

MEETING BRIEFS

Integrating the Biological Sciences in New Mexico

ALBUQUERQUE—Living up to its new name, the Society for Integrative and Comparative Biology (formerly the American Society of Zoologists) attracted a diverse audience, from neuroscientists and physiologists to taxonomists and ecologists, to its annual meeting, which was held here from 26 to 30 December 1996. The 500 talks and posters melded cellular, molecular, organismal, and ecological studies of organisms ranging from flying insects to ocean-bottom worms.

Exotic Deep-Sea Lifestyles

Life in the abyss is no picnic. With no sunlight or plants to provide food, some bizarre inhabitants of this watery underworld—the mouthless, gutless tube worms, for example—rely on a toxic gas, hydrogen sulfide, that seeps or jets up from the very rock on which they live. Symbiotic bacteria in the worms' tissues oxidize the hydrogen sulfide and use the resulting energy to convert inorganic carbon into carbon compounds that nourish the meters-long worms. But that arrangement doesn't end the worms' problems, because in many of the sea-floor sites they colonize, the water contains enough hydrogen sulfide to poison them—or so little they should starve.

As work reported at the Integrative Biology meeting showed, however, the worms are masters of making do. They deploy an array of tricks to "mine" for the gas when it is scarce or protect their tissues from it when it is not. Says one of the researchers, Shana Goffredi of the University of California, Santa Barbara (UCSB), "They just surprise us all the time."

For the members of the *Lamellibrachia* genus, which form football field-size communities in the Gulf of Mexico, about 750 meters deep, the problem is one of scarcity. Indeed, marine biologist Charles Fisher of Pennsylvania State University, who has been studying the tube worm for 10 years, has been unable to detect hydrogen sulfide gas in the surrounding water. But the worms are clearly flourishing. Although Fisher found that they grow only a few centimeters a year at most, the largest of them "are easily 100 or more years old," he says.

The worms apparently sustain themselves, he now reports, by sprouting "roots" that extend into the sediment on which they grow. When Fisher retrieved some tube worms that were still connected to their rock perches, he found that they had sent an impressive tangle

of tubes some 15 centimeters long and about 1.5 millimeters in diameter into the rock. Laboratory tests indicate that these buried tubes can absorb hydrogen sulfide, which was plentiful underground in 13 of the 14 sites Fisher sampled in the Gulf of Mexico.

The much larger and faster growing tube worms that live at the deep-sea vents found along midocean ridges have the opposite



Spaghetti roots. Blue bands (above) track tube-worm growth, made possible by the tubes the worms send into the sea floor (left).



problem. The water the vents spew into the ocean is so rich in hydrogen sulfide that it might easily poison both the worms and their bacterial partners. New data that Goffredi reported at the meeting suggest that this doesn't happen because the tube worms control how much hydrogen sulfide they take up.

Researchers had assumed that the gas simply diffuses into the tube worms' plumes, as do the carbon dioxide and oxygen that the worms must also procure for their bacterial guests. But laboratory studies by Goffredi and James Childress, an ecological physiologist at UCSB, indicate that ordinary hydrogen sulfide doesn't even enter the worms' tissues. Instead, they absorb a charged version of the molecule, HS^- , which is also present in seawater.

The charge prevents HS^- ions from entering the worm's surface membranes and may

require that transporter or channel proteins in the membranes actively ferry the ions into the animals. That arrangement would allow the internal concentration of the gas to be regulated, preventing it from getting too high, Goffredi explains. Because this result goes against the current dogma, Fisher calls it "a really neat, new finding." Goffredi and her colleagues have not yet figured out how sulfide is imported, however.

The worms also control their chemical balances in other ways, adds Childress. As the bacteria living inside the tube worms oxidize the sulfide and fix carbon, he explains, they generate excess hydrogen ions, which have to be eliminated to keep the worm's interior from becoming too acidic. In Childress's lab, Peter Girguis has found that energy generated by an adenosine triphosphate-splitting enzyme that probably lies in the worm's outer membrane likely enables that enzyme to pump the ions out into the seawater. "The rates of elimination are very high," he says. Adds Childress: "That probably is as important for the symbiosis as the uptake [of sulfide and carbon dioxide]." And it's probably far from the last of the worms' wily ways.

