EIGHT

A Sickle-Cell Safari

What may at first seem contrived or even ugly may be the best solution that natural selection could devise.

—Francis Crick, *What Mad Pursuit* (1988)

Upper Gilgil, Kenya was a very long way from the scientific and educational hubs of Europe and North America, but there could not have been a more perfect place to inspire a young naturalist or anthropologist. Perched at 8,200 feet, overlooking the Great Rift Valley, the surrounding landscape abounded with wildlife and was home to a variety of tribes, each with their own language and traditions.

It was also a perfect spot to farm *Chrysanthemum*, whose flowers produce the valuable natural insecticide *pyrethrum*. The plant, introduced into Kenya by the British, thrives on the rich volcanic soil, ample rainfall, and abundant sunshine in the highlands. It was on such a farm, at the edge of the forest, with views of the massive volcano Mount Longonot and glittering Lake Naivasha, that Tony Allison was raised.

Tony's father, in addition to establishing a successful farm, was well versed in natural history and encouraged his son's budding interest. A family friend,

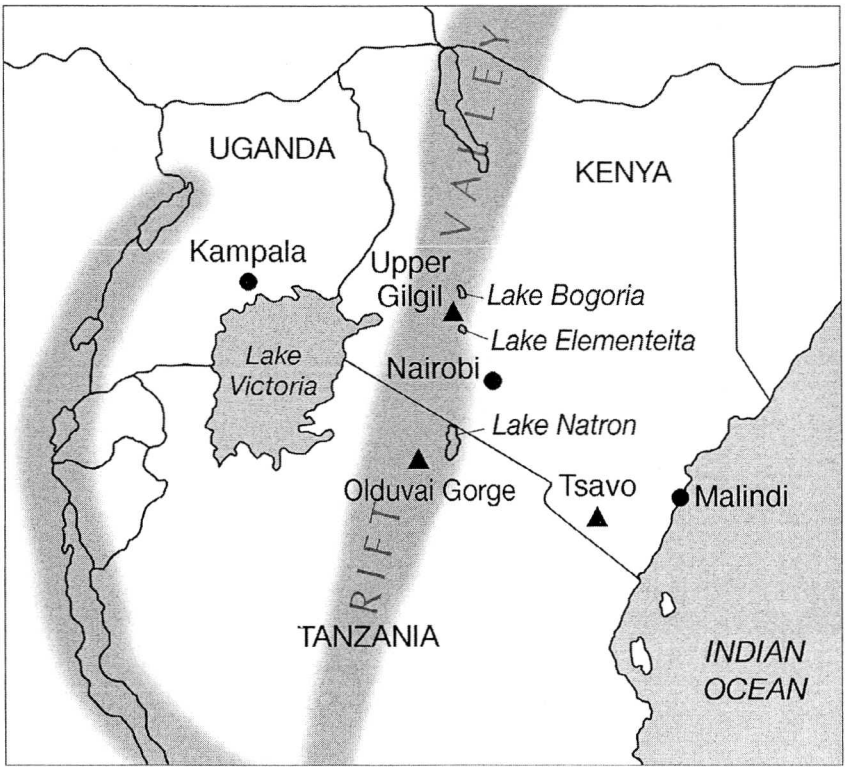

FIGURE 8.2 *Map of East Africa.*
The Rift Valley, prominent lakes and key locations are shown. *Drawn by Leanne Olds.*

Leslie Brown, was the preeminent authority on African birds. On school holidays he took Tony on two-week long birding safaris. Leslie and Tony hiked down the fault scarp and backpacked to the shores of the soda lakes of Rift Valley, such as Lake Nakaru and Lake Bogoria (see Figures 8.1 and 8.2), which drew very large numbers of birds. Opening the tent flap in the early morning, they would be treated to the spectacle of thousands of pink lesser flamingos wading in the glassy, still water just fifty feet away. (Leslie later discovered the elusive breeding site of millions of East African lesser flamingos [consistency] at Lake Natron in Tanzania.) Tony worked as Leslie's field assistant, helping with observations and the preparation of birds to be sent to the Natural History Museum in London.

