INFECTIOUS DISEASE

Fungi on the March

A strange fungal disease in Canada and the U.S. heralds a new threat to human health

By Jennifer Frazer

IN BRIEF

An airborne yeast that was found to have sickened apparently healthy people on Vancouver Island in British Columbia in 2001 became the first known human fungal pathogen to suddenly become more virulent in a place where the organism was previously unknown.

Determining where the yeast came from proved difficult. Eventually researchers realized that it was hiding out in surprising locations and that its emergence as a cause of serious illness in British Columbia may well have been prompted by climate change and land development.

The outbreak is expected to continue. Better diagnostic tools could increase preparedness. Stronger oversight of plant and animal transport across borders, as well as the development of more effective antifungal drugs and vaccines, also could help.

Photograph by Zachary Zavislak

FUNGUS
Cryptococcus gattii grows as cream-colored colonies on a laboratory plate.

Jennifer Frazer is a freelance science writer and blogger at The Artful Amoeba for the *Scientific American* Blog Network. She has written for *Nature*, Grist and *High Country News* and has received an AAAS Science Journalism Award.

IN 2001 DEAD PORPOISES WITH YEAST-PACKED LUNGS WASHED UP ON THE SOUTHEASTERN shore of Vancouver Island in British Columbia. The bloated organs were several times normal weight, with barely any room for air. The island's veterinarians had never seen anything like it. Cats and dogs there were having trouble breathing, too. In cats, the disease could cause a particularly gruesome symptom: weeping holes, produced when a yeast infection ate its way through the skull. At the same time, a few people on the island, located off Canada's Pacific Coast, also began falling ill with an unknown respiratory malady. They coughed constantly, their energy sapped, their sleep stolen. Chest x-rays revealed ominous lung or brain nodules. Biopsied tissue, however, proved the culprit to be not cancer but yeast.

Despite their varying symptoms, the pets, porpoises and humans all shared a single tormenter: *Cryptococcus gattii*. This fungus had never been seen on the island before, nor was it known to survive outside the tropics and subtropics. Now it was present in the environment, although no one knew where it had come from or how long it had been there. Most worryingly, no one knew how many would be sickened or how far the upstart yeast might travel.

There was good reason for concern.

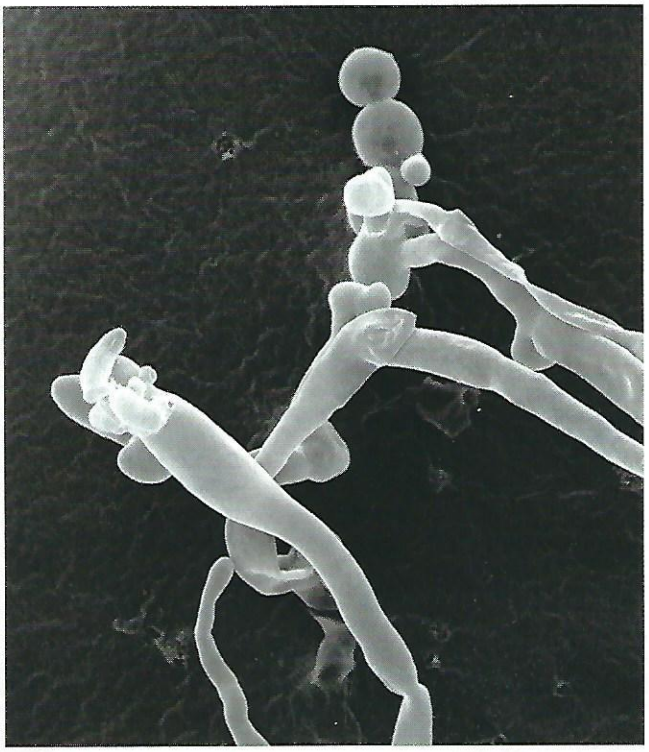
Fungi have long plagued plants—famously felling the towering elm and chestnut trees of the eastern U.S. and beyond. More recently, fungal epidemics have become alarmingly common among animals. From ponds in South America where frogs' fungus-clogged skin stops their heart to caves in the eastern U.S. where moldy, shivering bats drop pitifully from the ceiling, pathogenic fungi are running amok. Historically the fungi that infect humans have been known more for inspiring laughably bad commercials about trifling but irritating skin infections than for making people desperately ill. Our formidable immune system and torrid body temperature, too high for most fungi to tolerate, ensured that people in good health generally shook off serious attacks.

