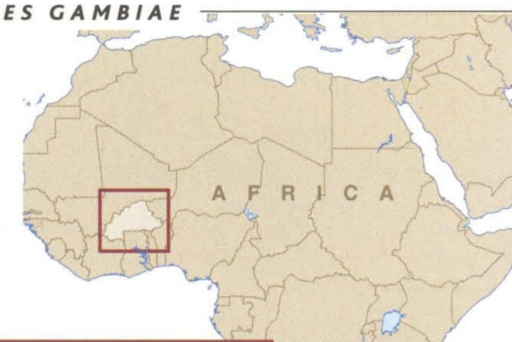
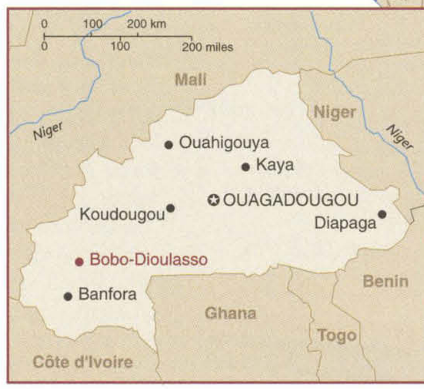
NEWS

In Pursuit of a Killer

Armed with microscopes, tweezers, and modified Shop-Vacs, researchers in West Africa are hunting malaria mosquitoes





Epicenter. Burkina Faso suffers from one of the heaviest mosquito burdens in Africa.

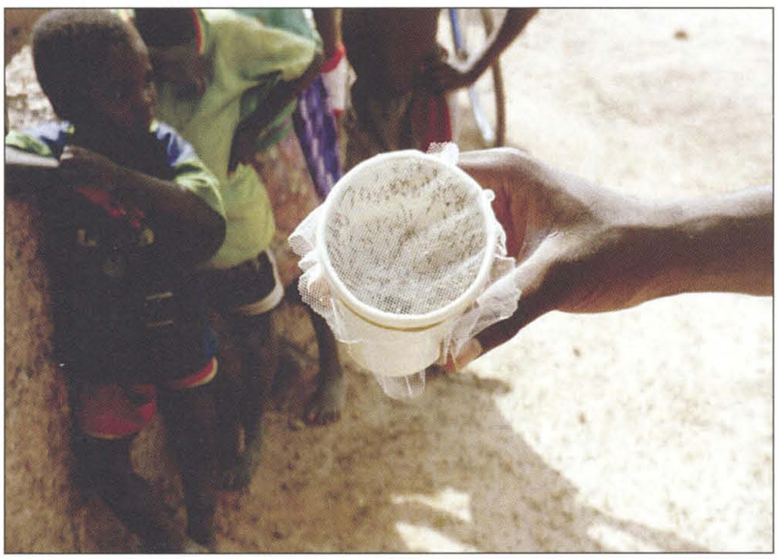
OUAGADOUGOU, BURKINA FASO—A basic rule of military intelligence is to know the enemy inside and out. Learn his habits, strengths, and weaknesses. The same holds true in the fights against malaria, dengue fever, filariasis, and West Nile virus. If public health experts are to have a chance at controlling these diseases, they need to understand exactly how the maladies' main ally—the mosquito—operates.

To deploy weapons effectively, from low-tech bed nets to high-tech transgenic mosquitoes, researchers have to understand the battlefield. They must know not only how many of these pests plague a community, but also which specific genetic types are prevalent, where they gather at feeding time, where their resting places are, and where they lay the eggs that produce the next generation. "Where malaria has been controlled anywhere in the world, it has been done by controlling mosquitoes, not through vaccines or genetics or anything fancy," says entomologist Robert Gwadz of the laboratory of parasitic diseases at the U.S. National Institute of Allergy and Infectious Diseases in Bethesda, Maryland.

Despite having research budgets that are a tiny fraction of those spent to sequence the mosquito genome (see p. 92), several teams of entomologists are in active pursuit of the world's deadliest animal. They are beginning to characterize a dizzying array of genetic subtypes, learning what makes them dangerous—and vulnerable.

