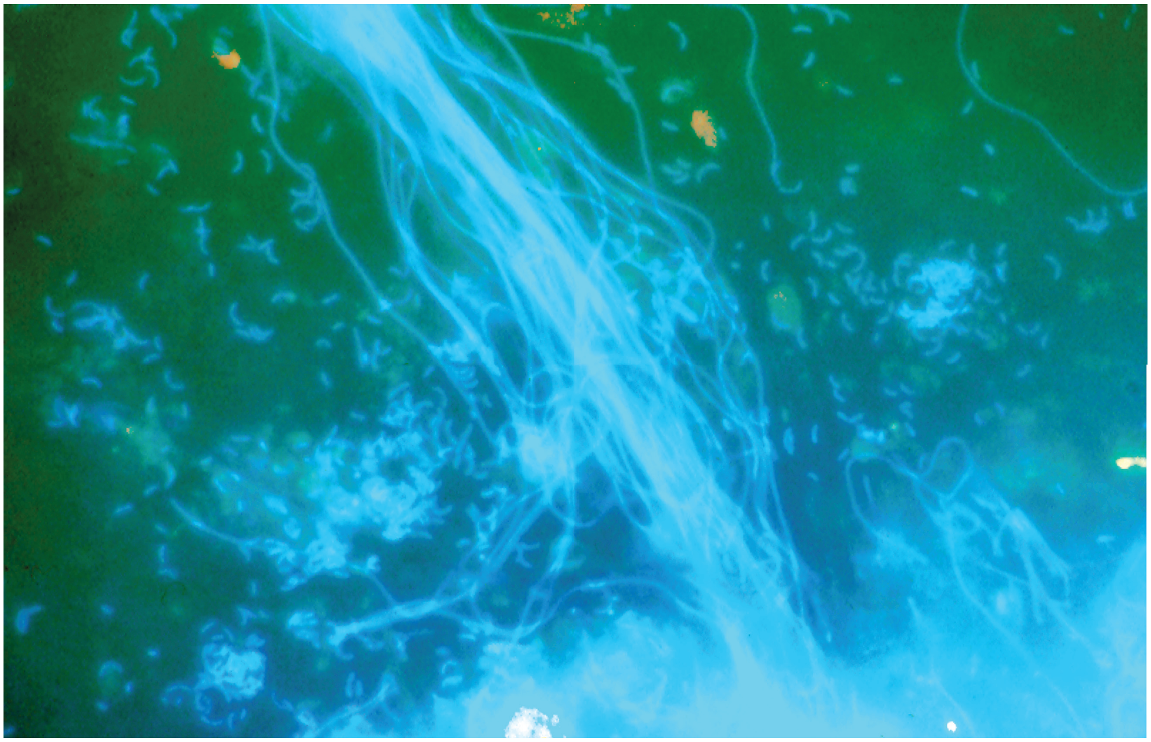
For the Love of Termites

By Elisabeth Nadin

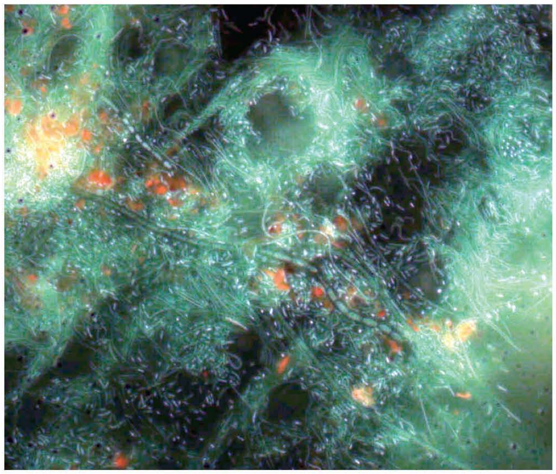
The strange world inside a termite's hindmost gut is inhabited by about 250 different species of microbes. Among them are millions to billions of long, filamentous bacteria and their shorter counterparts—lining the gut's outer skin as shown in this epifluorescence micrograph—which make methane from hydrogen and carbon dioxide.



From Leadbetter and Breznak, *Applied and Environmental Microbiology*, vol. 62, no. 10, pp. 3620–31, 1996.