Muscle-Bound Dragonflies

The thorax of the dragonfly would make any body builder jealous: Inside, it's all flight muscle. How that muscle develops during the dragonfly's lifetime is helping physiologists understand what makes muscles strong.

At the meeting, physiological ecologist James Marden of Pennsylvania State University described how a change in a single key protein, called troponin T, may help transform the relatively weak muscles of newly emerged adults into more robust muscles that contract at much greater frequencies. Marden's work suggests that the troponin-T change boosts the sensitivity of the muscle's contractile proteins to calcium ions, which trigger muscle contraction.

Although troponin-T structure changes during development in many organisms, including humans, the significance of the changes has been unclear. "It's some of the first data of this kind for any type of organism," comments Jon Harrison, a physiologist at Arizona State University in Tempe. "It's quite likely to be a general mechanism" for tailoring muscles to work best in their particular contexts.

Marden's interest in the dragonfly flight muscle was triggered by his observation that newly emerged adults spend relatively little time—about 2%—airborne, while older adults spend almost all of their day in flight. When he examined the muscle to see how it changes over time to accommodate these needs, he found that, as it matures, the young dragonfly's flat, white thoracic muscle fills

out and also reddens as more of the cells' energy-producing mitochondria appear.

These changes strengthen the muscle, allowing it to generate more lift per unit of mass, although the stronger muscle also generates more heat, thus changing the operating temperature of the muscle. Marden and graduate student Gail Fitzhugh then figured out that mature muscle works better because it's 10 times more sensitive than the young muscle to calcium ions. "The [mature] muscle is becoming much more excitable at high temperatures," Marden says.

He thought the explanation for the difference might be found in the troponins, because these proteins, which are attached at regular intervals to the filaments of contractile proteins, receive and relay the calcium-

ion signals to those filaments. Of the four types of troponins (designated C, I, H, and T), "there was a clear age-related change" only in troponin T, Marden says.

Melisande Wolf in Marden's lab then showed that the RNA copied from the troponin-T gene yields different forms of the protein through alternate splicing of the gene's coding regions. The protein from newly emerged adults has long strings of negatively charged amino acids at both ends, while that of mature insects has negative amino acids at one end, but positively charged amino acids at the other.

Previously, researchers thought that troponin T serves only to anchor other troponins to the contractile filaments. But Marden thinks the structural differences in these so-called isoforms of troponin T could

account for the altered calcium sensitivity of the older dragonfly muscles.

In young dragonflies, he suggests, the double negative ends keep nearby troponin-T proteins from interacting with one another. The insects don't fly much and instead need to devote most of their energy to growing. But in older insects, if the positive end of one troponin T is attracted to the negative end of another in an adjacent filament, then the two—or a whole series of troponin-T proteins—may line up and cooperate in initiating contraction. As a result, the muscle becomes more sensitive to calcium, maximizes its power output, and enables these dragonflies to do the high flying necessary to defend their territories and find their mates.

—Elizabeth Pennisi

CHEMISTRY

New "Guiding" Protein Discovered

Compared to nature's chemical sleight of hand, human chemists are all thumbs. Take molecules that can occur as mirror images of each other, or stereoisomers. Many test-tube reactions yield messy mixtures of stereoisomers, and because stereoisomers of the same molecule can have wildly different properties, chemists often have to put a great deal of effort into separating them. Yet somehow, the tiny chemical factories in cells manage to crank out just one version flawlessly.

tists believe their finding could help explain how cells fashion a host of phenolic compounds, from small molecules in plants, called lignans, which can have medicinal properties, to large polymers, such as those forming insect cuticle and lignin, the glue that holds together trees. And it could also be a boon for drug companies and lignin-intensive industries like paper-making, says Jeffrey Dean, a biochemist at the University of Georgia, Athens: "This is a seminal finding. It opens up a new door in a research area that has been stagnant for a while."

