Bright Ideas

"It now appears that Dos Pilas was a pawn *in a much bigger battle.*" —ARTHUR DEMAREST

Hieroglyphs Expose Mayan Superpower Conflict

Newly uncovered hieroglyphs in Guatemala are filling in an unknown period of Mayan history. Their translation is helping to explain the fracture of the Mayan empire into warring regional states and the eventual collapse of a civilization that once ruled southern Mexico as well as Central and South America.

Complete with references to piles of skulls and flowing blood, the glyphs were partially exposed during a recent hurricane at Dos Pilas, an ancient Mayan city isolated deep in the rain forest. "The hundreds of new glyphs fill a gap of unknown Maya history and clarify many of the political and military relationships of this critical period," says epigrapher Federico Fahsen, adjunct professor at Vanderbilt, who deciphered the text. Fahsen says the glyphs are extraordinary because they tell not only about the city of Dos Pilas's triumphs but also its setbacks and tragedies. He now believes Dos Pilas was part of a dramatic superpower struggle that may have contributed to the collapse of the Maya civilization.

Carved into an 18-step stone stairway, the hieroglyphs reveal that in the seventh century, the Maya world was divided between two superpowers — one under the control of the city-state Tikal, the other dominated by Calakmul. Tikal was located in what is now northern Guatemala; Calakmul was about 60 miles farther north in Mexico.

The glyphs — among the largest texts ever discovered — detail how Calakmul was involved in the wars that occurred in the ancient Maya world. Previously, Mayan scholars viewed the conflict between Dos Pilas and Tikal as a quarrel between two brothers.

The glyphs, however, reveal a very different story. It's one that begins with the birth of a king, Balaj Chan K'awiil, in 625 A.D., and Tikal's creation of Dos Pilas as a military outpost in 629. Dos Pilas was important for its proximity to the middle stretch of the Pasión River, the superhighway of the Maya world. It was strategically important because it allowed Tikal to control trade routes between the highlands and lowlands.

As told by the glyphs, Balaj Chan K'awiil was installed as ruler of Dos Pilas by Tikal at the age of 4. "Balaj Chan K'awiil became a very big warrior," says Fahsen. "He almost never stopped fighting and for many years was loyal to Tikal. When the king was in his early 20s, Calakmul attacked and defeated Dos Pilas. After capturing Balaj Chan K'awiil, Calakmul became a "puppet king" who kept his land in exchange for allegiance.

"When I read those glyphs, I had to blink to make sure I was reading correctly," he says. "I had never heard of Calakmul actually invading and defeating the king of Dos Pilas. We thought that, at most, they might have had a weak alliance."

The record continues to describe how Balaj Chan K'awiil, now loyal to Calakmul, launched a decade-long war against Tikal that ended in his victory. His forces sacked Tikal and brought its ruler — his own brother — and other Tikal nobles to Dos Pilas to be sacrificed. "The west section of the steps was very graphic," says Fahsen. "It says, 'Blood was pooled, and the skulls of the 13 people of the Tikal palace were piled up.' Following the victory, Dos Pilas embarked on a campaign of conquest with Calakmul's backing and became a major regional power."

"Rather than being an independent actor as previously thought, it now appears that Dos Pilas was a pawn in a much bigger battle," says Arthur Demarest, Ingram Professor of Anthropology at Vanderbilt. "In today's terms, Dos Pilas was the Somalia or Vietnam of the Maya world, used in a war that was actually between two superpowers."

Arthur Demarest, left, and Federico Fahsen have discovered hieroglyphs that change scholars' notions of Myan history.