There were a few exceptions: in the U.S., inhaled diseases such as valley fever in the Southwest and histoplasmosis in the Midwest and Southeast have long quietly stalked healthy people. For reasons not completely understood, valley fever exploded eightfold within its usual range between 1999 and 2011. In recent decades fungal infections have also unsurprisingly surged as the immune systems of millions of people became impaired by infection with HIV or by immunosuppressive drugs given to protect transplanted organs or to treat disease. When defenses are down, pathogens will thrive. Overall, however, fungal attacks affecting many healthy people at once have been rare and largely caused by fungi within their usual ranges as they encountered favorable environmental conditions.

C. gattii is different. Until it emerged on Vancouver Island, it had occasionally sickened healthy people elsewhere but had never before caused an outbreak—a burst of unexpected infections. Its appearance in Canada seemingly also marked a jump into new territory with a much cooler climate, where the microorganism had inexplicably become more harmful. Between the outbreak's start and the end of 2012, 337 British Columbians were reported infected, of which two thirds were Vancouver Island residents, says Eleni Galanis, an epidemiologist at the

British Columbia Center for Disease Control. And by 2005 *C. gattii* had started making people sick farther south, in the U.S. Pacific Northwest. Since then, at least 100 people in that area have been infected, and 25 to 30 percent of them have died. "It's a fairly high mortality rate for an environmentally acquired fungus," says Joseph Heitman, director of the Center for Microbial Pathogenesis at Duke University. For the most part, although these are not AIDS patients, about half had weakened immune systems from prescribed drugs or illness, and many of the rest had common ailments that can weaken immune systems to a lesser extent, such as diabetes, or lung, kidney or heart disease. But 20 percent or more were healthy prior to infection. "Many of these patients were completely healthy, spending a lot of time outdoors, and suddenly they were very ill," Heitman adds.

Today the *C. gattii* outbreak is giving every indication that it will continue to move south. Immunologist Arturo Casadevall of the Albert Einstein College of Medicine thinks the yeast—fungi growing as single cells instead of long filaments—will ultimately reach Florida. Indeed, the events in British Columbia and the U.S. Pacific Northwest constitute a landmark in the history of human disease: the first known outbreak of a disease caused by a fungus that had suddenly and unexpectedly evolved markedly increased virulence. And another first—it did so in a place where the organism was previously unknown. The *C. gattii* story thus raises a disturbing prospect: healthy humans can no longer assume they are immune to life-threatening outbreaks of newly virulent fungi. Indeed, as global temperatures rise, we may be inviting more.



REPRODUCTION: A few sausage-shaped *C. gattii* spores cluster around the club-shaped structure that produced them (*left*). The spores grow in long chains; here the chains have been disrupted.

COURTESY OF DEBORAH J. SPRINGER AND JOSEPH HEITMAN *Department of Molecular Genetics and Microbiology, Duke University Medical Center*

EVERYWHERE AND UNSTOPPABLE

THESE INSIGHTS WERE STILL FAR IN THE FUTURE on Vancouver Island in June 2001, where public health officials were about to be blindsided. For disease investigator Murray Fyfe, then at the British Columbia Center for Disease Control, the first sign that something was amiss was a call from the provincial veterinarian informing him of an unusual increase in *Cryptococcus* infections among dogs and cats on the island. Local physicians confirmed a similar rise in human cases, and tests indicated that the culprit was not the usual *Cryptococcus neoformans* but a different fungal species—*C. gattii*. The team raided the center's culture collections to see if *C. gattii* had been infecting people on Vancouver Island all along and had simply been misidentified as *C. neoformans*. That turned out to be the case for some infections starting in 1999 but not in any years previous to that.