The archeological opportunities were just as rich as the bird watching. The great anthropologist Louis Leakey had established excavations close by, near Lake Elmenteita. Tony visited the dig site several times. Even though Tony was just a teenager at the time, Leakey was very polite and patiently explained his efforts to understand prehistoric Kenyan culture. Tony even got the chance to work on sorting out some of the stone tools and other artifacts of the so-called "Elmenteitan" culture that Leakey was then unearthing from a series of caves. Tony become fascinated with questions of human origins: Who were Kenya's earliest people? How were present-day tribes related to them and to each other? Tony dug into and was duly impressed by Darwin's *The Origin of Species* and *The Descent of Man*.

School vacations also meant trips to the Kenyan coast, which offered a dramatic change from the highland scenery. It was a two-day journey to Malindi. Tony and his family camped overnight at the Tsavo game park, under the watchful eye of its infamous lions, whose ancestors once had a taste for railway workers. The long, lovely beaches of Malindi offered great swimming, fishing, and surfing, but the coast also held some risks not present in the mountains. During one holiday, when he was about ten, Tony contracted malaria. His first experience with the disease left a long-lasting impression, as did the kindly doctor who treated Tony and prescribed some medicine. With the disease rampant across Kenya and other parts of Africa, medicine seemed like a noble calling. Tony's ambitions turned toward medical school.

After World War II broke out, Tony enrolled in college in South Africa, at the University of Witwatersrand in Johannesburg, to pursue his bachelor's and master's degrees in medical science. The school choice was partly Leakey's suggestion. The professor of anatomy at the university was Raymond Dart, who had discovered the fossil called the "Taung" child in 1924. Dart's interpretations of this fossil skull as that of a young bipedal hominid were very strongly contested because most scholars at the time thought that Asia, not Africa, was the cradle of mankind. Dart thought otherwise, but it would take twenty years or more for his view to be accepted and for *Australopithecus africanus* to take its place in the story of hominid evolution in Africa. Robert Broom, who discovered the second specimen of *Australopithecus*, lectured in Tony's anatomy course. His

interest in the origins of humans as keen as ever, Tony took advantage of field expeditions to get some training in archeology and physical anthropology.

With heated debate accompanying the discovery of every bone, tooth, or stone, Tony thought that there must be better, more conclusive ways than conventional archeology and paleontology to get at the origins of humans and to work out the relationships between different peoples.

Out of Africa, and Back Again

In 1947, Tony enrolled at Oxford University to complete his medical training and soon became convinced that genetics offered a new way to get at human history. His teachers included some of the leading minds of population and evolutionary genetics. He was immersed in the mathematical works of R. A. Fisher, J. B. S. Haldane, and Sewall Wright, and impressed by Julian Huxley's account of the integration of genetics and evolutionary theory in *Evolution: The Modern Synthesis*. It was during his time at Oxford that Tony developed the notion that the use of genetic markers, such as human blood groups, would provide a better approach than linguistic or cultural traits to understanding human relationships.

His first opportunity to put his notion into practice came in the summer of 1949, between the completion of his basic science studies and the beginning of his formal medical training. Oxford University was mounting an expedition to Mount Kenya. While his colleagues would be studying insects and plants, Tony would pursue his anthropological interests by collecting blood samples from tribes all over Kenya. He hoped that blood types might reveal the genetic relationships among tribes. Before departing, he stopped in London for some advice on blood typing. An expert hematologist happened to mention that, in addition to the standard ABO, MNS, and Rh blood group tests, Tony should also test for the presence of sickle cells, as the frequency of that blood anomaly was reportedly unusually high in Africa. This casual suggestion would occupy Tony for the next decade.

