Lab Notes

The Methane-Eaters

"Once we know how these bugs work, we can really *use them to degrade toxic compounds"*

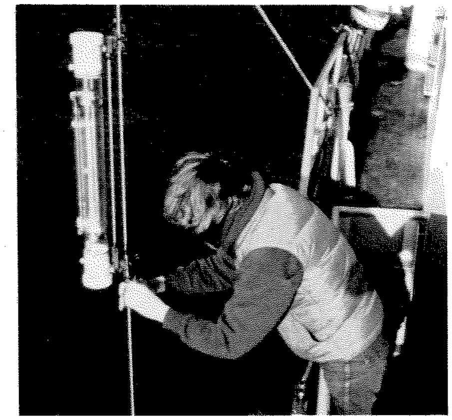
"People always ask me how a lab that's known for doing genetics fits into the Environmental Engineering Science Department," says Associate Professor of Applied Microbiology Mary Lidstrom. "To me, it's crystal-clear. You use the most sophisticated tools at your disposal to solve the problem at hand. It's unusual to combine environmental science and molecular biology, but people are beginning to realize that that's where the solutions to many environmental problems are going to lie."

Consider the methylotrophs, an obscure tribe of inoffensive bacteria who live on methane gas (CH_4). This little-studied family contributes to the natural order of things by removing methane from the atmosphere and converting it into multicarbon compounds that go back into the food chain. (All animals produce methane as a waste, humans more than their fair share by burning fossil fuels.) But although one-carbon compounds are the main course, it's been found that the methylotrophs can down a side order of chlorinated hydrocarbons simultaneously. Since chlorinated hydrocarbons such as trichloroethylene (TCE) are showing up in numerous cases of groundwater and soil pollution countrywide, researchers are looking to the methylotrophs for a biological solution to a chemical problem.

While the little guys have a limited diet, they aren't nearly as fussy about their lodgings. "You find them in soil, in lakes, and floating around in the ocean—just about anywhere," according to Lidstrom. "Other people have found that there is a small natural population in groundwater, and that its growth can be stimulated simply by injecting methane and air. But that's about as far as you can go with a black-box approach. We're taking a mechanistic approach, looking for the biochemical mechanisms and genetic regulators. Once we know how these bugs work, we can *really* use them to degrade toxic compounds in contaminated aquifers and soils."

There are two parts to discovering a mechanism: the first is determining which genes are involved, and where they lie in the array of chromosomes; the second is finding out what each gene actually does.

To find out which genes are involved in a given process, take a sample of bacteria, irradiate it to induce random mutations in the DNA of individual bugs, clone each bug into a colony, find the colonies where that process has gone haywire, and analyze those colonies' DNA to determine where the mutations occurred. But messing with the meth-



Far left: Dr. Lidstrom counting bacterial colonies in a Petri dish. Left: In the incubator room, where the colonies are grown. Right: Collecting wild bacteria in Framvaren Fjord, Norway.

ylotrophs' digestion turns out to be a tricky business. The methane-eaters are so specialized they can't survive on anything else—they starve on standard culture-dish fare. So mutations that interfered with methane metabolism promptly killed the bacteria, making them tough to study. Fortunately, their first cousins, who live on methanol (methyl alcohol, CH_3OH), can also get by on sugar, so work focused on them.

But now that you have a methanol-eater that can take it or leave it alone, how do you know whether your mutant has a defective one-carbon metabolic system? Methanol dehydrogenase, a crucial enzyme in one-carbon metabolism, also converts allyl alcohol (innocuous to these bugs) to allyl aldehyde (a toxin). Thus any mutants that survive a healthy dose of allyl alcohol have defective systems.

To find out where the mutations were, the researchers go to a "clone bank"—the entire genetic complement of a normal methylotroph chopped into random fragments and cloned. One fragment contains the original version of the gene that was mutated in the bacterium. Each fragment is inserted into a different sample of the mutant bug, using standard recombinant DNA techniques, and the bugs are put out to pasture in methanol. The sample that gets the original gene grows, and the fragment of DNA that went into that bug can be analyzed, the sequence of its amino acids determined, and its position in the set of chromosomes mapped.