It's evident by the way he cavalierly handles a slice of wood crawling with centimeter-long, fat, white termites that Caltech associate professor of environmental microbiology Jared Leadbetter has loved insects for a long time. Since he was four years old, in fact—that was when his big sister Briana, then nine years old, brought home mounted butterflies and beetles from her summer entomology course, and he decided that he would study insects when he grew up. But his love affair with termites began only after his junior year in college, during a summer course in microbial diversity at the Marine Biological Laboratory in Woods Hole, Massachusetts. During one lab exercise he found himself staring through a microscope at the insect's spilled guts and, he laughs, "it was basically love at first sight." It was the microbes living inside the gut that particularly fascinated him. "They're exciting to look at; they really catch your eye," he says. "And I thought, 'Aha! This is exactly the right project for me.'" Despite 100 years of investigation, very little was known about the workings of those microbes, further fueling Leadbetter's interest in the single microliter of material housed in a termite's hindmost paunch.

What's in a microliter? For starters, less than what can fill the volume under your pinky fingernail. But in that last of three of a termite's hindguts, a microliter hosts around 250 species of microbes, many of them oxygen-phobic, that digest



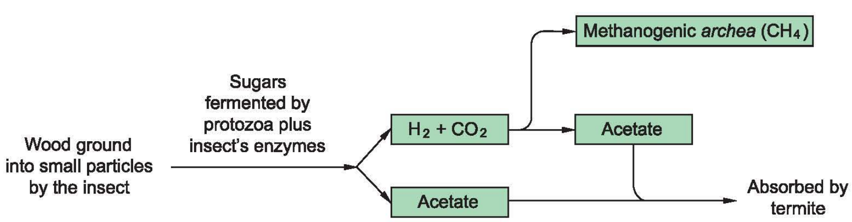
This view of the termite's hindgut microbe community turned Leadbetter on to the inner workings of the relationship that keep these symbiotes alive. The termite chews wood, but relies on its gut microbes to turn the particles into an energy form it can use.
Below: A soldier of the *Zootermopsis* genus doesn't gnaw on wood, but uses its pincers to chop off ants' heads.

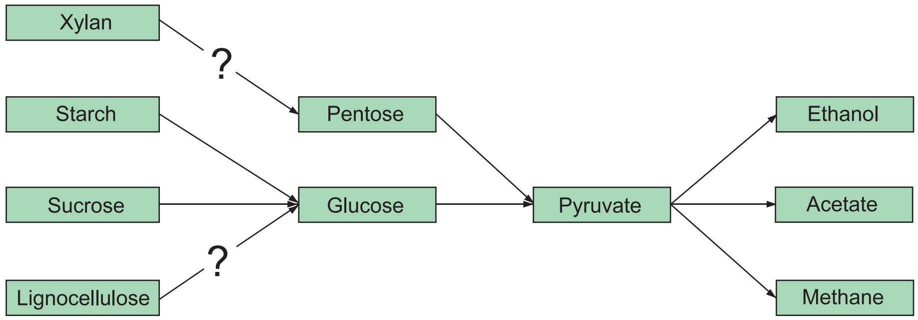
the wood that their host chews. This compensating mutualistic relationship keeps them all alive—termites can't digest their own food, and the microbes can't chew wood or survive outdoors. How the relationship evolved into the near-perfect symbiosis it is today is still an intriguing mystery. "It's one thing to consider how precarious any single life is, whether it's a microbe or an insect or a human. Then to have this interrelationship between two hundred different members—it seems doomed to failure. They're not even the same species, and they all have their individual needs, but they also have a shared need. To some extent, if one member goes they all go," Leadbetter remarks. This system may closely resemble its ancient origins. "We're looking at a community that has been passed down from termite to termite for over 100 million years. It's fascinating to think about that. What makes termites so successful now is a slightly improved version of what made them so successful then."

