Metabolism: feeding fruit flies

Vivien Marx

Measuring how much a fruit fly eats opens the door to studies of metabolism and aging. But the assays are hotly debated.

Scientists can choose from a wealth of assays to measure how much fruit flies eat. Such measurements are needed for experiments on metabolism, circadian rhythms, aging, social behavior, addiction or the neuronal underpinnings of feeding. *Nature Methods* checked in with a few fruit fly scientists about the strengths and weaknesses of feeding assays.

"They are a nuisance," says fruit fly researcher Linda Partridge about setting up feeding assays. "It's difficult to make these measurements." Partridge has a lab at University College London and also founded the Max Planck Institute for Biology of Ageing in Cologne, Germany, where she now spends the majority of her time. Making the measurements is a necessary nuisance, she says, because the data let one address many biological questions such as how food intake affects life expectancy[1], behavior, metabolism or the number of eggs a female lays.

Flies have become a model with which to explore and dissect complex behavior such as circadian activity, sleep and feeding behavior, says William Ja, a researcher at The Scripps Research Institute's Florida campus. Assays are needed to resolve the amount of food eaten and feeding differences, especially for experimental analyses of complex behaviors.

When doing experiments, biologists might give some flies a high-protein diet and other flies a high-sugar diet, and the data might show that high-protein diet reduces lifespan. But the conclusion may turn out to be wrong if measurements reveal that the flies are actually eating the same amount of protein in both diets, says Gil Carvalho, who is a postdoctoral fellow at the Brain and Creativity Institute at the University of Southern California. He did his PhD work in Seymour Benzer's lab at the California Institute of Technology, where Ja was a postdoctoral fellow.

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Feeding assays matter, but there is a bit of a fruit fly food fight going on about them.

Almost every factor known to influence aging is directly or indirectly related to nutrition, says Carvalho. Aging experiments can look at the effect of dietary restriction and other manipulations of food, the insulin pathway, or other pathways involved in metabolism. Mutant flies can show blatant or subtle changes in eating behavior, metabolism or sleep patterns. Inadequate feeding assays can lead to conclusions that can potentially mislead other researchers, editors and reviewers into a sense that feeding was addressed appropriately when it was not, he says.

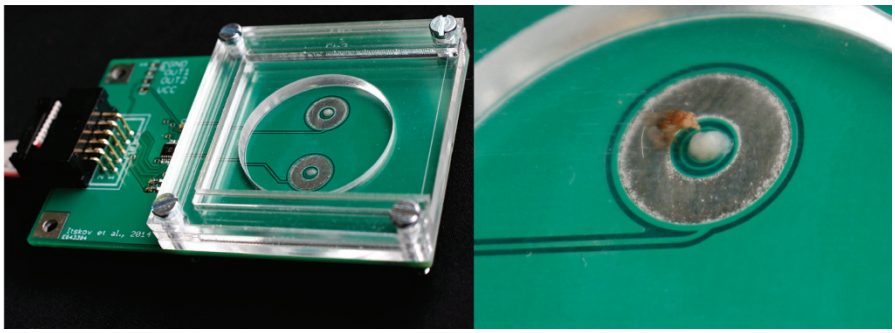
There is agreement on the importance of these assays. There is, however, vigorous discussion—one could even call it a fruit fly food fight—about the pros and cons of each method.

Measuring food intake and feeding behaviors is a hard problem, and many scientists are dissatisfied about the options and the ongoing discussions about methods, says the University of Michigan's Scott Pletcher, who was a postdoctoral fellow in the Partridge lab. "I hope that's changing," he says, especially now that there is growing interest in the neuroscience community to explore complex behaviors using the fly. These measurements will help to probe the complex control mechanisms of the fly brain and let scientists study the links between behaviors, metabolism and aging.

Dyes and tracers

Labeling food with radioactive tracers or dyes has long been a practice in fruit fly labs to measure food intake volume[2,3]. The materials that stay in the fly can be measured. But they don't stay in the fly's body forever: flies excrete.

Flies start defecating around 45 minutes after food intake, a fact researchers have to heed and which can help them calibrate



P. Itskov, Champalimaud Centre for the Unknown

The flyPAD detector tracks the interaction of flies with their food through capacitance changes.

their label-based feeding assay. "That's a lot of work," says Pletcher. For small numbers of flies, these radiolabel assays work, but it is not easy to have hundreds of thousands of radioactive flies in the lab. Also, some flies might retain more radiolabel, whereas others might excrete it more quickly, he says. The details of radiolabel absorption in the fly are not well understood.

