



Acquired inheritance. Instead of carrying eggs on their legs, Kammerer's midwife toads and their offspring started laying eggs in water.

HISTORY OF SCIENCE

The Case of the Midwife Toad: Fraud or Epigenetics?

Paul Kammerer has been called the perpetrator of one of the most celebrated scientific frauds of the early 20th century. He has also been defended as the victim of forgery by a lab assistant; some even say he was framed by political or scientific opponents. Now, a new analysis published this week suggests that his infamous experiments may have been the first demonstrations of a recently recognized phenomenon: epigenetics.

The story starts in the early 1900s. Kammerer, an Austrian biologist, argued strongly in favor of the Lamarckian view that traits acquired during an organism's lifetime could be passed on to future generations. He claimed to have observed Lamarckian inheritance in various organisms, including salamanders and tunicates, but his most publicized evidence came from the midwife toad, *Alytes obstetricans*.

Most frogs and toads mate in water and lay their eggs in aquatic environments. But midwife toads are landlubbers. Males and females copulate on dry land, and males subsequently wrap the strings of eggs around their legs, carrying them around until the embryos are ready to emerge as tadpoles.

Toads that mate underwater have special colored calluses on their forelimbs, called nuptial pads, that enable them to grasp a wet, slippery female. Given their terrestrial preferences, midwife toads lack nuptial pads—at least they did when Kammerer started his experiment.

He confined the toads to a dry, overheated environment, driving them to mate and lay their eggs in water. Most of the eggs died, but

the 3% to 5% of offspring that survived had lost the terrestrial habits of their parents. Even in cool, moist environments, they opted to mate and deposit their eggs in water, a preference that Kammerer said persisted for at least six generations, the amount of time Kammerer studied them.

Moreover, by the third generation, Kammerer reported a thickening on the forelegs that, two generations later, was a bona fide nuptial pad. Other traits useful for an aqueous existence appeared and became more pronounced: Eggs developed thicker jelly coats and reduced quantities of yolk; tadpole gills expanded in size.

Finally, when Kammerer bred the “water” toads with untreated toads, he saw these “water” traits appear in the proportions one would expect for Mendelian inheritance. According to science historian Sander Gliboff of Indiana University, Bloomington, Kammerer thought new genes were forming to pass on these traits.

Some of Kammerer's colleagues were aghast, and he came under attack from giants of the scientific establishment. The final blow came in 1926 when herpetologist G. Kingsley Noble of the American Museum of Natural History in New York City examined the last remaining specimen of Kammerer's “water” toads and noticed that India ink had been

injected into the toad's forelimbs. “We have proved conclusively that no pads are present,” Noble wrote in the 7 August 1926 issue of *Nature*. “Whether or not the specimen ever possessed them is a matter for conjecture.” Noble and his colleagues also discounted Kammerer's photos of pads and stained sections, arguing that they could not be verified as belonging to midwife toads.

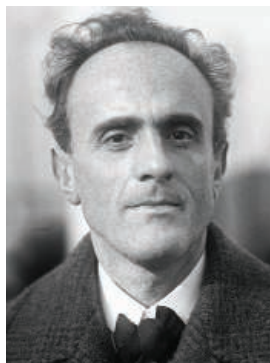
Less than 2 months later, Kammerer killed himself in the Austrian mountains. Some say he committed suicide because he was discredited; others blame unrequited love. Whatever the cause, Kammerer continued to spark controversy long after he was gone. A book about the case by Arthur Koestler, a TV documentary, and a Soviet movie all portrayed him as a victim. Kammerer is “either cited as one of the paradigms of scientific fraud, how a brilliant person can go astray, or by others as a hero for any form of antiestablishment cases in the sciences,” says Günter Wagner of Yale University.

Now comes Alexander Vargas, an evolutionary developmental biologist at the University of Chile in Santiago, who has taken a close look at the case from a 21st century perspective. In the *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, released on 3 September, he argues that Kammerer's experiments show signs of what are now well-known epigenetic effects. “There are potential mechanisms that can explain these observations that weren't

available at that time,” says Azim Surani, a developmental biologist at the University of Cambridge in the United Kingdom. Adds Vargas: It suggests “a tragic case of scientific incomprehension.”

Acquired traits can seem to be inherited because they result from chemical modification of DNA that can be passed on—even enhanced—in subsequent generations. The sequence stays the same, but the addition or removal of methyl groups silences certain genes. These changes are the guts of epigenetics.

Vargas points out that Kammerer also noticed a “parent of origin” effect in which a trait tends to appear only if it's passed on by the right parent. In Kammerer's experiments, if the father was a “water” toad, then 100% of the first generation and three-quarters of the next generation were “water” toads as well. But if the father retained the toad's terrestrial habits, then the reverse was true. This odd observation “only complicated the scenario for Kammerer, increasing suspi-



Victim of his time? Austrian biologist Paul Kammerer was discredited for work that may not have been wrong after all.

cions about his data,” Vargas writes. Similar effects have been noted in the inheritance of coat colors in mice, which have been ascribed to epigenetic changes in a gene variant called *Agouti variable yellow*, Vargas notes.

Vargas suggests that alterations in the jelly coat around the eggs of Kammerer’s toads could lead to abnormal methylation of some genes. All vertebrates have such a coating, and its removal just after fertilization can lead to a reduction in DNA methylation in very early embryos, Vargas points out.

Altered methylation patterns affect body size in mammals, particularly hybrids, and parent-of-origin effects influence egg size in

birds and may likewise have led to the large water toads and small eggs. “Kammerer could be the true discoverer of non-Mendelian, epigenetic inheritance,” Vargas concludes. And the intensification of these traits over subsequent generations could be a reflection of ever-increased amounts of methylation—akin to the increased darkening of mice carrying a more heavily methylated *Agouti variable yellow* gene.