This stagnation actually dates back to the 1930s, when chemists first postulated they could make artificial lignin by mixing a type of phenol, E-coniferyl alcohol, with enzymes called oxidases that link molecules together by oxidizing, or removing an electron from, a hydroxyl bond. Over the years, researchers experimented with a variety of oxidases, but no matter which they used, they inevitably ended

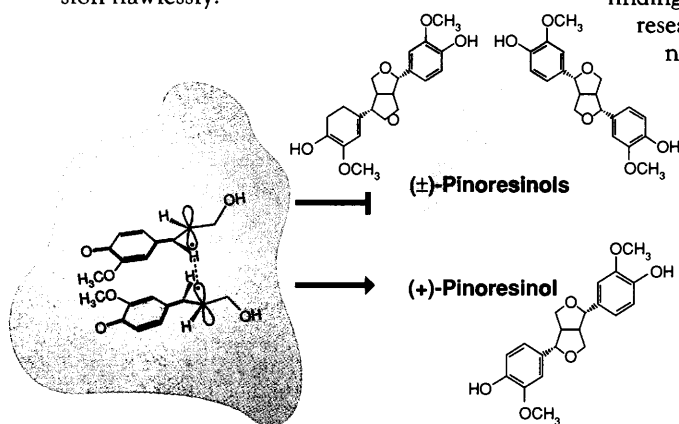
out those fractions capable of joining two E-coniferyl alcohol molecules.

The researchers purified the individual proteins in these fractions and, to tease out their role in lignan-making, mixed the proteins, one after the other, with E-coniferyl alcohol. To no one's surprise, several of the proteins turned out to be oxidases, which linked the alcohols into the usual mixtures of isomers. But one protein seemed to play an entirely different role: On its own, it couldn't join the alcohols. But when it was added to the test tube with one of the oxidases, the phenols combined into just one isomer—the one made naturally by the plant. Lewis dubbed the protein "dirigent" from *dirigere*, Latin for align or guide, because he thinks it clasps and steers two E-coniferyl alcohols so they come together in just one way.

"[Dirigent proteins] may really be a new class of enzymes," says pharmaceutical chemist Meinhard Zenk of the University of Munich in Germany. And if they are, their impact on the chemical industry could be enormous. With dirigent proteins, chemists might be able to synthesize improved versions of drugs derived from plants or bio-engineer trees with lignin that has a different stereochemistry, which might make it easier to degrade. That could make the paper-pulping process much cheaper and less polluting (although some chemists question how important stereochemistry is to the structure of lignin).

The researchers are now working to verify the protein's role in lignan-making by tracking down the gene that codes for it so they can actually make the protein, Lewis says. They're also hot on the trail of other kinds of dirigent proteins. Other researchers may well join the chase, says Dean. If so, chemists might find out that they're not so ham-handed after all.

—Jocelyn Kaiser



In charge. New type of protein appears to maneuver phenols so they form only one stereoisomer.

Now, researchers may have discovered how nature controls the stereochemistry in a major class of compounds: substances made from small, biochemical building blocks called phenols (see diagram). On page 362, chemist Norman Lewis and colleagues at Washington State University in Pullman and Simo Sarkanen at the University of Minnesota, St. Paul, unveil a brand-new kind of protein in plants that seems to grab and maneuver phenols so they will tack together in the proper orientation. The scien-

up with mixtures of various isomers that only vaguely resembled lignin. Clearly, some other enzyme or cofactor was involved, but nobody could figure out what it was.

To search for this mystery substance, Lewis's team studied a particular lignan—just two building blocks linked together in one of two possible stereo forms—that plants manufacture in various forms as defenses against pathogens and predators. Lewis's group crushed stems from a forsythia plant and extracted the proteins. They then screened