Fyfe, now a medical health officer at the Vancouver Island Health Authority, took several approaches to pinpointing where the fungus was hiding. In one line of attack, he assembled a team of investigators to look for new cases on the island and throughout British Columbia. The team interviewed patients and owners of infected pets, detailing symptoms and looking for commonalities and risk factors, such as previous illnesses or travel, and even investigating whether victims had eucalyptus trees in their yards. The fungus had been found living on such trees in Australia, where people had long been sporadically infected with *C. gattii*. They plotted cases on a map. They compared patients with individuals who did not get sick to see if any differences or trends emerged, a technique called a case-control study.

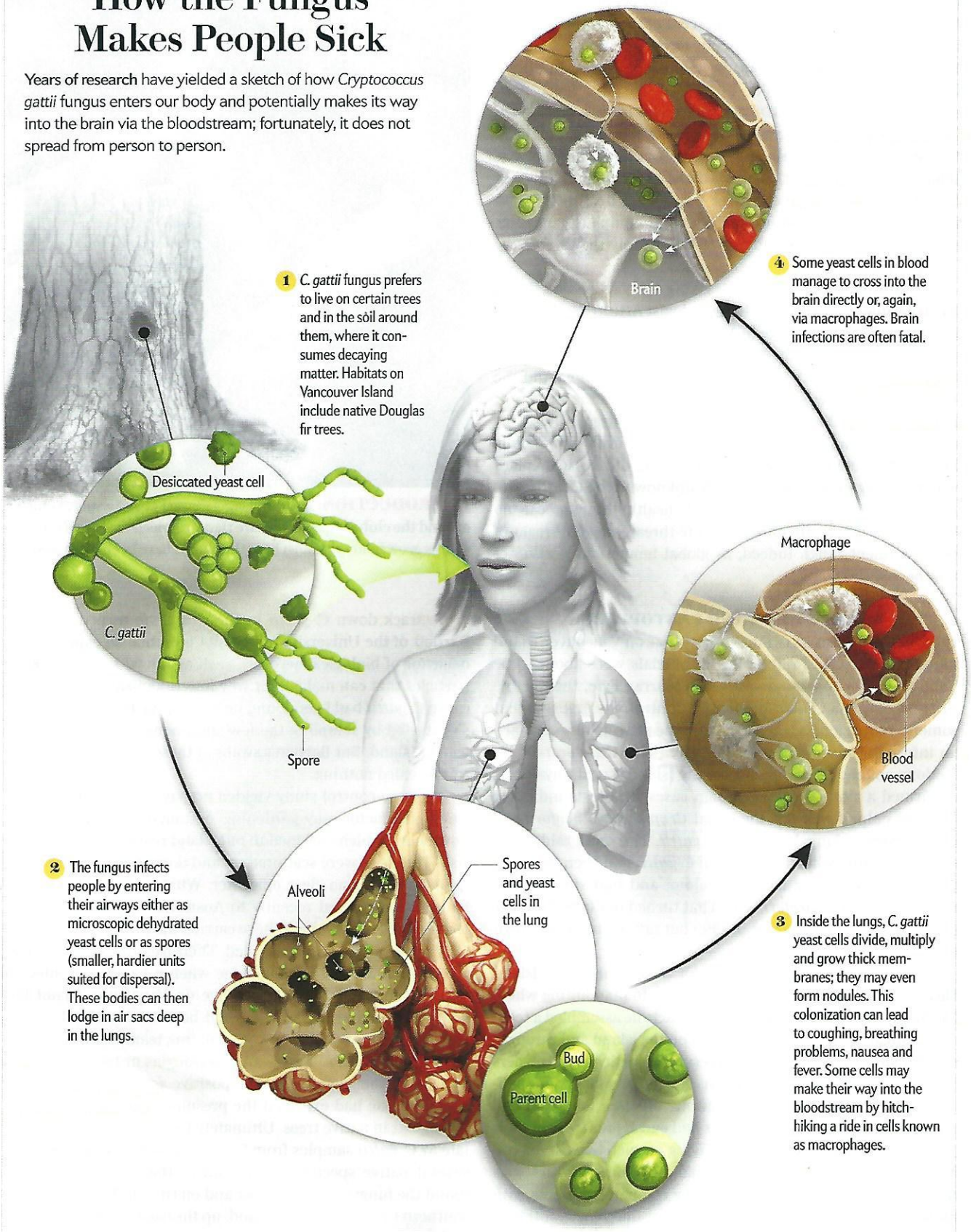
To track down *C. gattii* in the wild, Fyfe turned to Karen Bartlett of the University of British Columbia, an expert in the behavior of biological aerosols, such as fungal spores or other particles that can make their way into organisms' airways. Because *C. gattii* had been found on eucalyptus trees in Australia, she started by sampling the few specimens that grew on Vancouver Island. But Bartlett's swabs of these and other trees and soil revealed nothing.

