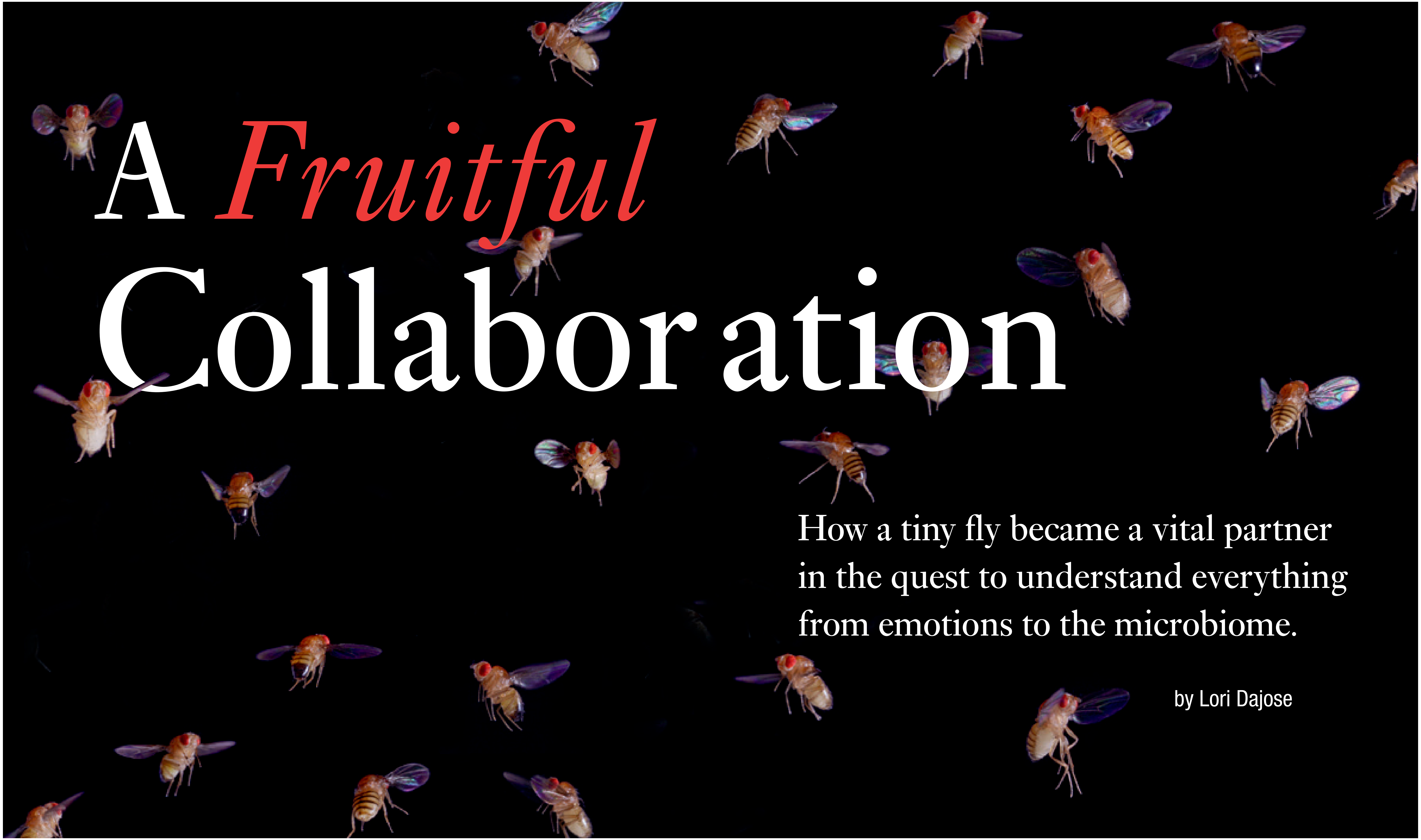


A *Fruitful* Collaboration

How a tiny fly became a vital partner in the quest to understand everything from emotions to the microbiome.

by Lori Dajose

Hours before dawn on a Thursday morning, the scent of caramel and warm bread drifts through the sub-basement of Church Laboratory.

It emanates from a bubbling vat of viscous golden liquid in a small corner kitchenette. Peering over the concoction is Aurora Ruiz Sandoval, a Caltech employee who has been preparing this mixture at least twice a week for the past 31 years. From the aroma, it might seem that she is creating some kind of treat for hungry graduate students, but the liquid is actually food for the fruit flies (species name *Drosophila melanogaster*) that Caltech scientists use for research.