Only the workers in a termite colony—comprising thousands to millions of individuals, depending on the species—have the specialized mandibles required to grind wood. Soldiers, whose jaw pincers are big enough to snip off the heads of ants or offending fellow soldiers, don't gnaw on wood, nor do molting juveniles or reproducing queens. They're fed a special cocktail by the workers, a nutrient-packed drop that exits through the posterior of the insect in a process called proctodeal feeding. "It's not feces. It's distinctly not that," comments Leadbetter. "Termites are exchanging this bolus, a drop of microbe-rich woodshake from a worker termite's rear end, periodically, all the time, so they're always sharing their microbes," he adds. That's how microbes populate the pristine guts of a growing young termite and proceed to digest its food. And digest it very well indeed.

When a wad of wood hits the termite's hindgut, the protozoa, spirochetes, and other microbes in there immediately get to work. They eventually convert more than 90 percent of the cellulose in their woody meal into the vinegar-like compound called acetate, which the termite absorbs and uses as fuel. On the way to churning out acetate, the

A termite's gut hosts a fairly efficient commune—almost 90 percent of the cellulose in the wood it eats is turned into its ultimate fuel, acetate. The process begins with protozoa, which ferment sugars into acetate. Other microbes then turn hydrogen (H_2) and carbon dioxide (CO_2) in the gut into either more acetate or into methane (CH_4).





Ethanol is society's desired biofuel, but for now, we can only make it from energy-poor sugars and starch in sources like corn or cane. Termite gut microbes turn energy-rich woody plant parts like xylan and lignocellulose into acetate, the termite's fuel, which society can't use. But the step from wood chips to simple sugars like pentose or glucose, which remains elusive, could be made through bioengineering.

microbes make a lot of hydrogen gas—about a third of the energy in the cellulose is released in this form—and carbon dioxide. Most of these gases are combined to form even more acetate, but the remainder is released as methane, which the insect can't use. Compare that to cows, for example, which vent as methane up to 20 percent of the energy in their microbially digested grassy meal. "Cattle lose basically a fifth of the nutrients in every mouthful as this energy-rich greenhouse gas," says Leadbetter. "Most termites release less than two percent, and oftentimes no methane at all." Still, multiply that by at least one quadrillion (picture 15 zeroes) termites on the planet, and it adds up. "It's important to understand termites because of their role in the global carbon cycle, both as degraders of plant material and producers of methane. In total, they still contribute a fair amount of methane globally. But understanding the details of why they don't emit more is extremely important to understanding the greenhouse gas budget," says Leadbetter.

Carbon dioxide is another byproduct of termite life, released as the insect burns its acetate fuel. Which brings us back to what's in a microliter. "To try to understand the current system on the planet, you have to understand what's going on in this one-microliter environment," says Leadbetter. Up to two percent of the global carbon dioxide budget comes from insects we don't think twice about unless they're eating our house.

"We must continue investing in new methods of producing ethanol—using everything from wood chips, to grasses, to agricultural wastes."—Bush State of the Union, 2007

The magical fruit (or log)