The case-control study yielded no pay dirt, either. No environmental factor—say, gardening, cutting down trees or laying down bark mulch—seemed to put island residents at increased risk. The cases were scattered up and down the eastern side of the island with no clear epicenter. What is more, the victims had not all traveled recently to Australia or any other exotic locale, whence they might have carried the fungus.

The researchers were stymied. Their frustration dragged on for six months. Their break came when a handful of infected patients surfaced who did not live on Vancouver Island but who had traveled there. A few had been to the island's Rathtrevor Beach Provincial Park. A sampling team was dispatched. At last, one of the samples—from a Douglas fir tree, a common species in the vicinity—tested positive for *C. gattii* in early 2002. No one had expected the presumed exotic fungus to be hiding out in native trees. Ultimately Bartlett's team would isolate 57 *C. gattii* samples from 24 trees in the park belonging to several native species. By the end of that summer she had found the fungus in soil and air and on trees in Victoria, on the southern tip of Vancouver Island, up the eastern coast to Campbell River, and west to the central island. It was the same area

How the Fungus Makes People Sick

Years of research have yielded a sketch of how *Cryptococcus gattii* fungus enters our body and potentially makes its way into the brain via the bloodstream; fortunately, it does not spread from person to person.



1 *C. gattii* fungus prefers to live on certain trees and in the soil around them, where it consumes decaying matter. Habitats on Vancouver Island include native Douglas fir trees.

2 The fungus infects people by entering their airways either as microscopic dehydrated yeast cells or as spores (smaller, hardier units suited for dispersal). These bodies can then lodge in air sacs deep in the lungs.

3 Inside the lungs, *C. gattii* yeast cells divide, multiply and grow thick membranes; they may even form nodules. This colonization can lead to coughing, breathing problems, nausea and fever. Some cells may make their way into the bloodstream by hitchhiking a ride in cells known as macrophages.

4 Some yeast cells in blood manage to cross into the brain directly or, again, via macrophages. Brain infections are often fatal.

Illustration by Emily Cooper

in which most of the population of Vancouver Island lived. Probably everyone would be or had already been exposed, and there was nothing that could be done about it.

