of openness and candor around significant dual-use activities. We are about creating light where there is darkness." U.S. officials disagree. "Illicit biological warfare work could easily be concealed or cleaned up, rendering it highly improbable that international inspectors would detect evidence of noncompliance," asserts the State Department's Edward J. Lacey.

Packaging death. Bomblet filler at Soviet bioweapons plant.

There's the rub, notes a U.S. delegate to the Ad Hoc Group. Weak challenge investigations might provide "a false sense of security," the participant warns. "Under the more restrictive protocol, would you ever really find a smoking gun? ... Is that false sense of security better than nothing?" But negotiators from other countries—and some U.S. experts as well—argue that protocol provisions can deter violators even if they don't necessarily provide access to ironclad evidence. "After all," asks Pearson, "what treaty ever provides such evidence?"

Political endgame

The U.S. is also sparring with its allies over the procedures for facility visits and mandatory declarations of potential dual-use organisms and technologies. Driven by the concerns of the biotech and pharmaceutical industries, the Bush Administration is worried about the inadvertent leakage of trade secrets—vaccines in development, for example. The Administration also fears that visits to government labs could compromise national security. A facility might take pains to ensure that nothing it declares or shows investigators would harm specific security interests. However, claims the U.S. Ad Hoc delegate, by looking at "aggregates of information," an enemy might add things up and find vulnerabilities in biodefenses of the U.S. or its allies.

Negotiators from other countries discount the U.S. objections as primarily politically motivated. When the Ad Hoc Group first began its deliberations in 1995, the British government pressed for more expansive measures that would give the protocol more teeth than the composite text. That put the United Kingdom at odds with the United States on several key issues, including the scope of declarations and site visits. In negotiations since, the British delegates have sought to find compromises that would be acceptable to the United States and other countries without undermining the protocol's effectiveness. The United Kingdom is now solidly in the protocol-enthusiastic camp. "The protocol, in my view, is a very fair compromise," says a senior U.K. official close to the negotiations.

But the protocol has some difficult hurdles to clear. If it wins a vote of confidence in the next few weeks—which will require at least tacit U.S. backing—it would go to a Special Conference in the autumn. If treaty signatories approve it there, the protocol would then go to each country for ratification.

That could be problematic in the United States. Arms-control agreements have not been popular among Republicans in the U.S. Senate, where a two-thirds majority is required to ratify international agreements. It would take a remarkable change of attitude in the Bush Administration to line up enough votes to get the protocol approved.

If the Ad Hoc Group reaches an impasse next month, one U.S. participant doesn't think the years of talks will have been all for naught. The discussions have brought weapons experts from so-called rogue nations that are BWC state parties into the fold and allowed experts from former adversaries to forge more open relationships. "It's much more difficult, in the end, to make a weapon against someone you know," the delegate says, "unless your government compels you to or unless you're truly evil." Of course, without a way to ensure treaty compliance, it will be hard to know who really is evil—until it's too late. **–RICHARD STONE**

INFECTIOUS DISEASES

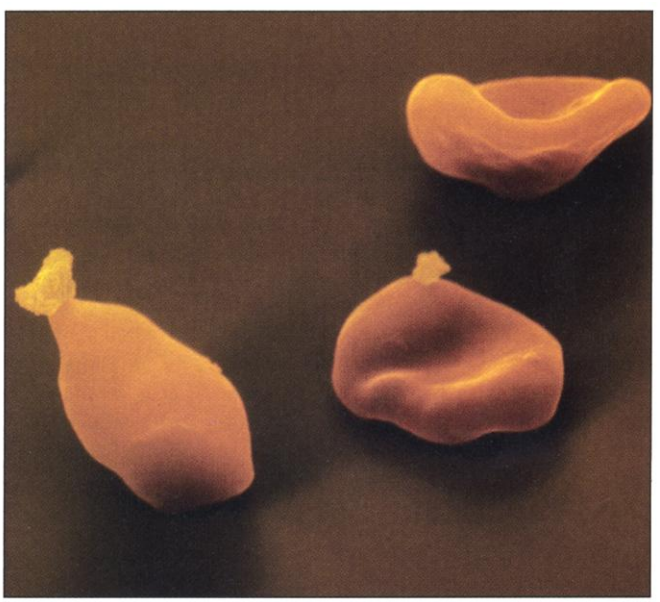
Malaria's Beginnings: On The Heels of Hoes?

By analyzing DNA of the parasite that causes malaria, researchers are trying to determine the role agriculture played in promoting this deadly disease

How long has the deadly malaria parasite *Plasmodium falciparum* plagued humanity, causing wrenching disease and epidemic death? Since hominids and chimps first went their separate ways? Or only after agriculture created the right conditions for malaria's spread? Scholars have debated this question for decades, but only recently have data become available that might turn theory into fact. By comparing DNA among the various *Plasmodium* species and strains, researchers are attempting to reconstruct when modern *Plasmodium falciparum* first emerged. But, depending on where they look, the DNA is providing different answers—and resolution is nowhere in sight.

Both camps agree that *P. falciparum* has been around in one form or another since the human branch of the primate tree split off from chimps about 8 million years ago. One new analysis, reported on page 482, supports the notion that epidemic malaria traces back to a small population of *P. falciparum* that suddenly expanded exponentially about 20,000 years ago. But another, in press at the *Proceedings of the Royal Society*, suggests the parasite has been common for hundreds of thousands of years, and that malaria took much the same toll on our ancestors on African savannahs as it does today across the globe. "This work is all so new that opinions are unsettled," says Daniel Hartl, a population geneticist at Harvard University.

Although the prevailing view has long



Persistent parasite. After attaching to red blood cells, *Plasmodium falciparum* (yellow) enters the cell, where it differentiates into a gametocyte that is ready to be taken up by the mosquito.