Sickle-cell anemia was first described in 1910 by Chicago physician James Herrick who observed sickle-shaped red blood cells in the blood of an

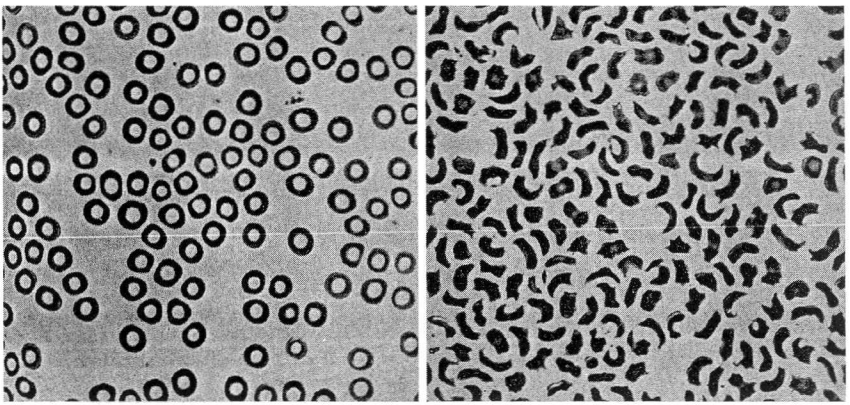

FIGURE 8.3 *A blood smear of an individual with the sickle-cell trait.* This individual is heterozygous for the sickle-cell mutation. *Left*, under normal oxygen concentrations, the red cells appear disc-shaped as normal. *Right*, but when deoxygenated, the cells assume the sickle shape. *Photo from A. C. Allison (1956)* Scientific American *195:87–94, used by permission of author.*

anemic dental student. It was subsequently discovered that sickling of carriers' blood samples could be induced by allowing it to stand for a few days, or by treating the samples with the reducing agent sodium metabisulfate (Figure 8.3). By 1949, it was widely thought that carriers of sickle cell were heterozygous (carried one allele that coded for sickle cell and one allele that did not) and that individuals with sickle-cell anemia were homozygous (in whom both alleles coded for sickle cell). And in 1949, Linus Pauling's group at the California Institute of Technology demonstrated that hemoglobin from patients with sickle-cell anemia (Hemoglobin S, or HbS) had an altered charge, and thus sickle-cell was a "molecular disease." Heterozygotes generally had a mixture of wild-type hemoglobin A (HbA) and HbS, and their genotype was designated AS, while the genotype for homozygotes was designated SS.

Tony collected blood samples by visiting hospitals all over Kenya, from Lake Victoria to Mombasa and Nairobi. These samples included specimens from members of the Kikuyu, Luo, and Masai tribes. Overall, the blood group marker data from Tony's survey and those of other workers were both surprising and a bit disappointing. With the exception of the Masai, blood

TABLE 8.1 *Frequency of HbS in Selected Kenyan Tribes*

Tribe	Ethnic Affinity	District/Region	% HbS
Luo	Nilotic	Kisumu (Lake Victoria)	25.7
Suba	Bantu	Rusingo Island	27.7
Kikuyu	Bantu	Nairobi	0.4

group frequencies were fairly uniform among East African tribes and did not reveal informative differences.

However, Tony did notice great variation in the prevalence of the sickle-cell trait. In tribes living on the coast or near Lake Victoria, the frequency of sickle-cell carriers often exceeded 20 percent, but in tribes living in arid Central Kenya or in the highlands, it was usually less than 1 percent (Table 8.1). These associations held across regions with different languages and cultures, and were entirely independent of the blood group types he had documented.

The findings were puzzling for two reasons. First, because sickle-cell anemia is frequently lethal, how could it be that the frequency of the AS genotype was so high? And second, why would it be high in some areas and not others?

Tony had a flash of insight. Lake Victoria and the Mombasa coast were low-lying humid regions plagued by very high levels of malaria, which is carried by mosquitoes and caused by the parasite *Plasmodium falciparum*. Nairobi was at a higher elevation, had fewer mosquitoes, and thus did not have a high incidence of malaria. Because the parasite multiplies within red blood cells, perhaps the HbS allele, in heterozygotes, conferred some degree of resistance to malaria?

