A disease's genetic puzzle

GENES from D1

ing progress.

With some skillful genetic manipulation, they suppressed the mutant insect's ability to pass on the rare disease to its offspring. And curiously, they found the disease shares a genetic kinship with others that attack the brain and nervous system, including a common type of dementia and Lou Gehrig's disease. It suggests they might all be treated with a single drug.

"All of these diseases may be different manifestations of the same underlying problem," said Taylor, 45, who has moved from Penn to St. Jude Children's Research Hospital in Memphis, Tenn. "And many researchers may have been unwittingly working on the same thing."

He is convinced the humble fruit fly will help crack the case: cheap to raise, fast to reproduce, easy to tweak the genetic recipe.

The insect, formally called *Drosophila melanogaster* (dark-bellied dew-lover), has been extensively studied, providing the foundation for much of modern biology in the past century. Yet its use to study disease in humans is more recent, and is still viewed skeptically by some.

The project in Taylor's lab, which The Inquirer followed for three years, shows how science can be a plodding affair, marked by frustrations and unknowns in between glimpses of understanding.

For the ambitious scientist and his earnest graduate student, the quest began in a small white room, with a jar full of insects and a paintbrush.

CLEM MURRAY / Staff Photographer

J. Paul Taylor, a professor of neurology, and his graduate student Gillian Ritson discussing their research inside the laboratory at the University of Pennsylvania School of Medicine in 2007. Taylor has since moved to St. Jude Children's Research Hospital in Memphis.

Muscle, bone, and brain

You've never heard of the disease that Ritson and Taylor are studying, and your doctor almost certainly hasn't, either.

It's a mouthful: inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia. IBMPFD for short.

It is caused by mutations in one gene, yet patients can have symptoms of up to three separate diseases reflected in the name: withered muscles, aching bones and dementia.

Just a few hundred people have been found to have the disease, though likely many more remain to be identified, said Virginia Kimonis, professor at the University of California Irvine. She was part of a team that discovered the disease in 2000. In 2004, she found the gene that, when mutated, would cause it.

But finding the genetic culprit for a disease is just a first step in a tedious process. Physicians can't "turn off" or replace individual genes in people, for the most part, nor would they want to without figuring out exactly what the gene does. Genes tend to have multiple functions and do not operate in isolation.

Instead, researchers seek to identify the "cascade" leading to a disease: a series of molecular events involving the interaction of many genes. By looking at various points "upstream" and "downstream" of a flawed gene, they hope to find a spot where it would be easy to dam up the river — without unwanted side effects.

That was the unglamorous task ahead of Gillian Ritson, a Philadelphia native who grew up in England, picking up a trace of an accent even as her father taught her to love Eagles football.

She came back to her native city to pursue her Ph.D., and was in her third year at Penn when Taylor gave her the fly project. It would take many months of work with a microscope, in a room barely larger than a Ping-Pong table.

Taylor hoped they could find a clue that would be useful not just against IBMPFD, but also the more common maladies that share its symptoms.

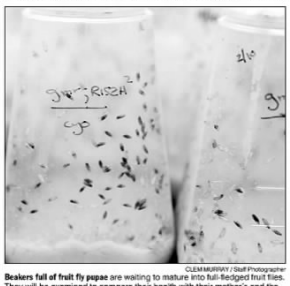
First, Ritson needed flies with the disease. She made the disease gene in the lab, using enzymes to cut up some DNA and insert the desired mutation. She then sent it off to a company that injected a few hundred insect embryos with the defective gene — a hit-or-miss process that yielded about 10 flies that were sent back to Philadelphia.

She tweaked the recipe so the flawed gene was "expressed" in the insects' eyes, causing them to appear grossly abnormal — or "rough." It would serve as a readily visible marker, like a warning light on a car's dashboard. Just from the eyes, she could tell whether a fly was sick.

The goal was to find other genes that interacted with the one that caused the disease. But it would take far too long to check the fly's 13,500 genes, one by one. So Taylor had her break the task into chunks.

She ordered 270 additional varieties of flies from Indiana University in Bloomington. Each was missing a different section of its genetic code, the gaps typically consisting of a few dozen genes.

The key to the puzzle came with breeding. One by one, Ritson mated each of these 270 "deficiency" flies with her diseased flies, and waited to see what happened.



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Beakers full of fruit fly pupae are waiting to mature into full-fledged fruit flies. They will be examined to compare their health with their mother's and the results compared with other groups with various genetic alterations.

Ordinarily, any offspring that inherited a copy of the defective gene from the diseased parent would also have the rough-looking eyes. But if the eyes looked better — or worse — than those of the diseased parent, that meant the chunk of missing genes from the other parent was somehow important.

It meant one gene from that chunk, or maybe more than one, was involved upstream or downstream in the disease cascade, in a way that made the disease better or worse.

It was the purest form of exploration. Ritson had no hypothesis, no idea what she'd find.

She hoped the screening would lead to multiple "hits." She would then narrow it down, identifying specific genes from a few of the hits that seemed to play a role.

Eventually she, or someone after her, might find a point in the flow of genetic instructions that would be a good target for a drug.

Taylor, who as head of the lab was both boss and teacher for Ritson and half a dozen others, urged her on. When she hit obstacles, he guided her to the answer — though sometimes he taught by allowing her to make mistakes.