Variability with radiolabels will depend on label type, says Ja. Different molecules can be labeled—sugar or deoxycytidine triphosphate (dCTP), for example—and different atoms can be used, such as ^{14}C or ^{32}P. ^{14}C from radiolabeled sucrose might be metabolized quickly and 'exhaled' as CO_2. But Ja and his group tested dCTP labeled with radioactive phosphorus ([^{32}P]dCTP) and found that 90% of the label is retained during a 24-hour period, which makes it measurable and a reasonable reflection of food intake, he says. It is not clear how either of these labels is metabolized, but what matters for a feeding assay is what is retained in the body of the fly and is measurable. Radioactive labels, he says, are still generally better for measuring "true consumption" than dyes.

Unlike radioactive tracers in food, dyes are not absorbed by the gut, and as soon as defecation begins, the measurement starts to be inaccurate, says Carvalho. Eating habits come into play, too. Flies can go several hours without eating, which means a random 15-minute measurement window might show no eating at all. Although he worked with dyes as a graduate student, he says, "it quickly became clear that dyes just aren't good enough if you want a serious measure of feeding."

Dyes are a great way to determine whether flies have a clear preference for one food, says Pletcher. An experimentalist might want to see whether flies prefer pure sugar—labeled blue, for example—or pure yeast—labeled red. Dye assays are fast, and many flies, including specific fly mutants, can be screened. If, however, the flies don't have nearly 100% preference for a food, says Pletcher, "then you're suddenly trying to tell whether a belly is kind of red but purple—or is it blue but purple?—so it doesn't have resolving power."

Both labeling methods involve a food marker, and the key feature is to measure how much of the marker is retained in the body for a certain period of time, says Ja. And both methods likely deliver close-to-perfect data if the time period of measurement is 10–15 minutes, which is before excretion begins and before most nutrients are metabolized and subsequently lost. With dyes that have been tested, excretion starts 15–30 minutes after intake and the 100% retention begins to drop. With radioisotopes, variability will also depend on the molecule used and the atom that is labeled.

Both dyes and radiolabels require quick measurements, otherwise there is a risk of measuring only the capacity of a fly's gut or levels of homeostasis, says Partridge. An issue with dyes is that transferring flies to dyed food likely disturbs them. The flies will first busily explore their new food surface, she says. "If you're lucky they settle down and feed pretty quickly, but you can only make a very quick measurement."

There are many variables. The fly's environment can affect its metabolism, says Carvalho. Nutrients aren't absorbed randomly, and scientists are exploring how to determine which nutrients get absorbed and into which tissue. "The isotope assay allows a first look at this, but all kinds of interesting additional questions remain," he says.

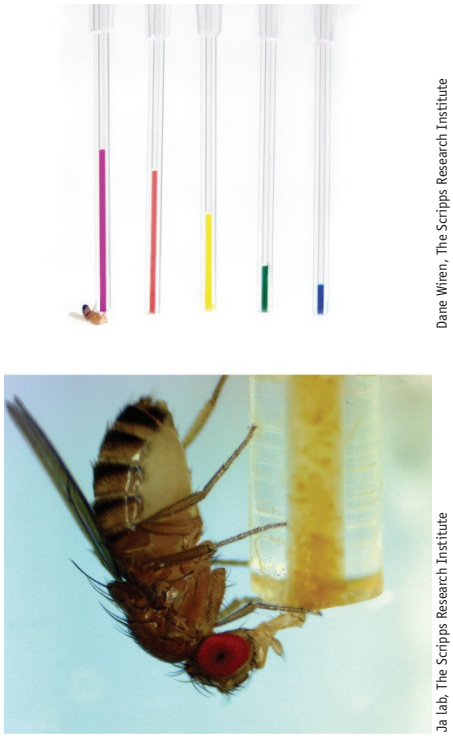
CAFE

The CAFE assay is a capillary feeder assay that Ja co-developed[4]. In this assay, flies drink liquid food from a tube hanging from the top of a vial, and the measurement is how much liquid leaves the tube. CAFE seems to be the most widely used and accepted approach right now, he says, as most labs seem to avoid using radiation.

The CAFE assay can directly measure food uptake, says Ja. But to assure that results are robust, researchers can validate CAFE results with radioisotope labeling[5]. Absolute feeding levels might be distinct because of the differences between liquid food and solid food, for example. But if the relative results are similar, such as when testing whether a mutant obese fly overeats relative to a control fly in both assays, a researcher can more confidently say that "the mutant obese fly eats more than control," says Ja.

Eating liquid food for more than a week is not healthy for flies, but this type of assay allows researchers to do measurements over multiple days, says Ja. Assay use should not be too brief, he says. Some groups use the assay for just a few hours to explore differences between a control and a mutant fly. That is too short a time span to see differences in feeding or caloric uptake.