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favored ancient origins, a few iconoclasts proposed a link between malaria and agriculture as early as 1958. More recently, a DNA-based study of the *Plasmodium* family tree, done in 1992, concluded that *P. falciparum* evolved from bird parasites after agriculture was established.

Curious about that result, Francisco Ayala, a population and evolutionary geneticist at the University of California, Irvine, decided to build his own family tree. To do so, he and his colleagues compared the genes for the small subunit ribosomal RNA of 11 *Plasmodium* species. As they reported in 1994, *P. falciparum*, the most virulent of the three *Plasmodium* species that infect humans, appeared to be about 8 million years old; in addition, it was more closely related to *P. reichenowi*, which infects chimps, than to either of the other human pathogens or the bird parasites.

But Ayala revised his thinking in 1998, after he and Stephen Rich from Tufts University in Medford, Massachusetts, did another analysis, this time assessing the amount of variation among DNA from different *P. falciparum* strains. (Typically, the older a species is, the greater the variation among strains.)

Using published data, they compared the DNA sequence of 10 genes in 30 *P. falciparum* strains. To their surprise, they found almost no variation—just a few differences that altered the protein-coding DNA. These data indicated that the 30 strains all came from a common ancestral population no more than 57,500 years ago. This conflicted, however, with earlier work by others that found considerable variation in the genes for antigenic proteins that are targets of the human immune system. Ayala and his colleagues concluded that the variation among those genes must have cropped up as they were rapidly evolving to evade the human immune system.

Skepticism was rampant. "My opinion was that [Ayala's result] could not possibly be true," Hartl recalls. Later that year, Austin Hughes, a molecular evolutionist at the University of South Carolina, Columbia, published a critique of the work. Among other points, he noted that the variation in one of the antigenic genes, *CSP*, indicated that the parasites had been around a very long time. He suggested that perhaps Rich and Ayala didn't analyze the right set of genes.

Seeking resolution, Dyann Wirth, a molecular parasitologist at the Harvard School of Public Health in Boston, and Karen Day, an epidemiologist at the University of Oxford, asked Hartl for help in deciphering the parasite's history. By that time, 2000, two of the parasite's 14 chromosomes had been sequenced and published, enabling the group "to take a much more systematic approach" than had Ayala and Rich, says Wirth.

They first identified eight genes that should have no evolutionary forces pressuring them to change at an accelerated or slowed rate. They decided to focus on the introns, the noncoding regions of the genes, because these regions mutate readily and so should be the first to accumulate differences. Thomas McCutchan, a molecular parasitologist at the National Institute of Allergy and Infectious Diseases (NIAID), calls that strategy "really smart." In all, they sequenced 25 introns from eight *P. falciparum* strains collected from all over the world, sequencing each multiple times for accuracy.

As they report on page 482, the Harvard-Oxford team found just three differences in the sequences from the different isolates, two of which were in an intron that might actually play a more important role in the gene than the researchers originally thought. Using those changes, they calculated the age of the genome as between 9500 and 23,000 years—about the time when early hunters settled down in communities and began to farm. "This may have been the time when human conditions, culture, and the [mosquito] vector came together to develop self-sustaining epidemics," explains Hartl.

Sowing sorrow. Farming helped increase the human population and create the right environment, with pooled water for breeding mosquitoes, for malaria to take off.

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Rich and Ayala are impressed. "[The study] is more complete than ours, as we were relying on stuff in public databases," says Rich. The new results also jell nicely with work by David Conway, a molecular parasitologist at the London School of Hygiene and Tropical Medicine. He, too, found little variation when he analyzed mitochondrial, as opposed to nuclear, genomes of various strains. "The implication, which I find convincing, is that virtually all of the existing sequence variation in the nuclear or mitochondrial genomes is of recent origin," Conway says.

Supporting evidence comes from other quarters as well. A new study, reported on page 455, suggests that the genetic mutation that confers malaria resistance in humans also arose recently (also see p. 442). To NIAID's McCutchan, the Harvard-Oxford group's new work "is another brick put in place," demonstrating the intertwined destinies of humans and *P. falciparum*: Agriculture enabled not only human populations to expand greatly but also *P. falciparum*'s.

That edifice is far from solid, however. Richard Carter, a geneticist from the University of Edinburgh in Scotland, for one, questions the underlying assumptions that the Harvard-Oxford team used to calculate the ages of the strains. Hughes, who advocates an ancient origin for *P. falciparum*, calls the conclusions "premature" because they looked at only two chromosomes in the genome. As evidence, he cites his work with Federica Verra of the University of Rome, reported in their upcoming *Proceedings of the Royal Society* paper. The two recently compared versions of 23 *P. falciparum* genes whose sequences were in public databases, looking for any base changes in them. They found plenty, enough to conclude that *P. falciparum* has existed in substantial numbers for at least 300,000 years.

Hartl calls the Hughes paper "extremely important and provocative." It might be right, he concedes—or not. One possibility is that the differences Hughes detected may simply reflect sequencing errors in the public database, although Hughes thinks that errors would account for only a small proportion of that variation. Alternatively, perhaps some parts of the parasite's genome are more uniform across strains than others—and Hartl, Wirth, and Day happened to study one of those regions. "That alternative has to be taken seriously," Hartl says.

Researchers in both camps agree that resolving whether modern malaria stems from a large, already diverse ancestral population of *P. falciparum* or a small, rather homogeneous one could reveal new ways to fight this scourge. If very little variation exists among all the parasite strains, says McCutchan, then "when you see a mutation, it probably means something and tells us that's where we should focus our studies." Those variations, in fact, could prove to be vaccine or drug targets. And that makes the ongoing debate more than academic. **–ELIZABETH PENNISI**