A physician and a Ph.D. neuroscientist, Taylor was drawn to the study of neurodegenerative disease after watching his grandmother succumb to Alzheimer's.

He told Ritson she might find many hits. Or none at all.

Distant cousins

Why use a fruit fly to study a human disease?

Rather than an inner skeleton, they have a shell. Instead of two legs, they have six, plus wings.

On the evolutionary tree of life, flies parted ways with the branch that gave rise to humans hundreds of millions of years ago.

Yet of the 2,000-plus genes identified so far in which mutations load to human disease, more than 70 percent have a counterpart in the fruit fly, according to a database at the University of California San Diego. Also, fly research was the key to identifying genetic mutations involved in most cases of colon cancer, said Alexis Schmid, a University of Utah geneticist.

Big drug companies mostly have not worked with fruit flies; Novartis had a fly program but stopped it in 2008. Yet several biotech firms are active in the field, as are university researchers.

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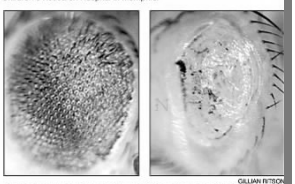
>
> **J. Paul Taylor**
> Associate member, St. Jude faculty

The insects are far easier to raise than lab mice, with a life cycle of weeks instead of years. As a bonus, flies do not draw the ire of animal-rights groups because they have "exactly zero Bambi coefficient," said Thomas Kaufman, co-director of the Indiana University facility that supplied Ritson's flies. "They're not warm and furry."

To mate her flies, Ritson put five of the diseased insects in a jar with five flies that had one of the gaps in its genome. The females soon hatched their eggs, and just 10 days later the offspring became adults.

Then, it was rating time. She knocked out the insects with carbon dioxide, then used a paintbrush to nudge them under her microscope so she could look at the eyes.

Each offspring got a rating from zero (meaning the offspring's eyes looked far better than those of the diseased parent) to 20 (a lot worse, to the point where the insects died). A 10 meant no change.



GILLIAN RITSON

Gillian Ritson evaluated the health of fruit flies by looking at their eyes. The fly on the left was healthier than its diseased mother. The one on the right, with whitish-colored eyes, was sicker than its diseased mother.

The hits started coming almost right away. She got five in the first few weeks: three genetic chunks that, when absent, caused the offspring to look sicker, and two that made them better.

But it was hard going. She had batches of flies in the incubator at all times, so she could evaluate some while waiting for others to hatch.

She worked 12-hour days, and often got pulled off for other projects with lab mates. At one point, she had to throw out the results of 50 matings after finding the parents had picked up an extra mutation.

"I cried," she said.

Sometimes her husband, a management consultant at the time, came in on weekends to keep her company.

"Everyone goes into their Ph.D. thinking they're going to have some magic 'eureka' moment," she said in 2007. "It just doesn't happen. For most people, you slog away, and you put in a lot of work, and you get a Ph.D. at the end."

After making her way through the fly genome, she came up with 74 hits. But which ones were worth pursuing?

Related diseases

As this was happening, scientists who study other brain diseases were making progress.

At Penn, the prominent husband-and-wife research team of Virginia Lee and John Trojanowski found a new clue about a type of dementia: the brain cells of patients were marked by unnatural clumps of a protein called TDP-43.

And the same clumps were found in people with Lou Gehrig's disease.

Other researchers checked brain samples from people who had died of IBMPFD — the disease Taylor and Ritson were studying in the flies — and sure enough, there was abnormal TDP-43.

Taylor was energized. "We knew we were on the same biological pathway," he said.

Most of these neurodegenerative diseases are marked by abnormal buildup of some protein. But it isn't always clear whether the clumps actually cause the disease or are merely a telltale sign.

So Ritson needed to identify a few of the individual genes from her 74 chunks, and find out what they did. With Taylor's guidance, she picked a few hits that looked interesting — including the section containing the gene with the recipe for TDP-43. She set out to check the genes in those sections one by one.

She ordered more custom flies, this time from a facility in Vienna that can silence individual genes with a new technique called RNA interference. Once again she bred flies, mating the Vienna insects with the diseased flies, to see which individual genes would, when silenced, make a difference.

The result was a paper in the Journal of Neuroscience, by Ritson, Taylor, and a slew of others, including Penn's Lee and Trojanowski.

They identified three genes that had an impact on the disease, the one that lays waste to brain, muscle, and bone.

One was indeed the gene with the recipe for TDP-43 — indicating that abnormal clumps of this protein were not just a mark of the disease, but a driver of it.

In healthy people, molecules of this protein spend most of their time in the nucleus of brain cells. But in those afflicted, the protein builds up in the cell's cytoplasm. And the authors reported that the very same thing was happening in their tiny insects.

Taylor is so keen on this avenue of research that he now has four or five people on it.

Kimonis, who identified the disease in 2000, praised the paper. "They are competitors, but they move the science forward," she said. "Paul Taylor is a fantastic researcher."

Both continue to work on this disease, as do a few others. Someday they aim to find a treatment, finding the right point to intervene in the fatal genetic cascade.

Ritson, meanwhile, is starting to look for a postdoctoral fellowship.

She defends her Ph.D. next month.

Contact staff writer Tom Avril at 215-854-2430 or tavril@phillynews.com