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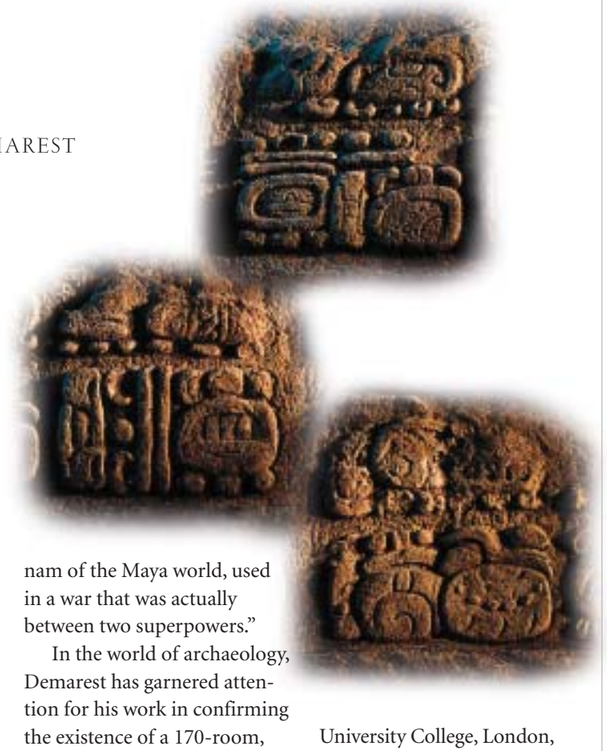


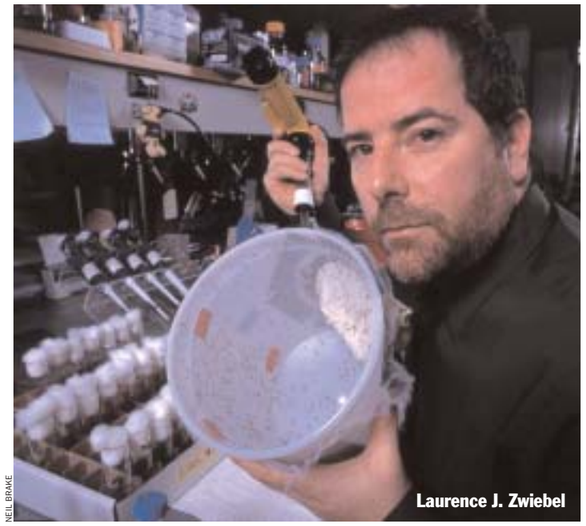
In the world of archaeology, Demarest has garnered attention for his work in confirming the existence of a 170-room, three-story, eighth-century royal palace in Guatemala in 2000. Located in the ancient city Cancuén (meaning "Place of Serpents"), the palace is believed to be one of the largest, most elaborate and best-preserved residences of ancient Maya kings.

Fahsen and Demarest contend the newly translated account at Dos Pilas supports the theory advanced by some Maya scholars who have asserted that this period in Maya history was a "long world war" between Tikal and Calakmul. Scholars previously characterized the conflicts between different Maya city-states as regional and unrelated. "The new evidence supports the more extreme versions of theories advanced by two Maya scholars — Simon Martin of University College, London, and Nikolai Grube of the University of Bonn," says Demarest.

After evaluating the new material, Demarest now concedes this was a time when the Maya civilization was on the verge of moving to a higher level of organization and consolidating into a single empire. "However, this didn't happen. Instead, the giant war went back and forth. After Tikal was sacked, it roared back and crushed Calakmul. And then the Maya world broke into regional powers, setting the stage for a period of intensive, petty warfare that finally led to the collapse of the Maya," says Demarest. By 760, Dos Pilas was abandoned.

The work in Guatemala was funded, in part, by Vanderbilt and the National Geographic Society.

Deciphering Mosquito Senses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Laurence J. Zwiebel

There's been a national buzz about mosquitoes this year, but the study of the genetic map of the species, *Anopheles gambiae,* by three Vanderbilt researchers has gained notice within the scientific community and may foster new ways to control this disease-carrying insect.

It seems that *A. gambiae* is something of a gourmet — its preferred meal is almost exclusively human blood. Its insect's preference for humans and its ability to seek them out, in fact, are the insect's hallmarks and have made the mapping of its genes an important subject of scientific study. The net result could lead to a more effective assault on the mosquito's ability to spread malaria, a disease that annually causes millions of deaths worldwide.

Vanderbilt researchers Laurence J. Zwiebel, A. Nicole Fox and Jason Pitts, along with scientists from the University of Notre Dame and the University of Illinois at Urbana-Champaign, have identified specific genes related to this species' ability to spread malaria. These genes play a critical role in almost every aspect of the insect's life cycle, including its ability to see, taste, touch and smell.

"This is an important step in our ability to first understand the mosquito's host preference and tracking system and then to interfere with it in a way that can save human lives in an economically feasible and environmentally benign fashion," says Zwiebel, an assistant professor of biological sciences and study leader.