Ja is now testing an automated version of the CAFE assay he and has team have built, in which a camera captures feeding behavior in the tubes, and which they are sending



Dane Wiren, The Scripps Research Institute

Ja lab, The Scripps Research Institute

The CAFE assay is a capillary feeder from which flies drink liquid food. It allows measurement of how much food leaves the tube.

out for other labs to try. He wants to use this assay to study genes related to feeding behavior in order to identify the neuronal circuitry connected to that behavior. Other labs are also building automated versions of this assay, he says.

Carvalho likes that the CAFE assay avoids killing the animals with each measurement and that it can be used for a few days and longer. It also avoids the need for labeled food, and he finds it easy to use, he says. The assay requires liquid food, which is unlike the solid food used in most labs, but there is an experimental advantage. "You can switch foods without disturbing the animals," he says.

Critics of the CAFE assay point out that flies need to feed in an upside-down position to drink from the tube. Ja says that he and his team have looked at the issues this might cause and found that the flies still show their typical circadian rhythms, such as eating more during the day than at night. The assay "definitely looks different than the standard lab vial, and you always need to consider whether that is an important consideration for what you're studying."

"It's a very accurate measure of how much goes away from the tube," Pletcher says of the CAFE assay. Its weakness, he says, is the behavior required of the flies to get to the tube. "They just can't get at the food as they get older," says Partridge. "They need a flat surface they can easily access and stand on." In these researchers' view, this means it is not an assay with which to measure the link between feeding behavior and lifespan.

Studying aging in fruit flies is difficult because their lifespan in the wild is not known. Researchers generally agree that lifespan is around one month in the wild. But aging happens quickly, says Partridge. Even at two weeks, flies are not as motile as when they first emerge from their pupa. "The locomotory impairment comes really quite quickly," she says.

One way to address whether the assay is changing behavior is to run CAFE in parallel with another assay that does not involve a hanging capillary with liquid food, says Carvalho. An experimenter will want to use the same flies and the same food components. "If the CAFE causes behavior changes that affect feeding, you'll see a difference between the two assays," he says.

Proboscis extension

A different class of assays measures the fly's extension of its proboscis. Developed in the Partridge lab, this approach measures food-related behavior and is an indirect measure of food consumption[6].

Pletcher has developed an assay in this class called the fly liquid-food interaction counter (FLIC) that he is giving to other labs to try[7]. FLIC is a set of feeding wells surrounded by an electronic pad. "When the fly stands on the pad and sticks its proboscis in the food, we get a signal," says Pletcher. Software collects, preprocesses and then sends data to a desktop computer for further analysis and visualization.

The assay tracks signals as short as 50 microseconds and measures a fly's continuous interaction with food. Although not a direct measure of consumption, says Pletcher, it can be combined with radioisotopes or dyes to collect information on food uptake. And it can help with analyzing feeding behavior such as circadian rhythms and interactions with food over time.

Another feeding-behavior assay, the fly proboscis and activity detector (flyPAD), developed by Carlos Ribeiro and his team at Champalimaud Centre for the Unknown and by Michael Dickinson and his group at the University of Washington, also tracks flies' interaction with their food[8] (Dickinson is now at California Institute of Technology). Beneath a food dish is an electrode, and the area around the food is equipped with another electrode. When a fly touches the food with either its proboscis or a leg, the change in capacitance leads to a detectable signal. The team developed software to parse these signals for calculating food intake.

Proboscis extension reflects a fly's motivation to feed, says Pletcher. A fly might be hungry and first use the many taste receptors on its proboscis to test the food. "If it tastes good, the rate of proboscis extension will increase," he says. Flies also have sensing receptors on their legs. The more favorable the environment, the more frequently a fly will extend its proboscis.

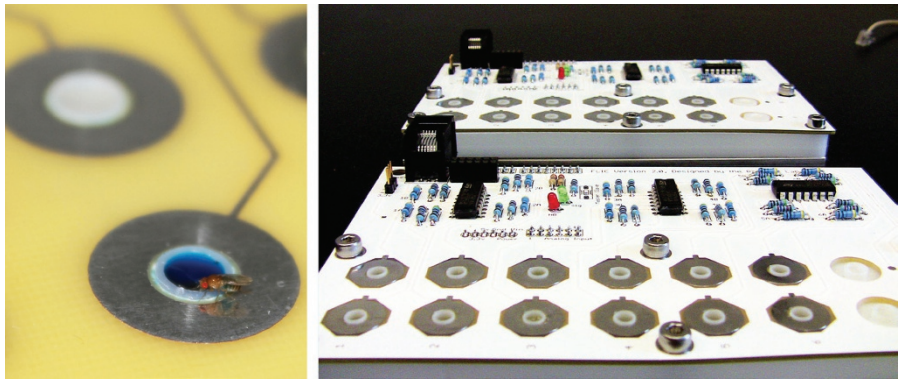
And proboscis extension is part of other behaviors still: a female fly might be sniffing for pheromones of other flies to see whether other females may have lain eggs in a given spot, says Partridge. Knowing whether there are larvae there will discourage egg laying because those other larvae will eat the new eggs. The flies might also be foraging for microorganisms, she says.