Once a gene has been sequenced and mapped, there are several ways to figure out what it does, but that's another story.

Lidstrom helped develop the techniques used to study the methanol-eaters while at the University of Washington in Seattle, before coming to Caltech in May 1987. When she left Seattle, the group had found 10 genes. One gene codes for methanol dehydrogenase itself. Three are involved in attaching the enzyme to its "cofactor"—another molecule the enzyme needs to do its job. One helps stabilize the enzyme and transfer it to where it's needed. One encodes a protein, called cytochrome c, that transfers the energy provided by methanol dehydrogenation to the cell's other metabolic machinery. Four regulate the other genes. The group had made little progress with the methane-eaters, however.

Lidstrom's Caltech group has found three more methanol-eater genes. "One is a previously unknown subunit of the enzyme, which is very interesting. One seems to be

In the experiment's simplest form, voters have no information whatsoever.

involved in regulation, and we have no idea what the other one does. We're in the process of making a mutation of it right now, and we'll see if the mutant can still grow in methanol."

The Caltech group has also been able to crack the methane barrier. According to Lidstrom, "The genes are similar enough that once we get them from the methanol-users we can use them to identify that same DNA in the methane-users. We've looked at five of these genes in the methane-users now. There would be no other way to get those genes."

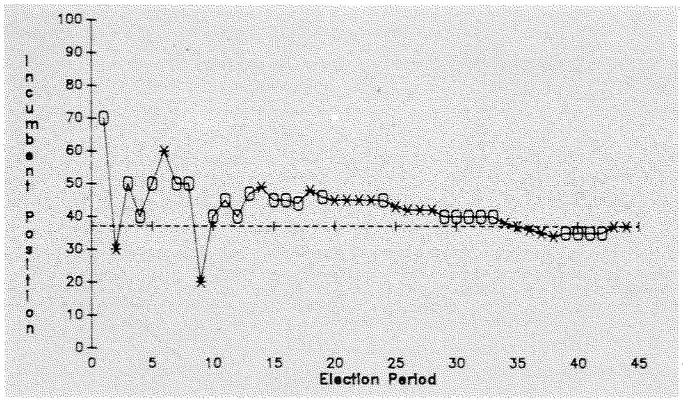
The genes can also be used to identify and count bacteria in the field. A soil or water sample is chemically treated to extract the DNA from any bacteria present. This DNA is matched against tagged DNA from the methylotroph genes by a process called hybridization. The tagged DNA can be counted in a detector, giving a number proportional to the number of methane-eaters in the original sample. The population data, when correlated with methane and TCE consumption studies at the same site, will show how the bugs behave in the wild.

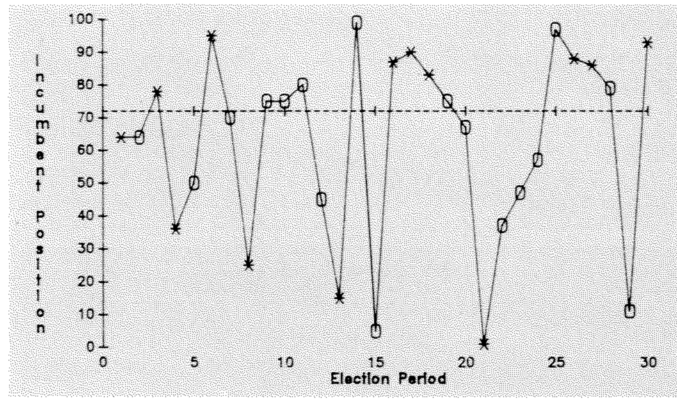
Says Lidstrom, "We've done some field studies already, just looking at population distributions in various environments. We should know enough about the mechanisms to be able to start field tests of methane and TCE consumption in about two years, and we'll have to see how closely our lab work fits with what we get in the field. But conservatively, we should see applications on-site in the next five years."□—*DS*

Voting in the Dark

Who are your Representatives in the State Legislature? What are their positions on acid rain? If you haven't the foggiest, you're not alone. But a democratic society depends on well-informed voters making rational choices, doesn't it? Think of the Pilgrim Fathers electing William Bradford governor, or the ancient Greeks meeting in the agora to discuss the issues of the day. How does the system work when voters know little or nothing about the candidates and issues? Does it work at all?