If true, Tony realized this would be the first ever demonstration of natural selection operating on a genetic variant in humans. Indeed, in 1949 there were no examples of natural selection in any species where a variant molecule was known. The link between malaria and the prevalence of the sickle-cell trait was a terrifically exciting idea, but Tony was in no position to test it. He had no time — his formal medical training was about to begin. He had no medical qualifications — he was still just a student. And he had no training — especially

in parasitology. He decided not to publish the sickle-cell data or his theory. He would sit on his idea until he could earn the necessary credentials and training to test it. That would make for an agonizingly long wait of more than three years. He would have to live on tenterhooks, dreading that somebody else would realize the same connection and beat him to the punch.

There was one tremendous benefit from the idea burning in his head: It gave him a sense of urgency and enthusiasm for some subjects that his fellow students often lacked. He could not get enough of genetics, and the course in tropical medicine, one of the least popular among his classmates, was his favorite. The course was taught by Lt. General William McArthur, former director of the Indian Medical Service and an expert in tropical diseases. Tony clung to his professor's side just as he had to Leslie Brown on safari and to Louis Leakey at his excavation sites. Tony was always a willing assistant in McArthur's class, handing out microscope slides for his classmates to look at, and then staying after class for hours of one-on-one tutoring in tropical medicine. He loved every minute of it.

Finally, in 1952, he received his M.D. degree. He had not yet been scooped. It was finally time to put his training into practice.

There was just one not-so-tiny problem — Tony had no laboratory or position. Fortunately, in the course of his blood typing studies, he had met Alan Raper, the director of the Medical Laboratory in Kampala, Uganda. Raper was impressed with Tony's work and generously offered him the use of his house, his laboratory, and even his cook, while he was away on leave in 1953. It was perfect.

A Sickle-Cell Safari

Tony established Kampala as his base of operations and spent most of 1953 testing his idea that sickle-cell carriers (AS heterozygotes) were relatively resistant to malaria. He realized early on that he would have to take two factors into consideration that might complicate the situation: the induction of immunity by exposure to malaria and the widespread use of malarial drugs. In order to detect the role of the HbS mutation, he would have to try to minimize these factors in the design of his studies.

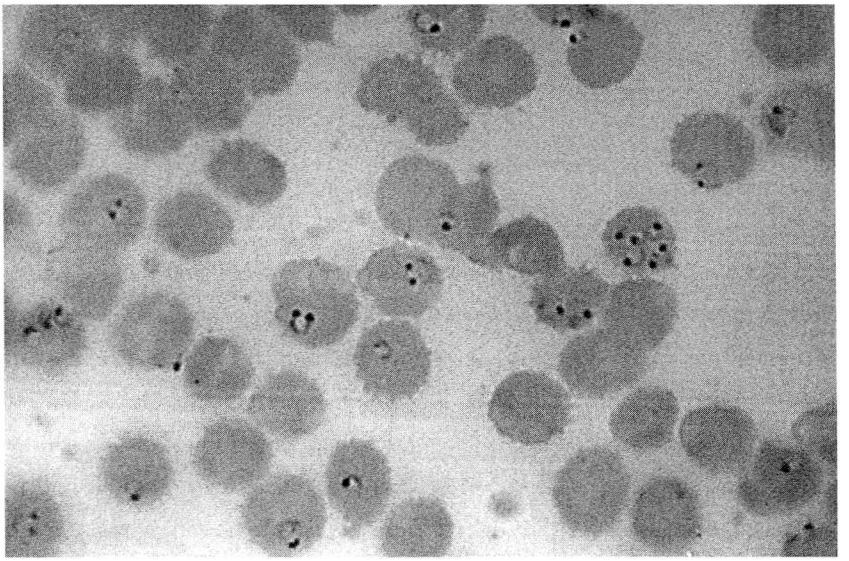

FIGURE 8.4 *A blood smear of an individual infected with malarial parasites.* A red blood cell sample was treated with Giemsa stain to reveal parasites within cells (black dots). *Photo courtesy of Dr. Mae Melvin, CDC Public Health Image Library.*