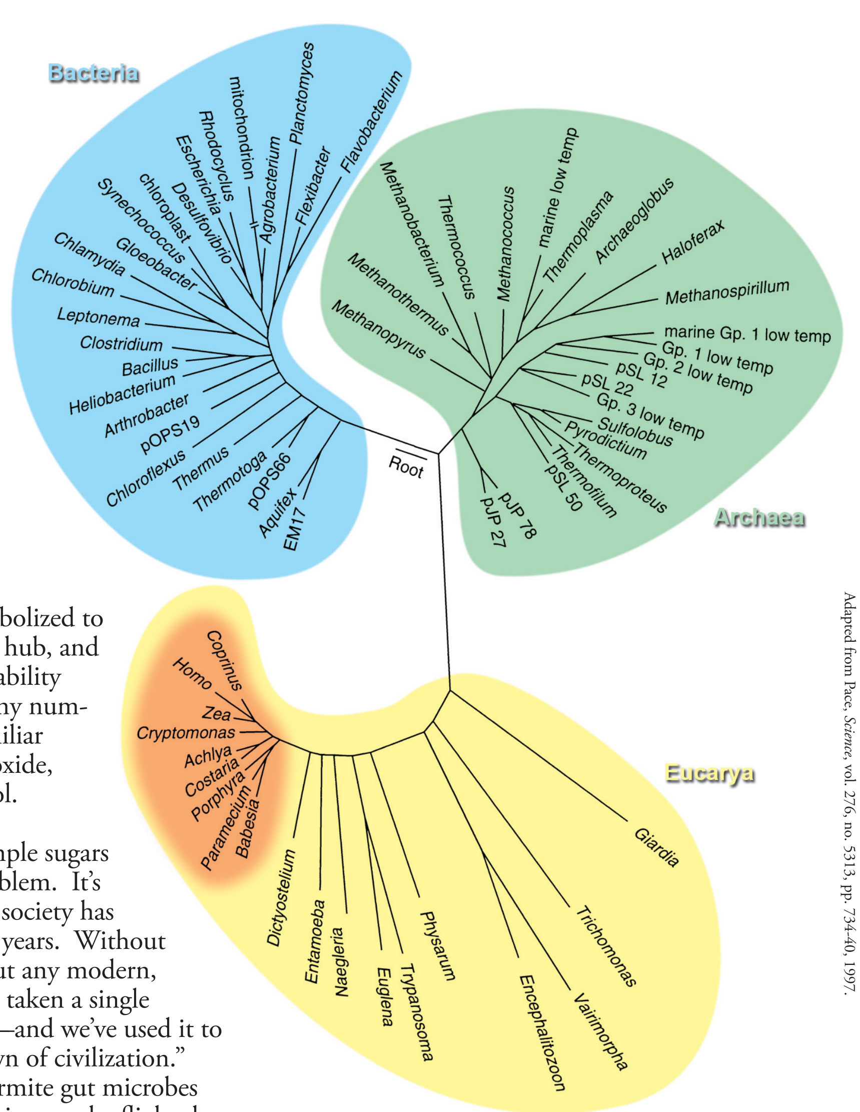
The same processes that provide energy to termites and ultimately lead to their portion of greenhouse gases may also help alleviate the human contribution to the same. Where Leadbetter sees microbes deriving fuel from a pine chip or a two-by-four for their hosts, many others see a potential bioalchemy that can fuel industries, homes, and cars. In his 2006 State of the Union address, President Bush announced that the government would begin funding research in "cutting-edge methods of producing ethanol, not just from corn, but from wood chips and stalks, or switch grass." In 2007 he reiterated, "We must continue investing in new methods of producing ethanol—using everything from wood chips, to grasses, to agricultural wastes."

Despite the promise of fuel from recycled sources, the primary source of ethanol is still corn. This summer will see the largest corn crop grown in this country since World War II, and, at 90.5 million acres, a 15 percent increase over last year's, states a U.S. Department of Agriculture report released on March 30. The cause for this boom, states the report, is the high demand for ethanol.

But the switch to renewable biofuels, or fuels derived from plants, comes with the usual problems and questions attendant to large-scale change. There are economic ones, like: Will farmers abandon other crops, like soybeans or cotton, in favor of profitable corn? Will the demand for corn-derived fuel then drive up the cost of food? In fact, we typically avoid consuming the most energy-rich plant parts—the woody fibers called lignocellulose—although these are potentially the best source for society's energy needs.

The route from wood chips, or any lignocellulose, to the final product of ethanol is short but crooked. Leadbetter equates it to an airplane flight with several potential hubs. From the plant source the route leads to simple sugars, such as

Life's diversity, once broken into only three kingdoms—Animalia, Plantae, and Fungi—is now recognized to be far more extensive. Bacteria and Archaea, which used to be lumped together, are as different from each other as they are from Eucarya, the multicellular organisms. Animals like humans (*Homo*), plants like corn (*Zea*), and fungi, together shaded in red, comprise what Caltech bioengineer John Doyle describes as only "a very nice hood ornament" on the car of life. Evolutionary distance, or how related one organism is to the next, is depicted by the length of the line that separates them.



Adapted from Pace, *Science*, vol. 276, no. 5313, pp. 734-40, 1997.