Professors of Political Science Richard D. McKelvey and Peter C. Ordeshook are exploring the gap between the traditional civics-text theory of well-informed voters and the reality of a poorly informed public. They work in Caltech's Laboratory of Experimental Economics and Political Science, where researchers investigate aspects of economic and political behavior through simulations in controlled settings. Volunteers play the roles of the entities under study: voters, committee members, corporations, or what have you. As an incentive to play their parts to the hilt, the participants are paid cash according to how well their entities did. A network of personal computers doles out information to the participants, records decisions, and handles all the bookkeeping needed to





Left: Election record for a typical experiment. The dotted line indicates the median policy. The 0 and * indicate which candidate is the incumbent at each election. Thus Candidate 0 is in office before the first election, but is promptly ousted. Both candidates pick more or less random policies at first, but learn from their mistakes. By election 10, they start to converge to the median. Note how the voters "test the waters" every few elections by electing the challenger. This may help drive convergence by showing what the other candidate has to offer.

Right: An experiment that never converged. Although the voters tended to re-elect candidates who stayed close to the median (Candidate 0 in elections 10 – 12, for example), the candidates didn't seem to get the message.

track the experiment.

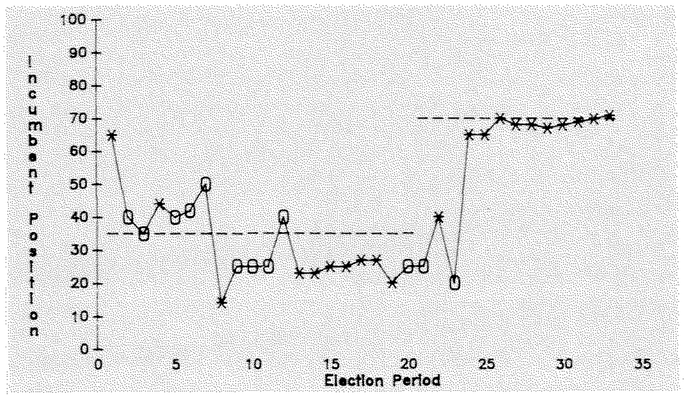
The electorate consists of up to 50 students. Two are candidates, one of whom is in office when the experiment begins. The incumbent selects a "policy" regarding an "issue." Neither has anything to do with the real world. The issue is a linear scale of, say, 0 to 100; choosing a policy amounts to picking a number in that range. Each voter is assigned a "payoff curve" (a plot of policy vs. payoff) that peaks at some random policy number; each curve is different. All voters are paid according to where the incumbent's policy falls on their individual curves: the closer the policy is to the peak, the larger the payoff. Once paid, each voter must decide whether to keep the incumbent in office or to vote for the challenger in the next election. At the same time, both incumbent and challenger select (but do not reveal) new policies. Then the election is held, and all voters are paid according to the winner's policy. The process repeats for 40 cycles or until time expires, when the voters get real money in proportion to the payoffs they have amassed. Candidates are paid in proportion to the number of elections they win.

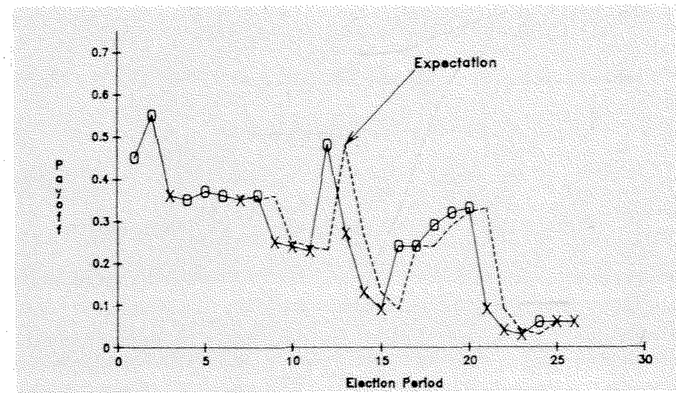
In the experiment's simplest form, voters have no information whatsoever about the candidates' policies, or where their own curve peaks. All they have is their personal history of payoffs under past administrations. Similarly, candidates know only their own policy selection, and who won the election.