Vargas suspects that epigenetics brought out the water traits and that the nuptial pads were a rare recessive trait connected with traits that enabled that small percentage of midwife toads to survive a water gestation. Eventually, two survivors, each with a recessive gene for

nuptial pads, mated, causing the pads to appear.

Vargas doesn’t explain the India ink that ultimately led to Kammerer’s undoing. Koestler and others have ascribed it to an overzealous lab assistant or even Kammerer’s scientific or political opponents.

Gliboff is not completely swayed by Vargas’s arguments. “It’s still hard to see Kammerer as anything but a failure as a scientist,” he says. But Surani’s curiosity is piqued. “It would be extremely interesting if someone did really try to repeat [Kammerer’s] experiment,” he notes. “I wouldn’t be surprised if he turned out to be right.”

—ELIZABETH PENNISI

HIV/AIDS RESEARCH

Potent HIV Antibodies Spark Vaccine Hopes

If HIV/AIDS researchers had a wish list, at the very top would sit a vaccine that could teach the body to make potent antibodies against the many strains of the virus. Despite 25 years of effort, no such vaccine is in sight, but now they are a step closer. A large team of researchers has identified the most powerful, broad-acting antibodies yet against multiple strains of the virus.

Finding good antibodies is a far cry from developing a vaccine that prods the immune system to produce them. But “broadly neutralizing antibodies” (bNAbs) are rare: Researchers have identified only a half-dozen to date. Now an international group funded mainly by the International AIDS Vaccine Initiative (IAVI) has discovered two new ones that have an unusual potency. “This has actually made me quite optimistic—for once,” says Dennis Burton, an immunologist at the Scripps Research Institute in San Diego, California, who led the research effort.

For many years, Burton says, he thought that if an antibody had a broader reach, it inevitably would be weaker. “I wondered whether there would be any antibody better than the ones we had,” he says. “Well, these are.”

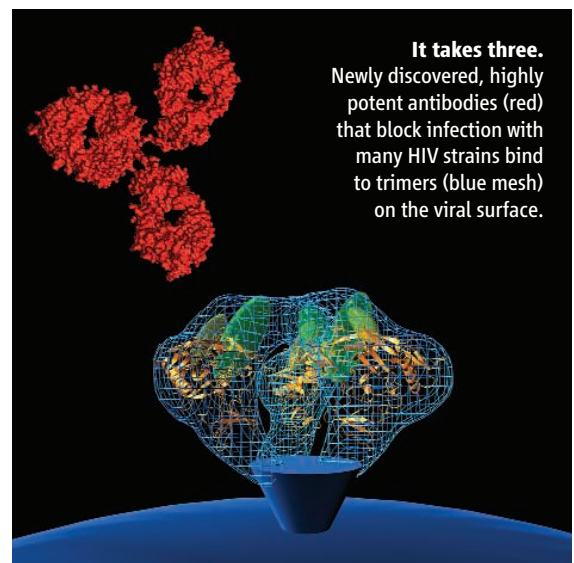
Burton, his graduate student Laura Walker, and 33 other researchers report online 3 September in *Science* (www.sciencemag.org/cgi/content/abstract/1178746) that the two new antibodies have unusual characteristics that open new avenues of AIDS vaccine research. “It’s a great paper that describes very novel antibodies,” says immunologist John Mascola of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland.

The researchers first collected blood from some 1800 HIV-infected people in Africa, Asia, Europe, and North America. Using novel techniques, they identified 10% who had antibodies that could derail more than a dozen different strains of the virus. This paper focuses on one sub-Saharan African donor; the person did not benefit appreciably from the antibodies, which are no match for HIV once an infection is established.

The researchers sifted through a staggering 30,000 antibody-producing B cells from the donor and isolated two monoclonal antibodies, dubbed PG9 and PG16, that could prevent infection in more than 70% of 162 viral strains tested in cell culture. Not only were they broad acting, but the antibodies worked at minute levels—a magnitude lower than the four best characterized bNAbs so far. “It’s an enormous amount of work—a tour de force,” says AIDS vaccine researcher Ronald Desrosiers, head of the New England Primate Research Center in Southborough, Massachusetts.

On a more sobering note, many researchers have tried to make vaccines that elicit previously identified bNAbs. “In the last 5 years, there have been intensive efforts, and no one has succeeded,” Burton says.

Still, Burton and others hope that understanding the unusual way that PG9 and PG16 stop the virus will provide new leads for AIDS vaccine designers. Specifically, HIV’s surface proteins attach to immune cells to establish infections. The surface proteins nat-



It takes three. Newly discovered, highly potent antibodies (red) that block infection with many HIV strains bind to trimers (blue mesh) on the viral surface.

urally occur in clusters of three, or trimers, and PG9 and PG16 work only against the trimer. Other bNAbs bind to trimers as well as single surface proteins, or monomers. So this suggests that if a vaccine can present the surface proteins to the immune system in the trimeric form, it may have extra punch. It might also help explain why several AIDS vaccines that contain monomeric surface proteins have performed poorly.

Wayne Koff, who heads research and development at IAVI, says PG9 and PG16 are the first of several new bNAbs that he predicts will help guide the field. In particular, researchers hope the antibodies might help crystallographers finally elucidate the structure of a trimer, which occupies another slot on the wish list. “The machine is built and ready to crank out a lot more—and it’s very likely to,” says Koff.

—JON COHEN