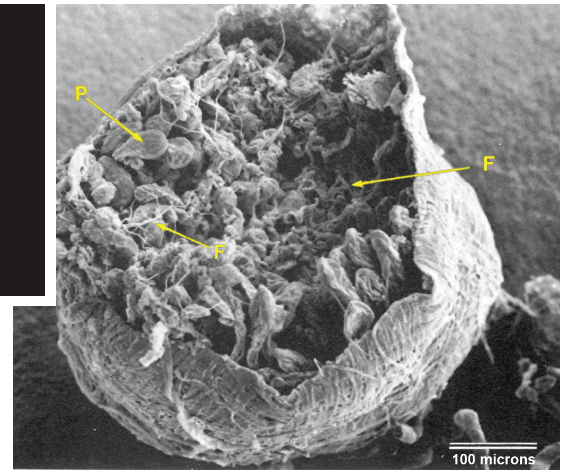
glucose. This sugar is metabolized to pyruvate, which is the next hub, and depending on oxygen availability the next flight can follow any number of paths to become familiar compounds like carbon dioxide, acetate, methane, or ethanol. According to Leadbetter, "We can convert simple sugars into ethanol at will, no problem. It's a modification of a process society has been performing for 3,000 years. Without genetic engineering, without any modern, sophisticated science, we've taken a single organism—the wine yeast—and we've used it to make ethanol since the dawn of civilization."

It just so happens that termite gut microbes are more interested in hopping on the flight that eventually lands at acetate, not ethanol. But they start from wood rather than a simple sugar, which is where Leadbetter's research comes into play. "There are many options at the level of pyruvate. It's been demonstrated several times over the last 25 years that we can engineer organisms to make ethanol from pyruvate. But what we're sorely lacking is the segment that might take us from a pine chip or rice hull or some other low-value plant lignocellulose source, to the level of a simple sugar or pyruvate. So we have to go to nature to come up with solutions to dismantle wood into its components."

Playing with Bugs

You probably learned in grade school that there are two, maybe three, kingdoms of organisms—plants and animals, and maybe fungi—but it turns out, as you might imagine, that this is a gross oversimplification of life's diversity. In the early 1960s, here at Caltech, Emile Zuckerkandl and Nobel Laureate Linus Pauling (PhD '25) revolutionized this staid view by introducing the field of molecular evolution, which allows scientists to map through genetic analysis the evolutionary history and development of complexity in organisms. Now, instead of three kingdoms, we see an array of branches on the tree of life, each one corresponding to "evolutionary time." On this tree, says Leadbetter, you see fungi, plants, and animals, but also a lot of other lines that are generally as long as those between plants and animals and fungi. When you zoom in, those three kingdoms, he says, "are what John Doyle here at Caltech described as being only 'a very nice hood ornament.' The car, of course, is the rest of the tree. The difference between *E. coli* and some of these other bacteria, for example, is as great as the difference between corn and ourselves. By extension, you might imagine that what these organisms can do, in terms of their physiology and their roles in the environment, is also very diverse." Life therefore clumps into three domains: eukaryotes (which include animals, plants, and fungi), bacteria, and

Photo by Tina Salmassi (MS '98, PhD '01).



From Breznak and Pankratz, *Applied and Environmental Microbiology*, vol. 33, no. 2, pp. 406–26, 1977.

A view of *Zootermopsis* and its guts (above). The open gut (right), only 100 microleters in volume, is chock full of wood particles, protozoa (P) and bacterial filaments (F).

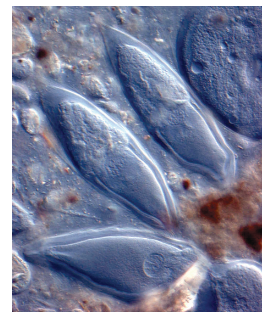
***Zootermopsis nevadensis* is Leadbetter's termite of choice in California because it accessible and its gut microbes can be cultivated in the lab. Above, grad student Elizabeth Ottesen and Jian Yuan Thum (BS '04) collect specimens from Mount Pinos, in the Angeles National Forest.**

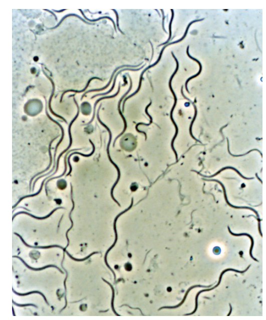
archaea, which were once lumped in with bacteria but are now understood to be as clearly different from bacteria as they are from eukaryotes. "We live on a microbial planet," says Leadbetter. He calls the termite hindgut "a playground of microbial diversity."

