COMPARATIVE PHYSIOLOGY

Recharged Field's Rallying Cry: Gene Chips for All Organisms

Seeds of microarray technology help comparative physiology bloom again

Four years ago, comparative physiology was a discipline sorely in need of a push. Bogged down in traditional approaches that seemed to yield only incremental advances, researchers couldn't even make a case for holding their every-fourth-year international meeting, sponsored by the American Physiology Society. So they didn't have one.

But with researchers now eager to apply 21st century technology to the field, the study of how animals' bodies work and how creatures adapt to environmental conditions is coming out of its slump. "Comparative physiologists are really tapping into a lot of the advances in molecular technology to ask questions that they have been pondering a long time," says James Hicks, a comparative physiologist at the University of California (UC), Irvine. Adds physiologist Andrew Cossins of the University of Liverpool, United Kingdom, "It's shaking everybody up."

Last month, comparative physiologists met* for the first time in 8 years. Cossins's colleagues Andrew Gracey and Jason Podrabsky, both of Stanford University, and others wowed the audience with studies using gene chips—glass slides dotted with thousands of bits of DNA—they had created. When exposed to labeled RNA samples, the chips' dots grab hold of matching sequences and light up. Researchers can use the technology to track the activity of many genes over time or under different conditions.

Until recently, many physiologists bypassed the chips as impractical. Except for organisms such as yeast, *Caenorhabditis elegans*, and others whose genomes had been sequenced, there was very little genetic material to put on the chips. People realized that the technology "might be powerful," says Hicks, "but it seemed completely out of reach for the kinds of animals we were studying," such as minnows, turtles, and ground squirrels. But when researchers steeped in chips, such as Gracey, gave their presentations, "a light went on," Hicks adds. Everyone realized that "you can apply this kind of analysis to all sorts of animals."

Custom-made chips

Gracey gained his expertise early. In 1998, he arrived as a postdoc at Stanford just in time to get caught up in a gene-chip stampede set off by Pat Brown and his colleagues. They had not only developed a relatively inexpensive gene chip, called a microarray, but they had also distributed free instructions on how to build, use, and analyze the gadgets (*Science*, 15 October 1999, p. 444). Microarrays were showing up in labs all across campus, and Gracey tapped that expertise to build one for studying the longjaw mudsucker (*Gillichthys mirabilis*), a fish abundant in California estuaries.

New blood. Stanford's Andrew Gracey (left) and Jason Podrabsky (right) are adding genomics tools to comparative physiology.

CREDIT: GLENN JOHNS

The task was daunting. Previously, researchers had usually made chips with DNA from yeast or other well-characterized organisms, accessing public archives for the necessary genetic information. In contrast, "we had the sequence of only one gene out of about 30,000," notes Gracey's senior Stanford colleague, physiologist George Somero. "But we went ahead anyway."

To get the genetic information they would daub on chips, the researchers isolated RNA from mudsucker liver, brain, and muscle. Sequencing these RNAs gave them the code for genes active in these tissues. They put pieces of 5400 of these genes onto their array, which they planned to use to study changes in the mudsucker's genetic expression when the fish burrowed into oxygen-poor mud at low tide.

As Gracey, Somero, and their colleagues reported in 2001 in the *Proceedings of the National Academy of Sciences*—a "proof of principle" paper, Somero says—"there are widespread changes [in gene activity] that we really wouldn't have predicted." Some changes mirrored what happens in mammals when their oxygen is restricted; others did not. The results showed differences in how each tissue responded. To their surprise, Gracey and colleagues also found revved-up tumor suppressor genes, which likely retard cell growth to cope with low oxygen, says Gracey.

In more recent studies, Gracey has subjected mudsuckers to a wide range of stressors, including water with very low or very high salinity. He has even removed them from water entirely. (The fish can breathe air.) "What we are seeing is a common battery of genes" that are either turned up or down, no matter what the stressor, he notes. These preliminary observations suggest that the fish have a standard set of responses for whenever there's trouble. Researchers studying yeast are finding the same to be true, Gracey adds.

Stanford's Podrabsky and Somero also looked at genetic activity in a different fish under other challenging conditions. They made a microarray with DNA from the annual killifish (*Austrofundulus limnaeus*), which lives in temporary ponds with widely fluctuating daily temperatures. The researchers tested fish that were either kept at constant temperatures or subjected to daily fluctuations of 15°C and looked at gene expression.

As they reported at the meeting, out of 5000 genes tested, only about 200 "were changing in an important way," says Podrabsky. Some changes promoted mitochondrial activity. Genes for lipid metabolism also became more active—much as they did in Gracey's experiments looking at low-oxygen conditions. And a gene whose proteins influence the structure of the DNA-protein complex in the nucleus was downregulated, suggesting how many genes can be affected at once. "It was a beautiful demonstration of how an organism changes its physiology to fit its environment," says Liverpool's Cossins.

Cossins, too, is now using microarrays to learn more about how gene expression in carp (*Cyprinus carpio*) enables this species

* "The Power of Comparative Physiology: Evolution, Integration, and Application" was held 24 to 28 August in San Diego, California.

to withstand seasonal temperature shifts from 4°C to 30°C. His team had already examined some likely targets, "but we were running out of good candidate genes," he says. With a microarray, "we knew we could develop a broad overview of how huge systems of hundreds and thousands of genes are working together." Cossins was Gracey's graduate adviser at Liverpool, and as soon as Gracey got a microarray up and running at Stanford, he headed back to his old lab to help Cossins do the same.

