.

Ephydatia muelleri is a transparent, globally available species that must withstand its freshwater habitats freezing around it each winter. To do so, the adult tissue dies back, leaving behind minuscule capsules of stem cells called gemmules. Researchers can collect those in the field and keep them dormant in refrigerators. Whenever you're in need of a sponge to study, simply place a gemmule on a coverslip and in four or five days, you'll have a new, clonal animal ready to go.

Many in the sponge community have agreed to more concerted efforts to develop tools and standardized methodologies for *Ephydatia,* says Leys. "It's probably like being in a time capsule of how other model systems started," she notes. She is currently working on sequencing its genome and establishing standardized protocols for working with it, and others like Nichols and Hill have also adopted the freshwater sponge in their own work.

But sponges could still use some more ultrastructure and cell biology work in the meantime, says Leys. "We jumped from electron microscopy through to molecular techniques," she says; it's valuable to know that genes are showing up somewhere, but limiting to not know exactly where that actually is in the animal. "The techniques are a little in advance of the understanding at this point," she says.

Tracy Simpson, in her 1984 book *The Cell Biology of Sponges*, compiled initial documentation of sponge ultrastructure and cell biology, as did Norbert Weissenfels (albeit in German) but then "we sort of leap frogged over that," says Leys, leaving gaps of knowledge about some of their basic attributes.

She hopes to rectify that with a sponge atlas, akin to what researchers might find with mouse or fly.

Combing for a ctenophore. An emerging ctenophore model, "the white rat of ctenophores" according to Martindale, is the sea walnut *Mnemiopsis leidyi*.

Most ctenophores spend their lives in open water, but *Mnemiopsis* is a coastal species. As a result, they do tend to periodically run into things, says Browne. That makes them just a bit more robust than other ctenophores out there, and more amenable to life in the lab.

They still need their space though. Browne says he keeps about 10 to 15 animals in a 90-gallon tank filled with sea water and modified so that it slowly circulates to keep the animals away from the hard glass. They'll reproduce in the lab too—he's now up to eleven generations—and he's noticed signs of domestication going on as a result. "At this point the laboratory strain is doing better under crowding conditions than wild-caught animals," he says.

Mnemiposis leidyi's genome was sequenced in 2013[10], and there are published protocols to work with primary cell cultures, a requisite to cell biology studies as well as for spawning new ctenophores from wild-caught adults and fixing embryos for immunohistochemistry and whole mount *in situ* hybridization work.

Don't miss them: Freshwater sponges in Colorado. Credit: J. Mitchell

When Martinedale's not busy running the Whitney Laboratory for Marine Science at the University of Florida, he's currently looking to improve microinjections and other ways to manipulate gene expression in ctenophores. Equipment needs to be modified for use with them, and it's currently a slow process given their embryo's small size but large yolk mass, he says. He's had initial success in applying electroporation techniques to up the throughput from the ten or so embryos he's able to manage currently, and he's also able to manipulate some gene expression with Morpholinos. CRISPR is likely coming for ctenophores.

Ctenophores though are self-fertilizing hermaphrodites, "which from a genetic standpoint is a bit of a double-edged sword," says Browne. *Mnemiopsis* may be fertile in the lab, but isolating which eggs and which sperm came from what animal in a standardized way to enable crossing, and thus maintain specific genetic strains once CRISPR inevitably arrives, is a puzzle he and his colleagues are still looking to solve in order to get a fully tractable model.

Time to reflect

The communities studying these three phyla are small and no sponge, ctenophore, or placozoan may ever dethrone the traditional models like the mouse, but putting the time and effort into working with less traditional animals has its place. "We need to keep that breadth, and it's sort of jolting," says Leys. "It helps people understand things that are not always typical in mammalian systems."

Mice of course tell us a lot of important things, says Hill, "but if we only work on a couple of model systems, I think that we're at risk of missing out on all the ways that the animals have and possibly can do things, on the tissue level, on the cell level, and on the molecular level." Having worked with murine models, she notes a tendency in the community towards studying minute details, while sometimes forgetting that at the end of the day, mice aren't people either.

It's important too to think about what the word 'model' means, says Dunn. "If we study flies and they do one thing one way and then we study mice and they do it another way, well, what does that tell us?" he asks. Looking elsewhere might just help fill in the gaps.

Sequencing tools are improving, which means "we're going to have really high-quality, full-length genomes from a broad diversity of animals," says Dunn. An exciting prospect, but then it's going to become about the organisms again, he continues, as those genomes inevitably raise new questions about development, morphology, and physiology.

Every organism has its advantages and its limitations. And everyone, even those working with novel, non-traditional animals, should try to avoid overgeneralizations, says Nichols, and remember to pause sometimes to appreciate the diversity that's out there.

Cheers to the entire animal kingdom. ❐

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