This particular day happens to be an easy one for Ruiz Sandoval: Caltech's scientists have ordered only 3,650 vials of fly food. Usually, she makes six or seven thousand vials in a single day to feed hundreds of thousands of flies.

For most people, "feeding" fruit flies is accidental, with some forgotten food on the counter summoning the tiny unwelcome guests to the kitchen. But in the laboratory, *Drosophila* are encouraged to multiply so that researchers can use them to unlock the astounding wealth of scientific information the flies hold.

Over the decades, *Drosophila* have been used in dozens of laboratories across campus as models for studying the brain, behavior, body development, flight mechanics, genetics, and more. An eighth of an inch long with round brown bodies and vivid red eyes, they may seem so far from our own species as to be irrelevant, but their simplicity makes them a powerful model organism. For one thing, they are straightforward to breed: just put male and female flies into a test tube and, 10 days later, there are new flies. Their DNA sequence consists of about 15,000 genes, all well studied and characterized. And, most importantly, a century of research on fruit flies, much of it spearheaded by Caltech researchers, has led to the development of myriad genetic tools that allow scientists to precisely manipulate individual fly genomes.

For comparison, a bacterium has about 4,000 genes while a human has over 20,000, making the fruit fly a good middle ground.

THE MAKING OF A MODEL ORGANISM

Drosophila melanogaster's relationship with *Homo sapiens* began around the time that humans were migrating out of Africa and cultivating yeast in abundance by fermenting grains and fruit to make beer and wine. Soon, wherever humans could be found drinking alcohol, the yeast-loving *Drosophila* could be found, too.

After a few millennia, the fruit flies repaid the humans, however unwittingly, by contributing their bodies to science. In the early 1900s, a biologist named Thomas Hunt Morgan sought a convenient model for studying genetic inheritance and developmental biology. He came to realize that there were few models more convenient than *Drosophila melanogaster,* which could be housed in a milk bottle with a bit of mashed banana for food. Thus began a long and fruitful research collaboration.

Morgan, who arrived at Caltech to establish its Division of Biology in 1928, used the flies to discover basic principles of genetic inheritance. He had observed that one of his male flies had white eyes rather than the usual bright red eyes. Curious about how this characteristic would be passed to offspring, Morgan conducted several series of breeding experiments. He found that a fly's eye color was linked specifically to its X chromosome. Thus, he realized, a male fly (with one X and one Y chromosome) must inherit the white-eye trait from its mother, which would give the fly its X chromosome. He then reasoned that if this one trait was physically linked to a chromosome, others likely were as well.

For his discovery that certain traits are linked to chromosomes, Morgan was awarded the Nobel Prize in Physiology or Medicine in 1933. His work on simple eye colors set the stage for the understanding of inherited X-chromosome-linked diseases such as hemophilia while also establishing *Drosophila* as a powerful model organism in the emerging field of genetics.