Malaria is spread when a human is bitten by an *A. gambiae* mosquito carrying the protozoa that causes the disease. This single-celled entity spends part of its life cycle in humans and part in the mosquito. The disease produces a severe fever and, in some cases, potentially fatal complications affecting the kidneys, liver, brain and blood.

In the Oct. 4 issue of *Science*, Zwiebel, Vanderbilt graduate student Fox, and research associate Pitts and colleagues reported the identification of 276 genes in the *A. gambiae* genome. These provide the blueprints for proteins central to the mosquito's sensory systems, including its ability to find and feed on humans. In particular, Zwiebel and colleagues found 79 genes that appear to be involved in the mosquito's sense of smell and 72 involved in taste. Previous studies have shown that both of these chemo-sensory senses play a major role in the mosquito's strong preference for human blood.

The identity of many of the genes was determined from their structural characteristics and their similarity to genes found in a particular species of fruit fly. "In addition, we found a large number of genes that are unique to the mosquito and, therefore, could very well be involved in mosquito-specific behaviors such as blood feeding, host preference and tracking," says Zwiebel.

The Vanderbilt researchers' paper on the topic is part of a special issue of *Science* that reports the sequencing of the entire *Anopheles* genome. Concurrently, the journal *Nature* is announcing the sequencing of the genome of *Plasmodium falciparum*, the single-cell parasite carried by *Anopheles* that causes malaria. These two achievements are providing the impetus for an assault on malaria.

Pancreas Cells Pave Way for Diabetes Treatment

Before the pancreas is a pancreas, it is just two tiny bumps — two groups of cells sprouting from a central tube. What makes these cells bud off from the main group? How do they go on to make all the cell types of the mature pancreas? These are the kinds of questions that drive the research efforts of Christopher V.E. Wright and colleagues. The answers could pave the way toward limitless supplies of pancreatic cells for transplantation therapy of diabetes.

"It has been established that islet cell transplantation can solve the diabetes problem," says Wright, associate professor of cell and developmental biology and director of Vanderbilt's Developmental Biology Program, referring to studies carried out in Edmonton, Canada, and elsewhere. "The problem is having a suitable and sufficient source of transplantation material."

Donated pancreases and the technical expertise required to isolate functioning islet cells — the pancreatic cells that produce insulin — will not meet the demand, Wright says. An alternative, he says, is to produce insulin-secreting cells from embryonic or other stem cells.

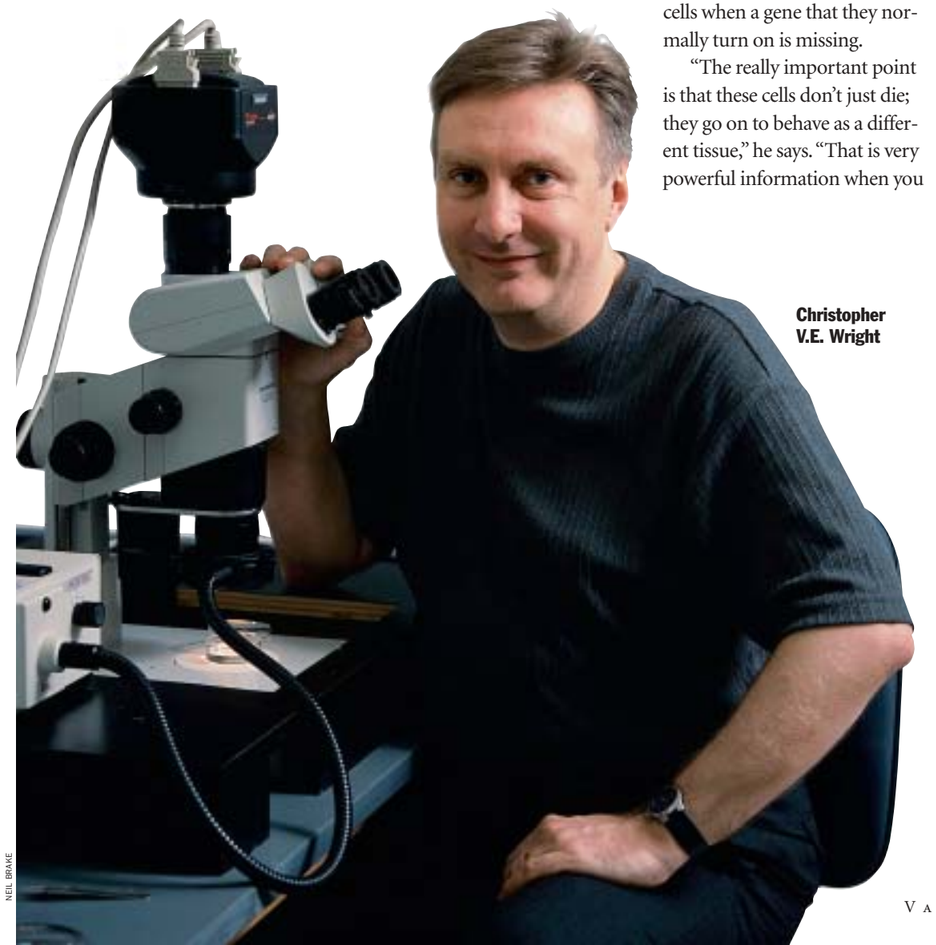
"If we can identify the factors that determine pancreatic cell fate, we might be able to coerce embryonic stem cells or other cells to turn into pancreas."