"Some of the time it may just be sampling its environment and not actually taking up food or not taking up very much," says Partridge about proboscis extension. To make the assay deliver data about food uptake, she recommends that an experimenter also use dyes and measure over a 45-minute period. Groups can be compared by correlating the amount of proboscis extension with the amount of dye uptake. When doing longer-term measurements, researchers can calibrate the proboscis-extension method with data from the dye assay, she says.

"It's certainly an interesting behavior, but to use it as a surrogate for food intake?" says Ja, "Okay, if it's the only assay available—but not anymore, in my opinion."

Proboscis extension, CAFE or other assays are fine, says Carvalho, if researchers are scoring interactions flies have with food. But in his view there are too many confounding factors when proboscis extension is used to infer food intake volume.

One challenge with all assays, Pletcher says—including his assay, CAFE, dyes and radiolabels—is that there is no gold standard against which to benchmark them. "When they disagree, we don't have any objective standard to compare them all to



Pletcher lab, University of Michigan

FLIC uses feeding wells and an electronic pad to track when a fly extends its proboscis into its food. Software is used to collect, process and visualize the data.

Table 1 | Comparison of fly feeding approaches

Feeding approach	Some pros	Some cons
Food labeled with dye	Easy to use; good for discerning clear food preferences	Imprecise for food intake measurements; owing to flies' rapid excretion of dyes, can skew longer experiments
Food labeled with radioactive tracer	High sensitivity	Impractical for experiments with large numbers of flies; choice of radiolabels can be a source of variation
Proboscis extension	Good for studying feeding-related behavior and aging	Needs to be combined with dyes or radiolabels to measure food intake
Capillary feeder, CAFE	Allows precise measurement of food intake; good for drug testing assays	Eating liquid food for more than a week is not healthy for flies; critics say older flies cannot use it and need to feed on a flat surface

say who's right and who's wrong, or who's closer and who's further," he says. The ideal assay with which to measure food intake volume has not yet been found, in his view.

In their assay choice, says Ja, scientists need to consider application and context (**Table 1**). He believes that the CAFE assay is most appropriate for measuring the quantity of food intake, whereas the other assays can be used to measure food-related behavior. "So if you're more interested in one of these other behaviors, then you should use the appropriate assay," he says.

Food favorites

One aspect that influences fruit fly feeding is the food itself. Recipes and food ingredients vary between labs, and, says Partridge, "I suspect it matters like hell to the flies." Most recipes include sugar and yeast and some include cornmeal, which is added to change the viscosity of the food, making the food softer for the flies. But food might include ingredients such as rolled oats, bananas or malt.

In 2013, Partridge and Pletcher along with colleagues in the UK, Portugal and China developed a holidic medium, that is, fly food with completely chemically defined ingredients[9]. "You can make it completely repeatably in different labs, because it's just chemicals—it's like making up a buffer solution or anything else," says Partridge. But from the fly's point of view, she says, it is not natural food, just as most of the fly food used in labs. In the wild, flies would not be eating as much sucrose as they do in the lab and would be eating microorganisms and wild yeast.

"There's a little bit of controversy about the yeast," says Pletcher. Many labs use brewer's yeast, whereas others use yeast extract, which has been depleted of many nutrients. Flies detect the difference, says Partridge, "and they hate yeast extract."

At times, a completely chemically defined diet is smart, says Ja, but in other situations a return to "good old yeast" is right. Yeast seems to contain one or several ingredients that influence fly metabolism, and scientists have yet to figure out that link. The chemically defined diet out of the Partridge lab is "the closest to getting the flies to full health and fecundity, though, and that is definitely an impressive achievement," he says.

When scientists' careers take them from one lab to another, they take their recipes with them, says Pletcher. When he first started his lab, he tried to get the community to agree on a fly food. "That just didn't work," he says. "People are wedded to a recipe." That is understandable given that diet shifts can draw data from past experiments into question, he says.

As scientists learn more and more about which diet components are best for flies, fruit fly diets in labs have converged, says Pletcher. There are fewer recipes overall, and they usually have two main components: some form of sugar and yeast.

With diet and with assays, "I don't think there is a wrong way," says Ja. "You just have to be honest about what you're doing, and super careful about how you ultimately control and interpret your own experiments."

All techniques are potentially a trade-off, says Partridge, between being able to measure food intake accurately and putting the fly in an artificial or even harmful environment that risks making the measurements meaningless. It is not so much that there are different assays for different questions, but rather, she says, "for different questions, you can get away with different assays."

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