More worrying news followed. Data collected between 2002 and 2006 eventually showed that the infection rate on Vancouver Island was 27.9 cases per million in the population—three times higher than the rate among humans in tropical northern Australia. North Americans could well have a more virulent pathogen on their hands, although a lack of previous exposure to the fungus might also explain the pattern; if the fungus was a new arrival, few if any people in the area would have encountered it and built up an immunity to it earlier in life. Tests performed on the organism were also unsettling. It could survive in freshwater. It could survive in saltwater. It could survive in air. It could survive for years in mud on shoes. Parallel work showed that *C. gattii* was on the move. Cases began appearing on the mainland of British Columbia in 2004 among people who had never visited Vancouver Island. Modeling showed that the organism preferred warm winters, low elevations and dry conditions. Points south seemed to offer fertile ground. In February 2006 an older man with leukemia taking immunosuppressive steroids who lived in the U.S. San Juan Islands off the coast of Washington State came to his doctor with a cough. His chest x-ray revealed a nodule; it was *C. gattii*. Genetic analysis by Heitman in collaboration with Kieren Marr, a physician-scientist then at the Fred Hutchinson Cancer Center in Seattle, revealed that the fungus was indistinguishable from a Vancouver Island strain. Although the man lived within a few kilometers of the Canadian maritime border, he had never traveled to Canada. The fungus had come to him.

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WHY NOW?

ALTHOUGH RESEARCHERS realized by the mid-2000s that little could be done to stop the spread of the *C. gattii* outbreak in North America, they still wondered how long the fungus had been in British Columbia and the U.S. Northwest, where it came from and what caused it to suddenly start making so many people sick. They found clues by analyzing its DNA.

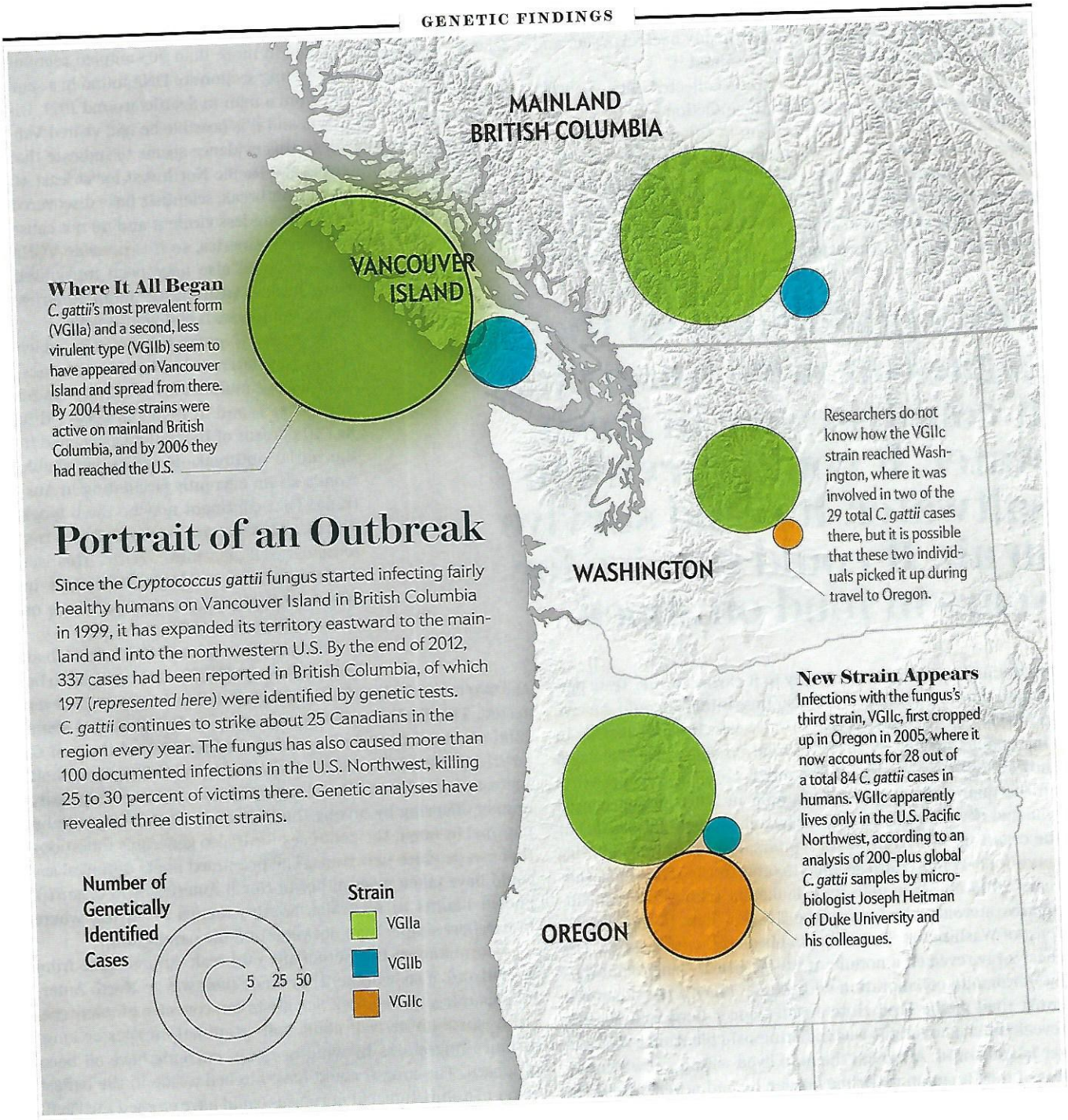
The genetic work revealed that the fungus may have been in the vicinity of Vancouver Island for up to several decades before 1999, Heitman says. The sequence of DNA "letters" in the major type of *C. gattii* ultimately found on the island—called VGIIa and responsible for 90 percent of infections on Vancouver Island—is indistinguishable in more than 30 sampled sections of DNA from the corresponding sections of DNA found in a sample of sputum collected from a man in Seattle around 1971. His travel history is unknown, and it is possible he had visited Vancouver Island. Regardless, this evidence seems to indicate that VGIIa has been present in the Pacific Northwest for at least 40 years. In the years since the outbreak, scientists have discovered that varieties of *C. gattii* that are less virulent and do not cause outbreaks also occur in North America, so it is possible VGIIa evolved there. But *C. gattii* could also have been introduced from Africa, Australia or South America, where the species is endemic as well.