The set of payoff curves has a median peak—the one where half of the curves peak to its right and half to its left. The median policy is the candidates' optimum position under majority rule. If a candidate should take a position to the right of the median, for example, all voters to the left of the median would prefer to vote for the median instead. If everyone were fully informed about policies and payoffs, the candidates would immediately adopt the median policy, or the electorate would quickly drive the candidates there by voting for whoever was closest. McKelvey and Ordeshook have found that, over time, candidates still move toward the median in the information-poor experiment described above. In other words, even though voters have incomplete information, the system is still able to function, albeit more slowly.

The experimental voters view their personal histories differently, McKelvey finds. "Some voters just go by the last period—am I better or worse off now? Others give the incumbent the benefit of the doubt. If the payoff drops a little bit, they'll still vote for the incumbent; they take a weighted average over the past few cycles, and only punish the incumbent if the payoff drops significantly. We are still working on a theoretical model for this."

Most runs converge to the median in 10 to 15 cycles. Some never converge, however, if candidates misread the voters' signals. "We also get deviations," McKelvey says, "because some individuals vote at random, or do crazy things. We think that in large electorates, these phenomena would disappear. Individual mis-





Left: When the median suddenly shifts during the experiment, the candidates flounder around their previously successful positions until Candidate * stumbles upon the new median.

Right: A single voter's history. A 0 represents voting for the incumbent, an X represents voting for the challenger. The dotted line shows the voter's payoff from the previous election—if the current administration gives a smaller payoff, this person votes for the challenger.

takes would tend to cancel out statistically, so the consistent behavior of a few people would tend to govern the whole system. Even ten percent of the electorate would be all you need if the rest voted randomly."

In some experiments the payoff curves are changed in the middle of the run, radically shifting the median. This generally throws the candidates for a loop, but only for a few cycles until one candidate stumbles upon the new median. Then both candidates rapidly converge to it.

Current experiments make additional information available to the participants. Voters may be told where their curve peaks, for example. Voters may buy information about the candidates' positions, or the experimenter may publicly announce which candidate's position is more extreme in one direction—equivalent to a special-interest group endorsing the candidate most in line with its position.

These runs also converge to the median. "Voters frequently know a lot more about interest groups and other voters than they know about the candidates," McKelvey notes. "So in real campaigns, you look at the endorsements. Take California. We have all these very complicated propositions on each ballot. Every voter gets a pamphlet with the full text of each measure. But very few voters actually take the time to read them and figure out what they mean, because in that same pamphlet are signed arguments for and against them. Who signs what tells you a lot about the proposition. Trying to dissect the propositions yourself is expensive, in terms of time invested, so you take the more cost-effective method. You read the endorsements, and ask your friends' opinions. And as long as some segment of the electorate opts to be informed, this works fine."

In the latest wrinkle, a candidate is in office for four "years" between elections. A policy is chosen each year, the voters are paid accordingly, and then a poll is taken: if the election were held immediately, would you vote for the incumbent? After four cycles of policy, payoff, and poll, the election is held in earnest.

This set is just getting under way, so it is too early to tell if the intermediate polls, by allowing incumbents to test several policy variations, help the candidates converge faster. "In the real world," Ordeshook remarks, "voters are continuously monitoring their own welfare, and the candidates are continuously polling the electorate. It would be much more realistic to have analog computers, with people turning policy knobs and approval knobs continuously, and then have an election after some period of knob-turning. But we're stuck in our digital age."

McKelvey concludes, "You have to be very careful in trying to extrapolate to the real world. These are very simple experiments. But we feel the convergences we have seen demonstrate that it is *possible* for electoral systems to work properly over the long term, even when individuals have access to very little information."□—*DS*