The first set of experiments he undertook was to see if AS heterozygotes were relatively resistant to new infections. A pharmaceutical company had established a laboratory in Nairobi to test antimalarial drugs, and volunteers were routinely inoculated with *P. falciparum* as part of these clinical studies. In order to try to minimize complicating factors, volunteers were selected that had not recently visited malarial areas nor received antimalarial drugs.

Fifteen AS and fifteen AA Luo tribesmen were exposed to malaria and followed for forty days (then given a course of antimalarial drugs to arrest the infection). Infection was established in fourteen out of fifteen AA individuals, whereas only two out of fifteen AS individuals showed signs of infection in blood smears (Figure 8.4). So far, so good.

Tony was concerned that these studies on adults were still affected by prior exposures, so he decided to examine the levels of parasitemia in children. Consultation with various experts convinced him that children aged six months to four years would be the most vulnerable to malaria infection and the most informative for testing the hypothesis of the advantage of AS

FIGURE 8.5 *Masai woman and child on market day. Photo courtesy of A. C. Allison.*

heterozygotes. In order to get an adequate amount of data for statistical purposes, he needed to examine a lot of children. Around Kampala malaria was at high levels and no preventative drugs were in use, so he could survey local children and use Raper's laboratory for the work.

On every weekday in the Buganda region around Kampala there were farmers' markets to which local women, accompanied by their small children, came to buy fresh produce (Figure 8.5). Every day Tony went out with a local pharmacist and offered a free check-up. He examined the children's eyes, chests, skin, and so forth and took a small sample of blood by pricking their finger or heel. Tony would then head back to the lab at 5:00 p.m. and

spend until midnight preparing and analyzing samples, looking for HbS and parasite levels. After six months of exhausting work, he had data for 290 children, and the results he hoped for.

The incidence of parasitemia was 27.9 percent in the AS heterozygote children and 45.7 percent in the normal AA homozygote children. Furthermore, parasite density in the AS heterozygote children was lower than in the AA homozygote children. These results suggested that AS heterozygote children had a lower incidence of malaria or were affected for shorter periods than AA homozygote children, and thus AS heterozygote children would have a selective advantage in regions where malaria was at high levels.

Again, so far, so good. But while the Luo and Kampala studies supported Tony's theory that malaria and sickle-cell anemia were connected somehow, he wanted to know if the association between the high frequency of the HbS allele and high levels of malaria held *everywhere*.

Although Tony had data that showed the correlation from his 1949 Kenyan expedition, he felt he needed to test more tribes throughout East Africa. He embarked on what might be best described as a "sickle-cell safari." The journey took him from the forests of Western Uganda to the coasts and highlands of Tanganyika (now Tanzania) and Kenya, and he even managed to pay a visit to Louis Leakey's ongoing dig at Olduvai Gorge. Tony used the facilities of district hospitals where he could; otherwise his camp was his laboratory, with the crucial piece of equipment being a battery-operated microscope inside his tent (Figure 8.6). Tony managed to test five thousand East Africans in total, representing three countries and more than thirty different tribes. He found HbS allele frequencies of up to 40 percent in some areas where malaria was hyperendemic, and frequencies as low as 0 percent in areas where malaria was absent.

He had all the confirmation he needed.

Disease and Natural Selection on Humans

Large differences in sickle-cell allele frequencies among Kenyan and Ugandan tribes had been noted in surveys conducted by other researchers.