By measure of both abundance and species diversity, termites are much more successful in the tropics, and comparatively few of their kind survive the harsh winters of northern New England and the Midwest. Leadbetter's group studies two from among 2,600 species of termite in detail. He started with *Zootermopsis*, which basks in year-round comfort in temperate California. He compulsively looks for them, even when hiking with his family in the Angeles National Forest. "I turn over logs and peel back bark and see what's on the other side. I think they find it annoying when I stop all the time. But they say you should never bring your work home with you," he laughs. He brings it to his lab instead, filling Tupperware containers with *Zootermopsis*, an especially handy termite because it doesn't mind lab life and its internal microbes are easily cultivated. "This allows you to not only pass through the intestinal zoo, but actually spend some time at some of the exhibits in a way you wouldn't be able to otherwise," he says. His group's studies of the genes and pathways underlying *Zootermopsis* bacterial metabolism has most recently been aided in large part by a gene inventory-taker, known as a microfluidic device, whose development they pioneered at Caltech (see *E&S*, 2006, No. 4, p. 3).

Half of a *Zootermopsis*'s weight is in its guts. Organisms from all three major domains of life reside in its hindgut paunch, which resembles our colon. A cross-section reveals what Leadbetter describes as "a cornucopia of microbes." Most of the termite's weight, therefore, is microbes and wood particles. Methane-making Archaea colonize the epithelium. In the gut fluid live seven species of protozoa that are found nowhere else in nature, and gene-based techniques have identified up to 100 different species of spirochetes, all of which happen to be closely related to *Treponema pallidum*, the bacterium that causes syphilis. But Leadbetter's research has shown that in termites they're symbionts, not disease agents. They're complex, for single-celled organisms—they flex and swim with the help of a flagellar motor (see *E&S* 2006, No. 3, p. 6). The spirochete's propeller, wrapped around the central region of the cell and encapsulated by an outer sheath, helps it generate enough torque to move in viscous environments that immobilize all other bacteria. "They hold the world record for being able to move at high viscosity," says Leadbetter. His group cultivated one species, thereby learning that it consumes hydrogen and fixes carbon dioxide to make acetate.

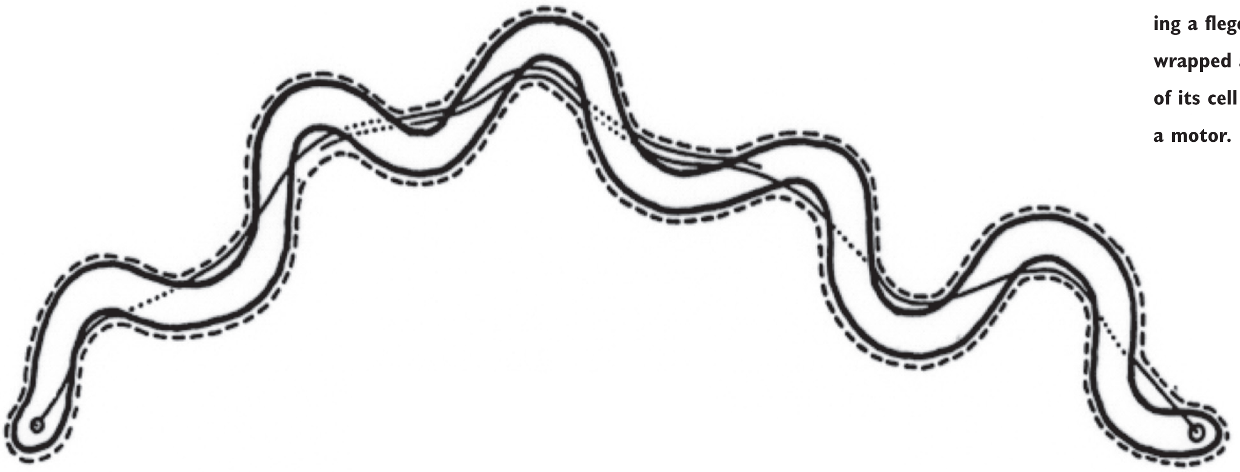
Although *Zootermopsis* microbes play interesting roles in wood degradation, and a billion dollars are spent on termite control and repair in South-





Symbiotic microbes, at least for termites—protozoa (top) and spirochetes (bottom).