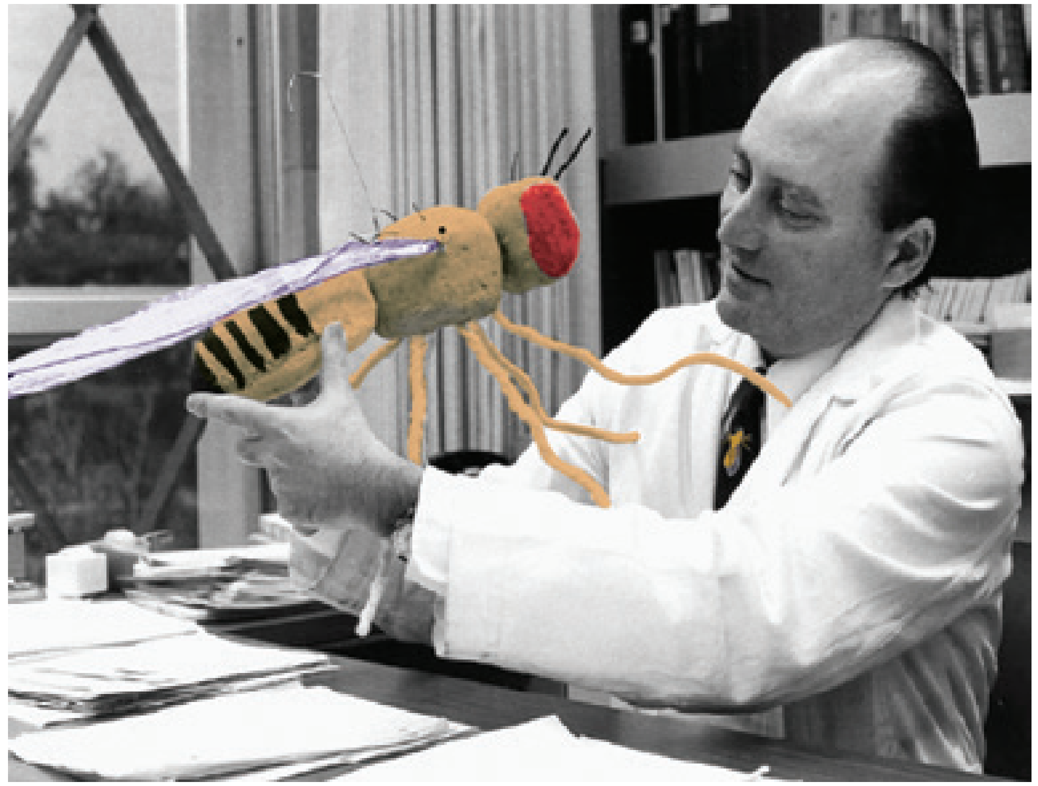
GENES AND BEHAVIOR

Caltech rapidly became known as a center for fruit fly research. In 1960, geneticist Ed Lewis (who had first come to Caltech as a graduate student to work on fruit fly genetics under one of Morgan's former graduate students) published a paper in an annual journal, *Drosophila Information Service,* detailing a recipe for fruit fly food that, he noted, resulted in quicker gestation and a higher yield of flies. The formula combined everyday kitchen ingredients, like yeast and cornmeal, with water, sugars, and a simple acid solution.

In 1988, Lewis hired Ruiz Sandoval to prepare this food. To this day, she says, the food preparation process has changed little from Lewis's original recipe.

Lewis followed in Morgan's Nobel footsteps, winning a share of the 1995 prize in Physiology or Medicine for "discoveries concerning the genetic control of early embryonic development." In that work, he had used *Drosophila* to study homeotic genes, which influence how an embryo differentiates to fit a specific body plan; for example, how the eyes, legs, wings, and other body parts form in their correct locations.

Floris van Breugel, who took the photographs of the *Drosophila* flying across these pages, was a Caltech postdoctoral scholar. He is now an assistant professor in mechanical engineering at the University of Nevada, Reno.



Biologist Seymour Benzer, who joined the Caltech faculty in the late '60s, in his office with an outsized (and colorized) *Drosophila* model.

Then, in the late '60s, biologist Seymour Benzer joined the Caltech faculty. Benzer, fascinated by the differences in personality between his two daughters, was motivated to examine how genes give rise to behavior. Eventually, Benzer's laboratory housed dozens of strains of *Drosophila*, each with one or more genes tweaked to give insight into a particular trait or behavior of interest. These included the *period* variant, which had an altered circadian rhythm; the *drop-dead* variant, a fly that would suddenly die; and the *dunce* variant, which was unable to learn to avoid danger.

"Experience thus far with the fly as a model system for unraveling the path from the gene to behavior is encouraging," wrote Benzer in 1971. "In any case, it is fun."

PROBING THE BRAIN

Today, much of the *Drosophila* research at Caltech focuses on probing the tiny insect's brain to understand fundamental principles of neuroscience. Building upon Benzer's work, several labs are examining how the neural circuits encoded by genes give rise to behavior. Fly brains are ideal for doing this kind of exploration because, unlike the brains of humans (which tend to be molded by our varying experiences of the world), fly brains are mostly hardwired by genes, making them constant from fly to fly.

Caltech neuroscientist Elizabeth Hong, for example, examines how scents and smells are encoded into the fly brain. "Primates like us tend to be very visual creatures, but the vast majority of the animal world relies on chemical sensing like olfaction to interact with their environments," she says.

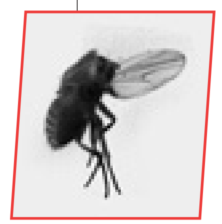
The core challenge in this area is understanding how the brain orders and encodes complex odors, from the fly-enticing fragrances of yeast growing on rotting fruit to the scents that signal the presence of a predator.