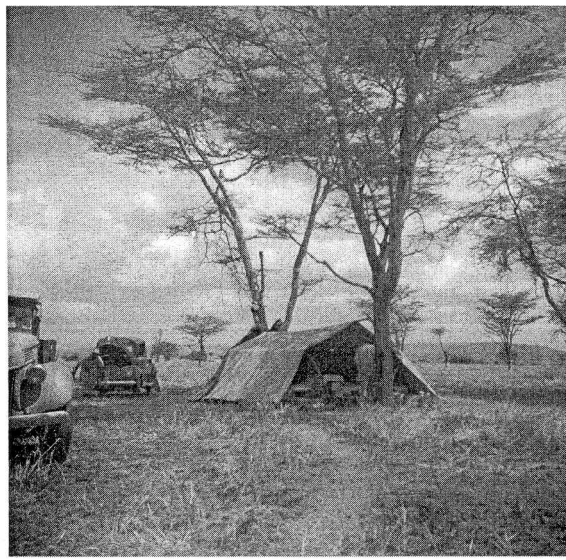

FIGURE 8.6 *On the sickle-cell safari.*
Tony Allison's camp under a fever-thorn tree (*Acacia xanthophloeia*), so-called because they grew near the water in malarious regions. *Photo courtesy of A. C. Allison.*

However, these scientists attributed the differences to different factors. Some researchers thought that the differences reflected tribal migration histories, or the degree of genetic mixing among tribes, or a very high genetic mutation rate, or even a higher rate of reproduction among families of sickle-cell carriers to compensate for child mortality caused by the disease. Only Tony had looked for, and found, a natural environmental explanation, one that cut across tribal lines.

With all of the pieces of the malaria and sickle-cell trait connection fitting together, Tony, in a series of three articles published in 1954, made the case for malaria as an agent of natural selection on humans. In an article in the *British Medical Journal*, Tony explained:

> The proportion of individuals with sickle cells in any population, then, will be the result of a balance between two factors; the severity of malaria, which will tend to increase the frequency of the gene, and the rate of elimination of the sickle-cell genes in individuals dying of sickle-cell anaemia . . . genetically speaking, this is a balanced polymorphism, where the heterozygote has an advantage over either homozygote.

How much of an advantage was the sickle-cell gene to AS heterozygotes? This was very important to determine in order to understand the exceptionally high frequency of the sickle-cell trait in some regions. To estimate the selective advantage, he leaned on his Oxford training in population genetics, and the help of a collaborator and outstanding mathematician, Sheila Maynard Smith. Tony and Sheila reasoned that if they could figure out the *disadvantage* of SS homozygotes, then from basic population genetic principles they could estimate the advantage AS heterozygotes must enjoy that would account for their high frequency in malarial areas.

Tony's firsthand experience with and data on the incidence of the sickle-cell trait in children and adults was crucial. In the Luo tribe, Tony had measured a frequency of 25.7 percent for the sickle-cell trait. Assuming this was a mixed group of AS heterozygote and SS homozygote individuals, and that all SS homozygotes survived, Tony calculated that the expected ratio of SS homozygotes to all sicklers was about 1:12. However, he found that the actual ratio was closer to 1:35, meaning that only about one-third of SS homozygotes survived to young adulthood with the potential to have children. Other scientists estimated from other data that about 20 percent of SS homozygotes survived and reproduced. The reduced fitness of SS homozygotes meant that the overall frequency of the sickle-cell allele would be rapidly reduced, unless there was some advantage to the AS heterozygote. Sheila Maynard Smith calculated that advantage to be about 26 percent. In other words, in high malarial zones, 26 percent more AS heterozygote children than AA homozygote children reached adulthood. That is a whopping selective advantage, on par with some of the largest selective advantages ever measured for any trait in any species.