Because of its location between rainforest and dry sahel, Burkina Faso has perhaps one of the heaviest mosquito burdens in Africa. Indeed, malaria is so thoroughly entrenched here that public health leaders in the 1960s quietly left it and its neighbors out of so-called world eradication strategies. Even eliminating nine out of 10 of the insects in some parts of the country would make little dent in disease prevalence, says Carlo Costantini, an entomologist at the University of Rome who has worked in the region since 1991. In the capital city of Ouagadougou, along streets lined with vendors selling mangoes, pottery, car parts, and cell phone cards, one of the most common billboards is for a popular brand of insecticide. A spray can looms victorious over a dead mosquito, legs in the air. "Creatures of the good Lord?" the caption reads. "Too bad!"

Not far from one such billboard, and just off a dusty main street clogged with bicycles, mopeds, and vintage Renault taxis, is the National Center for Research and Training on Malaria (CNRFP). There, funded by the National Ministry of Health and grants from the Italian, Dutch, and Swiss governments, a team of eight researchers and technicians is sorting out the mosquito tangle. The group is led by N'Falé Sagnon, who grew up in Banfora in southwest Burkina Faso. Sagnon became interested in entomology as a university student in Algeria when he joined a project studying the black fly, which transmits river blindness. In 1999, he earned his Ph.D. at the University of Rome working with entomologist Mario Coluzzi and Costantini; he returned to the center in Burkina Faso the next year to build a team of entomologists.



Captured quarry. As part of a mosquito census, researchers collect mosquitoes from different sites in the village.

Sagnon's team, working with Edith Ilboudo-Sanogo of CNRFP, Costantini, and Nora Besansky of the University of Notre Dame in Indiana, is trying to determine the behavior and genetics of the region's malaria-transmitting mosquitoes. Burkina Faso's bug demographics are some of the most complex in Africa, Costantini says. Local mosquitoes have adapted so well to the seasonal variations that in many areas, instead of appearing just in the rainy season, they are year-round pests. *Culex* species are a nuisance but pose little threat. Several others are killers: *Aedes aegypti*, which can transmit yellow fever and dengue, and the malaria vectors *Anopheles gambiae* and *An. funestus*. Two genetically distinct forms of *An. gambiae* "proper" are present. (*An. gambiae* proper is one of the seven sister species researchers identified in the 1960s that make up the *An. gambiae* complex.) These are the Mopti (M) form, which is especially adept at exploiting human-created water sources such as irrigation ponds, and the Savanna (S) form, which takes advantage of rain-triggered water sources such as puddles (see p. 80). *An. funestus*, the second malaria vector in Burkina Faso, might also be a complex of several distinct genetic types,

Giant
Toxorhynchites brevipalpis


credit: L. Munstermann

Huge, with a wingspan of almost an inch, this species is also a Methuselah, living up to 4 months. Because its larvae devour the larvae of other species, such as Aedes aegypti, *it is used in biological control.*

Distribution: Tropical Africa.
Breeding habitat: Treeholes and plant axils; also cans, tires, and other human artifacts.
Feeding: One of the few species in which females don't need blood proteins to produce eggs; they feed on plant nectar instead.
Diseases: Cannot transmit pathogens because it doesn't bite.

Toxorhynchites brevipalpis

recent research at the center suggests.

Hoping to target the most dangerous malaria transmitters, the team is sorting out behavioral differences among the various populations. Although they look the same, the different forms have distinct preferences in where they lay their eggs as well as their relative taste for human or animal blood. And the competition between them—for water in which to lay their eggs, shady resting places, or blood meals—means that some vector-control measures that hit one form especially hard might give its competitor an unintentional boost, notes Costantini. "The more you know about your vector, the better you will predict how it will respond" to different control strategies, he says.