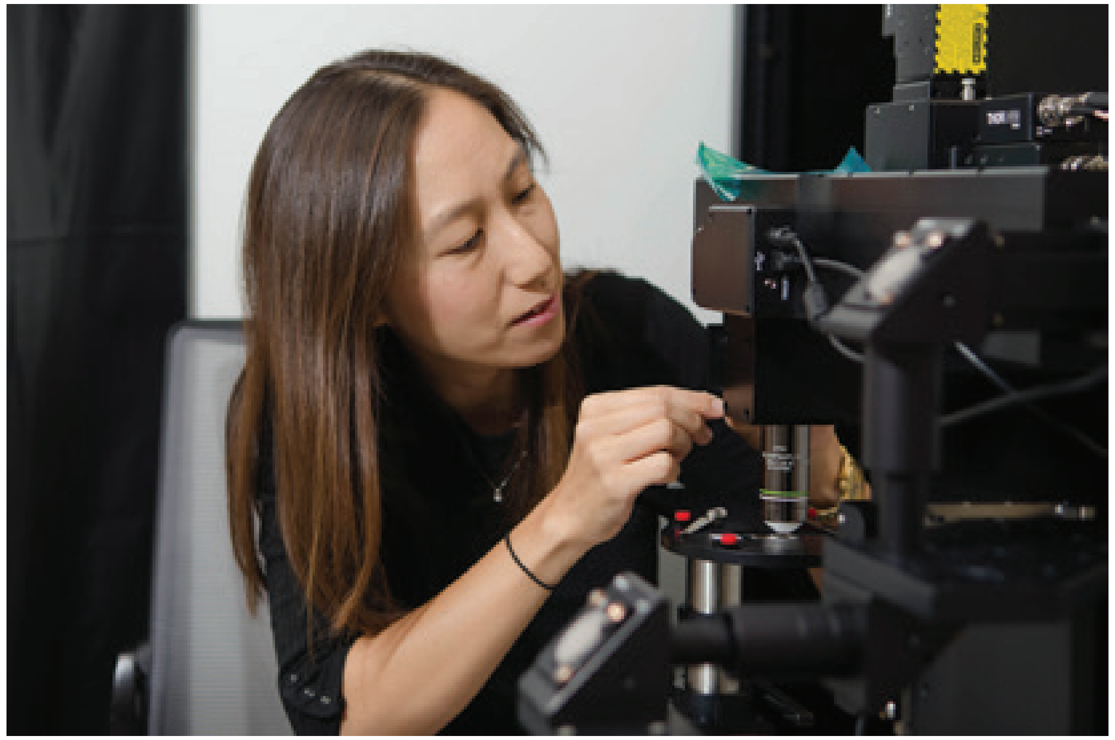
Drosophila are ideal models for studying this complex system because their brains have only about 50 unique scent receptors, the molecular detectors on neurons that recognize odors. Using the many genetic tools that have been developed for *Drosophila*, Hong and her team modify individual components of a fly's olfactory system so that they glow when activated; they then use a laser microscope that can peer deep into the living fly's brain to watch how, and where, it lights up when presented with various scents.

Compared to the mouse, which has over 1,000 scent receptors, this is a much more manageable system to work with.

Drosophila can also be used to understand the neural bases of different types of phenomena. Biologist David Anderson uses *Drosophila* in his laboratory to study how emotions and behaviors such as aggression, arousal, stress, and hunger are encoded into the brain.

Recently, the Anderson laboratory discovered a cluster of just three neurons within the fly brain that governs a so-called threat display, the specific combination of behaviors that male fruit flies exhibit when confronted with a male challenger. A fly making a threat display will throw out its wings, make rapid short lunges forward, and constantly reorient itself to face the intruder.





Neuroscientist Elizabeth Hong uses a two-photon laser-scanning microscope that can peer deep into a living fly's brain.

"These kinds of threatening behaviors are found throughout the animal kingdom, from fruit flies to lizards to aggressive guys at a bar, so humans may have a similar set of neurons in our own brains that generate these expressions," says Anderson. "*Drosophila* are a powerful place to start."

While a fruit fly has about 100,000 neurons, a human has billions.

Although researchers can already precisely probe individual neurons and circuits, teams of scientists at Caltech are currently working to create a map of the vast web of neural connections among the fly's 100,000 neurons to learn how information flows through the brain.

To advance that project, neurobiologist Carlos Lois recently developed a method that allows scientists to see, in real time within living flies, the flow of information between neurons. Dubbed TRACT (for TRAnsneuronal Control of Transcription), the technique uses genetically modified *Drosophila* whose neurons have been engineered to produce fluorescent proteins when sending an electrical or chemical signal. Any neuron that receives the signal and its accompanying protein will then produce its own differently colored fluorescent protein.