A second *C. gattii* type—VGIIb, which researchers later realized had begun causing illness simultaneously with VGIIa on Vancouver Island in 1999 and occurred in just 10 percent of the patients sickened in the initial outbreak—is indistinguishable from a strain currently circulating in Australia. That continent may be the b type's source. Oregon now harbors these two forms as well as a third: VGIIc. This last strain appeared suddenly in Oregon in 2005, although whether it arose there or elsewhere is unknown.

Worryingly, in mice at Heitman's lab at Duke, samples of VGIIa and c proved to be the two most virulent forms of *Cryptococcus* the group had ever tested. This finding and others suggest to Heitman that some kind of sexual reproduction among undetermined types of *C. gattii* generated the VGIIa and c strains and bestowed their increased virulence. Sexual reproduction promotes diversity among offspring by mixing the DNA of parents in new combinations. In fungi, the act of sex itself also generates mutations that can produce new traits. The presumed bouts of fungal sex could have taken place either in North America or in *C. gattii*'s known haunts in Australia, South America or Africa, where researchers simply have not yet found the parent fungi.

Also unknown is whether the outbreak strains came from abroad and, if so, whether they made their way to North America separately or together. Scientists can conceive of many scenarios for their arrival: plant, soil or animal imports or long-distance migrations by wind or ocean currents have all been proposed. The fungus could have hitched a ride in the ballast water of a ship. Infected porpoises could have crossed the Pacific on their own and released the organism to the soil or to scavengers when they died and their corpses washed ashore. Mud on vehicles or footwear could have carried the fungi from one place to another. The trip or trips that brought the fungi to North America may have taken place 10,000 years ago—or 43 years ago.