Where the streets have no name

Both *An. funestus* and the two forms of *An. gambiae* plague Goundri, half an hour northeast of Ouagadougou. An uninitiated driver could easily miss the turnoff for the village: a gravel slope off the paved road marked with a hand-lettered wooden sign. The rutted road winds through fields of millet, maize, and peanuts. Scattered over 2 square kilometers are more than 500 mud-brick and thatch-roof huts clustered into about 100 compounds. Goundri looks like thousands of other villages in central Burkina Faso, with one exception: Every structure, from the concrete-brick mosque to the simplest grain storage hut, has a large blue number spray-painted on its side—especially striking in a region where street numbers are rare. The numbers are courtesy of geographers from CNRFP, who last year used geographic information systems (GIS) data to map the precise location of every building. The GIS map is proving invaluable as the researchers collect mosquitoes from various locations and attempt to learn whether different genetic types have special preferences for residing indoors or outdoors, near water or near animal enclosures.

The tools for a mosquito census are fairly simple: a few long-handled dippers for collecting eggs and larvae, a type of modified Shop-Vac for capturing adults, paper cups, and a cooler filled with ice. Inside huts, team members search the walls and any protected corners for quarry, which are plentiful. The folds of hanging clothes swarm with mosquitoes when a visitor brushes up against them. The Shop-Vac—a hand-held vacuum fitted with a long nozzle and a net-covered cup instead of a dust bag—whirs as the researcher collects a dozen mosquitoes in a single pass. The paper cups filled with whining bloodsuckers are labeled with the collection time and place and then transferred to the cooler. In 10 minutes, the disarmingly fragile insects are dead.

Back in the lab, team members dissect the mosquitoes. Nothing from the tiny bodies goes to waste. Tests on the salivary glands determine whether the insects were carrying plasmodia, the parasites that cause malaria. The gut is used to determine whether the last blood meal was from a human, cow, goat, or other animal. Polymerase chain reaction (PCR) analysis on the rest of the body can determine whether the insects are M or S forms.

Over the last decade, mosquito censuses in Goundri and a half-dozen similar villages around the country have revealed a consistent pattern: At the beginning of the rainy season in July, the *An. gambiae* M form is most prominent—not surprising, perhaps, for a subtype that prefers relatively permanent bodies of water. A few months later, as the constant rains raise the water table and increase the number of temporary puddles, the S form's population increases. As the rainy season ends in October, *An. funestus* begins to appear in irrigated rice fields and artificial reservoirs, transmitting malaria even through the hot, dry days of April.

Tiny microbiologists

The availability of open water is what distinguishes those periods, and the researchers suspect that differences in water use are driving the genetic subgroups apart—a step toward separating into distinct species. "The larval stage is where competition [between mosquitoes] is felt most keenly" and therefore is a focus for speciation, says Notre Dame's Besansky.

The hunt. Researchers use a hand-held vacuum (*top*) to capture dozens of mosquitoes in their daytime resting places (*bottom*).

CREDITS: (MIDDLE AND BOTTOM) G. VOGEL/*SCIENCE*

The eggs' fate might depend on whether they are laid in a temporary or permanent pool, but how does the female mosquito distinguish between the two? The Ouagadougou team has found some early evidence that bacterial signals might be key. Wamdaogo Moussa Guelbeogo, a Ph.D. student at the University of Ouagadougou, collected water samples from long- and short-lived pools and cultured the bacteria in each. He and his colleagues identified 12 strains of bacteria, several of which were exclusive to either longer term or ephemeral pools.

To test female *An. gambiae*, he placed a single mosquito that was ready to lay her

300 or so eggs in a net cage containing shallow bowls of water seeded with bacteria—those typical of either temporary or more permanent ponds. When given a choice between the bacterial cocktail from temporary or permanent pools, the M-form females, he found, preferred to lay their eggs in the permanent one. S-form mosquitoes, on the other hand, preferred the temporary-pool bacteria. And when faced with only one option, the M-form females exhibited distinctive behavior. When they sensed bacteria from a permanent water source, they were more likely to lay all their eggs at once. But when they were given water laced with bacteria from a shallow pool, the insects tended to lay their eggs in several batches—perhaps ensuring that even if one water source dried up quickly, another might last long enough for the eggs to develop fully. "The mosquitoes are better at identifying bacteria than most microbiologists," says Coluzzi.