The chip Cossins and Gracey built sported 14,000 genes. They put some fish into ever-cooler water and exposed others to low oxygen. It took more than 500 microarrays to follow the gene-expression changes over the course of the experiment for each fish and each tissue, but the results made the effort worthwhile, says Cossins. They found that cold altered the activity of more than 10% of the genes—"multiple genes involved in multiple pathways," he notes. As Gracey reported at the meeting, the activity of some of the same genes—such as those involved in lipid metabolism—changed in the same way in all tissues; the activity of others went up or down depending on their location.

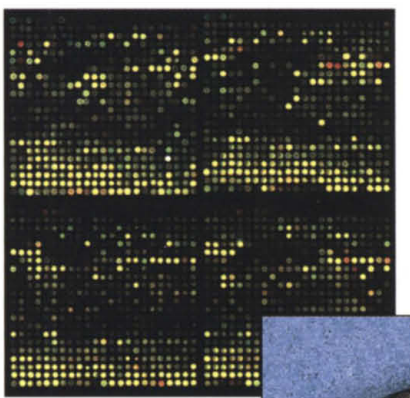
Another fish story

While Somero's and Cossins's physiology labs were gearing up to tackle microarray technology, evolutionary physiologists Douglas Crawford and Margie Oleksiak of the University of Missouri, Kansas City, were also taking their first steps into the world of gene chips. Their goal is somewhat different: They want to sort out genetic changes that contribute to adaptations and learn what prompts the evolution of new species. To this end, the two have been corralling genes involved in helping fish, particularly a killifish called *Fundulus heteroclitus*, adjust to different temperatures. They have focused on genes coding for metabolic enzymes.

Crawford hopes the gene-chip approach, which monitors thousands of genes, will allow the team to extend an earlier study based on a mere 11 enzymes. In that 1997 project, he and his colleagues assessed the role of natural selection in changing the enzymes' activity, which is presumably determined by DNA. They compared the concentration of these enzymes in four *Fundulus* species living at different latitudes along the Atlantic coast of the United States relative to similar species in the Gulf of Mexico. Within one Atlantic species, for example, northern fish live in waters as much as 12°C colder than those inhabited by their southern kin. In contrast, those in the Gulf of Mexico experience a fairly uniform temperature. In all four Atlantic species, three enzymes showed signs of having evolved to cope with different environments. And the genes for these enzymes were more active in northern than southern individuals (*Science*, 11 April 1997, p. 256). Later studies revealed that the three genes help the heart use glucose.

How fish cope. Biologists are learning what genes help the annual killifish (*Austrofundulus limnaeus*) live in temporary ponds.

To explore such evolution further, Crawford and his colleagues gathered DNA to set up their own microarray, eventually isolating some 9000 genes each from heart and liver. He likes to measure his progress in terms of the number of genes he can study at once: "In 1997, we measured 11 genes, in 2001 we measured thousands, and in 2002 we will measure 10,000." Already he and his colleagues have gleaned a few valuable insights. For example, they have determined that gene expression can vary quite a bit between members of the same species; in one population, 18% of the genes studied were significantly different among individuals. Some variations were unexpected, such as among genes for heat shock or cell cycle proteins. As for the 3% of genes whose activity varied systematically between different populations, they are "suggestive of evolution by natural selection," Crawford notes.



Gene-wise. Customized microarrays spotted with genes (*above*) are boosting studies of gene expression in carp (*Cyprinus carpio*).

Microarray fever

With these experiments, Crawford, Somero, Cossins, Podrabsky, and Gracey "are pushing the envelope in using gene-chip technology," Hicks notes. True, they still can't do as much with the animals they study as can their counterparts working with model organisms that have an extensive history of genetic and genomic studies. For lack of a genome sequence for carp or mudsuckers, say, far fewer of the genes observed have known identities. Furthermore, molecular biologists have learned how to turn individual genes on and off in mice, nematodes, and others, a boon for studying genes' functions. That's not the case for most animals studied by comparative physiologists. For these reasons, "it's more difficult to interpret the data," says Gracey. And with the amount of data being generated, "it's becoming quite a challenge to deal with all these genes," Cossins adds.

Nonetheless, these venturesome pioneers are making it possible for other comparative physiologists to join the microarray stampede. In November, Crawford will take over the University of Miami marine genomics center, which will provide sequencing and microarray facilities to a half-dozen visiting investigators a year, sending them home with ready-to-go gene chips. Cossins, too, expects that his lab will become a magnet for researchers who want to include microarrays in their experimental repertoire but lack the funding or expertise to build their own. Already he's helping other researchers build microarrays for projects that include looking at genes invoked by hibernating ground squirrels, rainbow trout subjected to stress, and embryonic fish exposed to dioxin.

This progress bodes well for the future of the field. "I think 4 years from now when this meeting occurs, we will see more [microarray work], and 4 years after that, a ton," says UC Irvine's Hicks. The technology should "accelerate the discovery of how genes work and how they affect metabolism." And that's really what these physiologists are all about. –ELIZABETH PENNISI

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