There is at least one clue, however, to how long these fungi have been in the Pacific Northwest. Fungi that have been in one place for a long time tend to diversify genetically. The three types of *C. gattii* causing outbreaks in the region are clonal—all the fungi of a given type, be it a, b or c, are genetically quite similar. "If it's been there 100,000 years, then you'd expect to find a lot more diversity in the population, and you



don't see that," Heitman says. "From my perspective, that may be the strongest case that maybe [these fungi] really [were] brought there 50 years ago or 70 years ago or 100 years ago" and not thousands of years earlier.

As for why *C. gattii* caused no outbreaks until recently despite being in the vicinity for at least 40 years, one possibility is climate change. The average temperature on Vancouver Island increased by a degree or two Celsius during the past 40 years, Bartlett says. That "doesn't sound like much, but it can be a huge difference to microorganisms," she adds. The years 1991, 1993, 1994, 1996 and 1998 all had above-average summer temperatures for Vancouver Island. Warmer temperatures may have allowed a subtropical organism that was previously teetering on the edge of survivability there to thrive. As the planet warms, Casadevall says, existing pathogenic fungi that like heat could extend their ranges into formerly inhospitable habitats. Indeed, plant-pathogenic fungi have already been found to be moving toward the poles in response to climate change at the particularly brisk clip of about seven and a half kilometers a year since 1960. Meanwhile hotter climates may encourage other fungi to evolve tolerance to warmer temperatures. The complex fungal genome—which is larger than that of bacteria and

SOURCES: "CRYPTOCOCCUS GATTII IN THE UNITED STATES: GENOTYPIC DIVERSITY OF HUMAN AND VETERINARY ISOLATES," BY SHAWN R. LOCKHART ET AL., IN *PLOS ONE*, VOL. 8, NO. 9, SEPTEMBER 3, 2013 (*Oregon and Washington*); BC CENTER FOR DISEASE CONTROL (*Vancouver Island and British Columbia mainland*)

Map by XNR Productions; Graphic by Jen Christiansen

viruses—gives its owners a variety of stress-response tools for adapting to heat that viruses and bacteria may lack. Even slight increases in heat tolerance might allow fungi that were on the cusp of pathogenicity to tolerate our high body temperatures and to proliferate, instead of dying, once they invade. If such scenarios do occur, it would be bad news for humans, given our reliance on body heat as a pillar of our fungal defense.

In addition to the warming climate, the late 1990s saw increased development on the eastern side of Vancouver Island. Forests were logged, an expressway was extended, soil was stirred up and subdivisions were built. Stirring up soil and cutting down trees could have released an organism from a previously small niche into the wider world, Bartlett points out. Emergence of *C. gattii* as a pathogen in that region may have resulted from a serendipitous confluence of factors: several years of warmer winters and drier summers; soil disturbance; and an area popular among both mobile tourists and retirees, who tend to be more susceptible to infection than younger people.

PORTENTS

TO A GREAT EXTENT, we have ourselves to blame for the growing menagerie of pathogenic fungi menacing plants, animals and people—and not only because we have had a large hand in climate change. Many fungi have a home range. Humans have relentlessly helped them escape those homes through international trade. Trade most likely brought the Irish Potato Famine to Europe, chestnut blight to North America and skin-infecting chytridiomycosis to amphibians worldwide. Our shipping addiction has created a de facto fungal dating service. Fungi, as a group, are enthusiastic sexual beings. When humans bring together fungi that were previously separated by geography but still able to mate, the resulting hookups can produce new and more virulent variants that are suddenly able to infect organisms that their ancestors could not or to thrive in new environments. Continuing or increasing trade will boost the odds both of existing fungal pathogens being introduced to naive hosts and of novel pathogens being created via fungal dalliances.