Adapted from Canale-Parola, *Bacteriological Reviews*, vol. 1, no. 1, pp. 181–204, 1977.



Spirochetes swim by flexing a flegellum, which is wrapped around the length of its cell and propelled by a motor.

ern California each year, this termite is only a bit player in the global carbon cycle. So Leadbetter took his research to the tropics, where termites rule. He and his collaborators delved into the microbial community of *Nasutitermes*, a native of Costa Rica, because, as he says, "both in number of species and number of individuals, they trump all other termites hands down." These he definitely can't bring home with him. "I have not even attempted to convince the USDA that this would be reasonable to do. In many places in the U.S., these termites would never make it through the winter, but I don't think you can guarantee that here," he says. So he studies the termites down there, and analyzes the data back in the U.S. Which is a good thing, because one Caribbean species of *Nasutitermes* recently made itself a happy new home in warm, humid Florida.

There are many zones in the guts of these Costa Rican termites, each with a different pH. They also host a completely different microbe population from that in *Zootermopsis*, most notably lacking the protozoa that digest wood in California termites. "So who's degrading the wood? Is it the bacteria? Is it the host?" Leadbetter wondered. To find out how it really works in *Nasutitermes*, Leadbetter joined forces with scientists Cathy Chang (BS '87), formerly of Diversa Corporation and now at the E. O. Wilson Biodiversity Foundation, Giselle Tamayo of the Instituto Nacional de Biodiversidad in Costa Rica, and Phil Hugenholtz and Edward Rubin of the Joint Genome

Leadbetter pulls open a *Nasutitermes* nest in a tree in Costa Rica (below). The streaks beneath his hand are termites jumping ship. *Nasutitermes* soldiers have nozzles instead of pincers (bottom). They spray their victims with terpenoids, an aromatic herbal substance that only annoys humans, but paralyzes ants.

Photo by Falk Warnecke, Joint Genome Institute.

Photo by Father Alejandro Sanchez.

Fluid from the guts of 200 termites (left) fills a tiny capsule whose genetic contents will be sequenced to determine the various roles of the termite's microbial community.

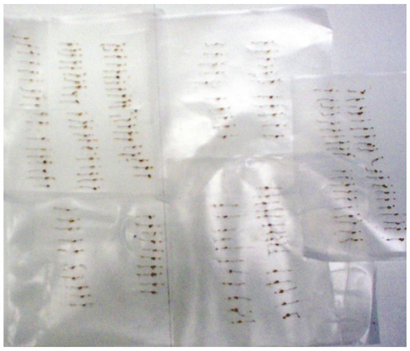


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Photo by Falk Warnecke, Joint Genome Institute.

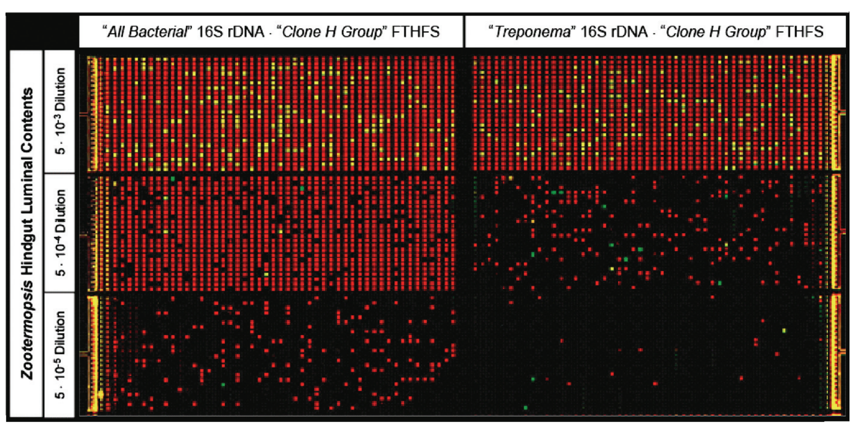
Institute. Together they are sequencing and analyzing the genes encoded by all 250 termite-gut bacteria species, most of which have never been studied before. They hope to eventually uncover what each is doing, catalytically speaking, especially when it comes to degrading wood. "This was a fairly risky project," says Leadbetter, because "the role of microbes in our local termites has been understood, to some degree, for about 100 years, but in these abundant tropical termites, there was no compelling evidence that their bacteria are involved in cellulose degradation."