One of these factors is a gene called PTF1p48 (p48 for short). Wright and colleagues reported in *Nature Genetics,* published online Aug. 19, 2002, that p48 is required for the development of the pancreas — both its exocrine cells (those that secrete digestive enzymes) and its endocrine cells (those that secrete insulin and other hormones).

Wright's team used what one reviewer of the paper called "a novel and powerful cell marking method" to track cells in the mouse that express the p48 gene, starting very early in embryonic pancreas formation. The method relied on genetic manipulations to introduce an inherited marker — a blue color that could be followed in cells that turned on the p48 gene, and in all the cells that came from those cells.

A simple way to think about the technique, Wright says, is to picture the crowd at a football stadium and to imagine that somewhere in the stadium, for a limited time, a man gave away unique blue hats and asked people to wear them. "Now we can follow the people who got hats, no matter where they go," says Wright. "Whether they go to get a hot dog or leave the stadium entirely, we can find them."

Using the technique, the investigators found and followed the cells that turned on the p48 gene — as if these cells were wearing blue hats. The cells that budded out to form the pancreas turned on p48; they were blue. And the cells of the mature pancreas were blue, too.


NEIL BRAKE

Christopher V.E. Wright

Wright's team combined this powerful method for tracing a cell's lineage with gene knockout technology. They engineered mice to lack the p48 gene, causing abnormal development of the pancreas. Cells in these knockout mice still tried to turn on the p48 gene, so the investigators were able to follow the blue marker in these cells.

They found that, with p48 absent, the cells that normally express p48 and go on to form pancreas became intestinal cells instead. And they became all types of cells in the intestines, including intestinal stem cells. It is the first time, to Wright's knowledge, that investigators have tracked what happens to cells when a gene that they normally turn on is missing.

"The really important point is that these cells don't just die; they go on to behave as a different tissue," he says. "That is very powerful information when you

are thinking about manipulating stem cells in the laboratory. Because you know now, at least for some genes, that you can put them in or take them away and you don't kill the cells; you manipulate what they're going to become. And that's exactly what we want to do therapeutically."

Wright believes that linking lineage tracing and gene knockouts will become increasingly common. "It adds extra depth to understanding cellular behavior," he says. He is also enthusiastic about fluorescent variants of the lineage tracing technique that will allow investigators to follow living cells as they change fates.

And he is excited about his group's ongoing studies with p48. The team is currently introducing the p48 gene into cells that would normally become intestinal cells to see if they change their fate and become pancreatic cells instead.

"If we can do that," he says, "we're a big step further towards knowing that p48 is one of the gene triggers you might want to put into an embryonic or other stem cell to make pancreas."

Those "other" stem cells could be circulating-blood stem cells or even cells within the pancreas that could potentially regenerate the organ, so-called pancreatic stem cells. They appear to exist in mice, which are capable of pancreatic regeneration, explains Wright. It is not so far-fetched, he adds, to believe that human beings harbor such cells. Identifying the genes, such as p48, expressed by pancreatic progenitor cells forwards efforts to find pancreatic stem cells.

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