As word of Tony's discovery began to spread, leading evolutionary biologists wanted to hear more about it. Tony was invited to speak at the 1954 Cold Spring Harbor Symposium — the most influential meeting in the then just blossoming field of molecular biology (at the previous year's meeting James Watson and Francis Crick explained their model of DNA structure). It was Tony's first trip to America and a golden opportunity. Many of the current and future figures of population genetics and evolutionary biology were there,

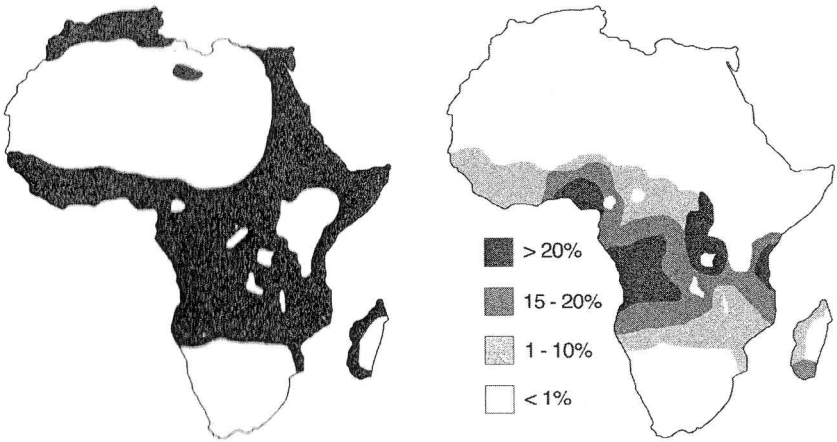

FIGURE 8.7 *The geography of sickle-cell hemoglobin and malaria.* These maps show the close correspondence between the distribution of malaria (*left*) and the frequency of the sickle-cell trait (*right*) across Africa. *Maps are based upon A. C. Allison (2004) Genetics 66:1591. Redrawn by Leanne Olds.*

including Theodosius Dobzhansky, Ernst Mayr, Sewall Wright, James Crow, E. B. Ford, and Motoo Kimura.

Tony showed the audience how the frequency of the sickle-cell trait and the incidence of malaria coincided on a map of East Africa. In an otherwise math-heavy meeting, Tony's clear demonstration of the selective advantage of AS heterozygotes was a welcome change and very well received.

Such a large advantage, Tony went on to point out, also explained the high incidence of the sickle-cell trait outside of Africa. In the Lake Copais area of Greece, the trait reached a frequency of 17.7 percent, in part of India it reached nearly 30 percent, and it was also common in parts of Italy. Tony argued that these high frequencies and those in parts of Africa could not be due to ancestral relationships. Blood type markers did not support genetic relationships among these populations. So what did all of these populations have in common? They lived in regions notorious for a high incidence of malaria. That correlation could be mapped onto not just East Africa, but across all of Africa, southern Europe, and southern Asia (Figure 8.7). These maps would become textbook illustrations of natural selection on humans.

The Sickle-Cell Mutation

Tony speculated that the sickle-cell trait in different populations could result from each of those populations acquiring genetic mutations independently. But there was no way to test that idea at the time. The structure of DNA had only just been revealed, the genetic code was unknown, and methods for analyzing protein sequences were in their infancy.

Tony, and many others around the world, wanted to know the precise molecular defect in HbS. When Tony returned to England from his sickle-cell safari, he happened to meet Linus Pauling, whose team had demonstrated that HbS was different from HbA. Tony told Pauling about his not-yet-published discovery, and Pauling invited him to Caltech to work on the HbS protein.

There were few, if any, greater minds or greater personalities in twentieth century science than Linus Pauling. His seminal work on the nature of chemical bonds and his solution of the alpha-helical structures within proteins earned him his first Nobel Prize, in Chemistry, in 1954. His leadership in the opposition to atmospheric testing of nuclear weapons earned him his second Nobel Prize, for Peace, in 1962.

Tony's year in Pasadena was an eye-opening experience. He would often have breakfast at Pauling's house, which enjoyed a great view of the surrounding hills, then meet again later in Pauling's office to report the results from the day in the lab, and to have Pauling explain what they meant.