When the results started coming in, "we breathed a big sigh of relief, because it turned out to be a gold mine in there," he says, and the details of the study will soon be published. "At least now we have a lot of genetic information—we don't know exactly what these genes do, but from similarities with other organisms in nature we can deduce their roles in one process or another. For the first time, we actually have a menu. We don't know how the meal is going to look when it's served, or how it's really going to taste, but at least we know the possibilities encoded in these genomes. It gives us some ideas, at the microbial level, of where they fit in the process of ligno cellulose-into-acetate conversion. And very simply, we didn't know that at all before," says Leadbetter.

Into the Future

The next step in producing ethanol from wood is more like a giant leap. Lignocellulose might be bioengineered into ethanol either by inserting ethanol-synthesizing genes into wood-degrading microbes, or by inserting lignocellulose-degrading genes into ethanol-producing yeasts or other organisms. It's too early to predict what will work in the end, says Leadbetter, "but at this

Leadbetter and his group pioneered a gene inventory technique that separates communities into single cells. This approach pinpoints the presence or absence of certain genes, like those that participate in making acetate (shown in green). At left is a full gut community, and at right the spirochete *Treponema*.



From Ottesen, et al., *Science*, vol. 314, pp. 1464-67, 2006.

stage, the idea is to think about how we might start assembling the components and organisms in new ways. Genome sequencing of microbial communities gives us many more possible components to work with," he continues. "If there's investment both at the university and the industrial level, I think there's no reason why we as a society cannot do this. This modularity in converting the ingredients into different products is real. It may be that within five years society will be able to convert some small amount of a pile of lignocellulose into some amount of ethanol at some rate. But what we really need is the ability to convert a large fraction into a large amount of ethanol or some other biofuel at a fast rate. And to be able to do so in really large volumes."

Despite its promise, Leadbetter is also concerned about complications that could arise. Is producing and burning biofuels rather than fossil fuels really better for the environment? The smell from modern ethanol production plants in the Midwest has been described as rubbing alcohol mixed with burning corn, and this pungent air is laced with carbon monoxide, carcinogens like formaldehyde and acetaldehyde, and smog-forming particles. Not only is corn a water- and fertilizer-hungry crop, ethanol production is itself a water-hungry process. A Minnesota study found that it takes more than four gallons of water—to ferment the corn meal and to cool the machinery—to make a gallon of ethanol. The wastewater, laced with hydrogen sulfide from the processing, is often dumped into nearby rivers. "It's a very complex issue, and I don't think the challenges we face with biofuels has been discussed all that realistically," says Leadbetter. "What will be the environmental impact of biofuel fermentation refineries? What will be the water demand? How sustainable will this really be?" Ultimately, he thinks several partial solutions will be necessary.

Leadbetter's immediate goals lie in a more focused direction. "I want to know everything there is to know about termites, their gut microbes, and how they make biofuels for their own use," he says. "To me it's mind-boggling: 250 species of gut microbes. Why not one? Why any at all? That's a teleological question, so you can turn it around and say 'What is the benefit for a system to have so many species? What are the mechanisms to come up with the best set of species—to kick out the losers, to acquire the winners, to improve? What has been the impact, on the organisms and the processes they catalyze, of over 100 million years of refinement of this system?' I think we're easily 25 years away from having what I consider to be a fundamentally sound understanding of this one-microliter environment, and that may be optimistic, to be honest." □

PICTURE CREDITS: 25, 28 — Jared Leadbetter; 28 — Andreas Brune