Although very little can now be done to stop *C. gattii*'s spread, we can take steps to reduce the chance that fungi will race through and wreak havoc on unsuspecting populations and to be better prepared when an outbreak occurs. A good place to start would be to improve monitoring and diagnosis of fungal diseases. Because fungal diseases are uncommon in healthy people, doctors may not test for them, leading to a delay in diagnosis and more intractable symptoms when treatment begins. Meanwhile the available diagnostic techniques for many fungi lack specificity or sensitivity, or both, and may be too expensive in impoverished countries. The World Health Organization has no fungal infection program, and few public health agencies, with the exception of the U.S. Centers for Disease Control and Prevention, currently monitor fungal infections.

Another line of defense would be heightened plant and animal biosecurity. Because human pathogens often also live in soil and on plants, increased oversight of the entry of such materials into our country—testing shipments of agricultural products or animals for fungi known to be important human pathogens, for instance, or being more diligent in airport customs about cleaning muddy shoes or outdoor equipment or preventing import of plant material by international travelers, as countries such as Australia and New Zealand do—could not only fight disease outright but also reduce the risk of illicit fungal sex.

More money could be invested in developing new and improved antifungal drugs. A major hurdle is our relatedness to fungi—we are practically first cousins in the tree of life. Fungi and animals parted ways more recently than any other major group of organisms. That kinship not only makes yeast excellent models for mammalian biology but also makes yeast and other fungi more challenging to treat when they infect animals. "The fact that they're [so closely related] is a huge problem because much of their machinery is shared with our own," Heitman says, "so it's harder to find unique targets for antifungal drugs." The drugs we do have are often only modestly effective in reducing deaths from invasive fungal disease, can produce toxic side effects or may interact badly with other drugs. Few new antifungal agents are in the pipeline. Antifungal vaccines offer another line of defense. Few have made it to clinical trials, however, and none are currently medically available for humans. Developing fungal vaccines would be a good insurance policy should the worst come to pass and could help many who are already at increased risk of fungal disease.

The story of *C. gattii* has an ominous footnote. VGIIa and b have now both spread to Oregon, but VGIIc first emerged there in 2005, before the Vancouver Island VGIIa and b had even appeared in the U.S. Extensive tests on samples from Vancouver Island show VGIIc has never been seen there. Genetic analysis of the Oregon VGIIc strain clearly shows it is not the offspring of a simple mating between VGIIa and b. These observations suggest not one outbreak of new hypervirulent *C. gattii* in the Pacific Northwest but two.

"This looks like an outbreak within an outbreak, which may be of independent origins," Heitman wrote in a recent report to the Institute of Medicine. "It is as though two pebbles have been dropped into a pond at different times, one earlier than the other, and they have generated concentric waves that are now expanding outward and intersecting." In other words, the nearly simultaneous outbreak of VGIIa and b on Vancouver Island and of VGIIc in Oregon may be an astounding coincidence, although perhaps both were favored by the same conditions. Such outbreaks were not known before 1999, and now two had occurred within just seven years. This revelation underscores one of the few certainties in the tale of coughing humans, molding bats and blighted trees: underestimating the fungal talent for migrating and thriving on new hosts in our warming and shrinking world is a very bad bet. SA

MORE TO EXPLORE

Global Warming Will Bring New Fungal Diseases for Mammals. Monica A. Garcia-Solache and Arturo Casadevall in *mBio*, Vol. 1, No. 1; April 2010.

Sexual Reproduction, Evolution, and Adaptation of *Cryptococcus gattii* in the Pacific Northwest Outbreak. Joseph Heitman, Edmond J. Byrnes III and John R. Perfect in *Fungal Diseases: An Emerging Threat to Human, Animal, and Plant Health.* National Academies Press, 2011.

Hidden Killers: Human Fungal Infections. Gordon D. Brown, David W. Denning, Neil A. R. Gow, Stuart M. Levitz, Mihai G. Netea and Theodore C. White in *Science Translational Medicine*, Vol. 4, No. 165; December 19, 2012.

SCIENTIFIC AMERICAN ONLINE

Learn more about the how fungi have evolved to elude our immune systems at ScientificAmerican.com/dec2013/fungal-infection