Permanent accommodations. Lakes such as this one outside Ouagadougou provide year-round habitats for mosquito larvae, one reason why the mosquito burden is so high.

In the short term, such knowledge might be useful for building effective mosquito traps—both for research and for vector control, says Guelbeogo (see p. 90). In the long term, he says, researchers might be able to develop a genetically altered strain of bacteria that could both attract egg-laying females and produce a toxin that would kill developing larvae. The idea is futuristic, Costantini admits, but he points out that *Bacillus thuringiensis israelensis*, a bacterium that releases toxins that are safe for humans but lethal to mosquitoes, is already in use as an antilarval pesticide.

Cellular bar codes

This year, CNRFP entomologists are targeting the more elusive *An. funestus*. Relatively little is known about the species, first described at the beginning of the 20th century, except that it is not particularly research-friendly. It grows poorly in captivity, rarely reproducing for more than four generations. Its larvae are extremely difficult to capture: Females prefer to lay their eggs in water with plenty of vegetation to provide hiding places, and as soon as a shadow passes over their pool, the larvae dive for the bottom and remain hidden for up to 30 minutes.

Costantini and Sagnon have preliminary evidence that this species, too, is really two distinct genetic subtypes, which they have dubbed Folonzo and Kiribina for two of the villages where the studies were done. Like the *An. gambiae* subtypes, the two potential subspecies look exactly the same even to an expert. And researchers do not yet have PCR-based markers that can distinguish between the two, as they do for the *An. gambiae* subtypes. Experts can identify different types of *An. funestus* by examining light and dark patterns in oversized chromosomes, which under the microscope resemble a sort of cellular bar code. Like M and S, Folonzo and Kiribina are characterized by specific chromosomal inversions: regions where the band pattern is flipped.

In mosquitoes, these polytene chromosomes appear only in certain cells in the maturing ovary. To isolate them, researchers must capture "semigravid" females: insects that have digested half of their blood meal, usually about 18 hours after feeding. Males—or females that have not recently fed—are useless for genetic typing.

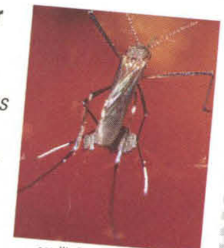
The team hopes that will soon change. Besansky and her colleagues in Indiana are working to identify DNA markers that correspond to the chromosomal inversions. If they succeed, researchers will be able to identify all collected mosquitoes by genetic type, not just the recently fed females. That will make it possible for the first time to analyze patterns of distribution of the two genetic types throughout their life cycle. Unfortunately, *An. gambiae* and *An. funestus* have diverged enough through evolution that this week's completed *An. gambiae* genome is unlikely to help the *An. funestus* effort, Besansky says.

The early chromosome-based evidence suggests that gene flow is restricted between the Kiribina and Folonzo types—even among *An. funestus* populations living in the same village or the same hut. "We're not saying they're different species, but the chromosomal markers we're using are not mixing the way they would if they were completely interbreeding," Besansky says. And Sagnon and Guelbeogo's latest work suggests that the Folonzo type might be more involved in malaria transmission. Those mosquitoes are more likely than their Kiribina cousins to have bitten humans and to be found indoors—a ripe target, perhaps, for public health's best weapons.

–GRETCHEN VOGEL

Protective mother
Trichoprosopon digitatum

Females lay their eggs in rafts and guard them until they hatch, up to 48 hours later.



credit: P. Lounibos/UF/FMEL

Distribution: From Central America to Brazil.
Breeding habitat: Plant-held water reservoirs, such as this empty cacao husk, and human-made containers.
Feeding: Various mammals, including rabbits, mice, and humans.
Diseases: May transmit several viruses, including Venezuelan equine encephalitis and St. Louis encephalitis.

Trichoprosopon digitatum

CREDIT: (TOP) G. VOGEL/SCIENCE