With this technique, researchers can observe and map the colorful glow of neural connections and how those relationships change over time as the fly grows, moves, and experiences its environment. TRACT, Lois says, could be used to determine how different diseases perturb the brain's circuits or to monitor how these circuits deteriorate with age.

Lois collaborates on this project with Hong, as well as with Caltech biologist Kai Zinn, whose work uses *Drosophila* to determine how genes control the wiring of the brain.

"In mammals, the brain has a basic initial scaffold laid down by genetics, and then, over time, there is a lot of complicated experience-dependent rearrangement," Zinn says. "Fly brains, on the other hand, don't change much in response to experience, which makes them powerful models to understand how the intrinsic properties of neurons determine the synaptic connections they make."

DEVELOPMENT, FLIGHT, AND THE MICROBIOME

Drosophila research at Caltech is not limited to the fly's brain, however. Developmental biologist Angelike Stathopoulos examines fruit flies while they are still tiny bags of nuclei, before they have separated into individual cells. Within a few hours, a complex choreography of cellular differentiation will begin to form the pattern of the fly's body. Stathopoulos's group studies how genes trigger and orchestrate this developmental process and how they work together in a so-called gene regulatory network to do so.

Michael Dickinson sees *Drosophila* not so much as a simplified model for studying humans, but as a kind of paragon of efficiency that can shed light on the study of the biomechanics of flight in the same way that a cheetah would be the ultimate example for scientists studying running. He and his team have pioneered new techniques for recording signals from cells in the flies' brains as they navigate within an electronic flight simulator. As a fly steers itself within a simple virtual reality environment, the researchers use a fine glass electrode to record the activity of single neurons and also employ imaging techniques to watch the activity within specific regions of the fly's brain.

"It's best to understand the brain in real time while the organism is behaving as normally as possible," says Dickinson.

Microbiologist Sarkis Mazmanian and his colleagues primarily use mouse models to study the microbiome, the communities of bacteria that reside in the intestines of almost all animals, and how they affect the health and behavior of the whole organism. But, a few years ago, one of Mazmanian's graduate students examined the microbial communities in the tiny *Drosophila* gut, discovering that not only does the fly's microbiome keep its metabolism running smoothly, but it also influences the fly's locomotion. Indeed, flies without a microbiome exhibited the same erratic walking behaviors found in starving flies, even though these flies were well nourished.

"*Drosophila* offer an excellent model system to make rapid and detailed discoveries on a cellular and molecular level," Mazmanian says. "We are now investigating if the novel findings from flies translate to mammalian systems, like mice, to advance the research toward potential future applications in humans."

FULL CIRCLE

The fruit flies on Caltech's campus have been part of a long history of scientific discovery spanning fields across biology, engineering, and the social sciences, and stretching back to some of Caltech's earliest days.

Ruiz Sandoval feels both the pull of that history and the importance of the work today as she prepares the flies' healthy concoctions.

"Your days are very busy," she says of the process. "But it feels good when somebody gets recognition for their research, and you think, 'I had something to do with that. I made the fly food.'"

David Anderson is the Seymour Benzer Professor of Biology; the Tianqiao and Chrissy Chen Institute for Neuroscience Leadership Chair and the institute's director; and a Howard Hughes Medical Institute Investigator.

Michael Dickinson is the Esther M. and Abe M. Zarem Professor of Bioengineering and Aeronautics.

Elizabeth Hong (BS '02) is the Clare Boothe Luce Assistant Professor of Neuroscience.

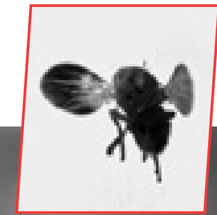
Carlos Lois is a research professor of neurobiology.

Sarkis Mazmanian is the Luis B. and Nelly Soux Professor of Microbiology and a Heritage Medical Research Institute Investigator.

Angelike Stathopoulos is a professor of biology.

Kai Zinn is the Howard and Gwen Laurie Smits Professor of Biology.

Anderson, Dickinson, Hong, Lois, Mazmanian, and Zinn are affiliated faculty members of the Tianqiao and Chrissy Chen Institute for Neuroscience at Caltech.



Aurora Ruiz Sandoval is in charge of making food for the fruit flies used in research in labs throughout the Division of Biology and Biological Engineering.