Tony soon decided that determining the sequence of the HbS protein was not yet feasible, so he focused instead on trying to figure out why HbS caused sickling. Pauling thought that HbS was aggregated in solution, while Max Perutz (a future Nobel laureate) thought that HbS actually crystallized inside red cells. Tony found out that Pauling was right, again, and that HbS aggregated into long rod-like structures.

Later, when back in England, Tony provided samples of the HbS protein to researchers who were using new techniques to determine protein sequences. In 1957, it was finally determined that HbS differed from HbA at just one amino acid, a valine in place of glutamic acid. Once the genetic code was deciphered and methods for the isolation and sequencing of DNA

were developed, HbS was found to be due to a single base mutation (GAG → GTG) in the glutamic acid codon. Subsequently, the sickle-cell genes from different regions of Africa, southern Europe, and India revealed that this same mutation arose at least five different times, confirming Tony's hypothesis of the independent origins and spread of sickle cell in different malarial zones.

The Road from Gilgil

In the 1930s and early 1940s, paleontology, systematics, and population genetics became integrated into what was called the "Modern Synthesis" of evolutionary theory. One might think that many examples of natural selection on specific genes would have been known at the time, but that was not the case. Natural selection had been demonstrated in various ways since Darwin's original work, but there was nothing known about the identity or function of any genes involved. There was not a single "integrated" example of natural selection where the agent of natural selection was known, the effect on different genotypes could be measured, the genetic and molecular basis of variation was known, and the function of the gene or protein was understood. Who would have thought that with all of the talent and brainpower in evolutionary theory then populating the upper crust of academia, the credit for the first such example would go to a newly minted doctor and former farm boy from the Kenyan highlands? And that it would be demonstrated in humans, no less.

That first flash of insight during the 1949 Kenyan expedition carried Tony far, both geographically and professionally, in the ensuing six decades. After his stint in Pasadena, he spent twenty years directing a laboratory in England, then several years running a tropical disease laboratory in Nairobi, and then moved to California to join the pharmaceutical industry. There, he and his wife Elise Eugui played key roles in the development of what is today the leading drug used to prevent the rejection of transplanted organs.

Tony's discovery of the link between sickle cell and malaria resistance still stands out as a leading example of "evolution in action." This is because the

agent of natural selection is known and still active, the degree of resistance is measurable and is clearly most important early in life, there is a simple genetic basis for resistance, and the association has been so well documented both geographically and clinically.

It is also a very important example because it challenges commonly held notions about mutation and evolution. Mutations are often perceived to be harmful, as is the HbS mutation under most conditions. However, as Tony put it, "the sickle cell mutation shows that mutation is not an unmixed bane to the human species . . . other mutant genes that are bad in one situation may prove beneficial in another." In fact, a few years after his HbS studies, Tony and David Clyde found that an enzyme deficiency caused by mutations in the gene encoding glucose-6-phosphate dehydrogenase (G6PD) affords considerable protection against *P. falciparum* malaria in Tanzanian children, a finding confirmed many times since. This explains why the enzyme deficiency is common in areas where malaria abounds.

Malaria has had a profound impact on human genetics and evolution that continues today. Over 40 percent of the world's population lives in malarial areas and over five hundred million cases occur each year, causing one to two million deaths. G6PD deficiency, HbS, and many other mutations now known to be associated with malarial resistance reveal that the battle against malaria has made a very strong mark on human evolution. Those mutations also demonstrate how natural selection works with whatever variation is available in the struggle for survival, and not necessarily by the best means imaginable.

We'll see in the next chapter how some species take even more radical measures in modifying their blood in the struggle to adapt to the challenges of their environment.

CHAPTER QUESTIONS

1. How did Tony Allison's early life experiences in Kenya prepare him to make the discovery of the sickle cell–malaria link?

2. What makes the sickle-cell mutation a balanced polymorphism?

3. Why was the demonstration of human resistance to malaria important to evolutionary biology?

For more on this story, go to the *Into The Jungle* companion website at www.aw-bc.com/carroll.