CHAPTER 3

THE MICROEVOLUTION
OF SICKLING

In contrast with the dramatic steps in the evolution of humans from their primate ancestors, the events that led to sickling represent a more limited process, yet one that changed hemoglobin molecules for the first time in some 5 million years, as far as we can judge, for a significant fraction of the human population in Africa. We shall now try to discover how hemoglobin that had remained constant for so long could appear in an altered form in millions of people in a relatively short period of time.

The critical events in the rise of the sickle mutation almost certainly occurred during recorded history—a time scale in which centuries once again assume importance. To bring us back to this perspective, we only need note that it was just somewhat more than a century ago (1864) that a German scientist, Felix Hoppe-Seyler, named the red substance from blood “hemoglobin.” Observations with the earliest microscopes had established in the 1600s that the red color of blood was packaged in multitudes of tiny cells or “globules.” The name hemoglobin reflects the source of the material from globules (hence “globin”) and from blood (hence the prefix “hemo,” from the Greek word haima, meaning “blood”).1 Hoppe-Seyler’s laboratory in Tübingen achieved renown for these studies of hemoglobin and also as the setting for the first isolation of DNA by his colleague Friedrich Miescher. In contrast, another scientist working at this time in what is now Czechoslovakia went largely unnoticed. His name was Gregor Mendel.

Mendel, an Augustinian monk, performed as a sideline to his monastic duties extraordinary experiments that laid the foundation of modern genetics. His findings were published by the Brno Society of Natural Science in 1865 in a paper entitled “Experiments on Plant Hybrids.” However, the Journal of the Brno Society was not in the mainstream of scientific publications. Although 120 libraries received copies of the journal, the significance of Mendel’s article was not appreciated in scientific circles until it was rediscovered at the turn of the century. Once his findings were thoroughly exposed to the scientific community, Mendel was recognized as an exceptionally gifted scientist. Unfortunately, the recognition came too late to provide satisfaction to Mendel, who died in 1884.

Mendel carried out his experiments with the common garden pea. He worked with different strains that possessed well-defined characteristics: some plants produced round peas and others produced wrinkled peas. He noticed that in his many crosses between the plants with round peas and those with wrinkled peas, the first generation hybrid always produced round peas. The following year, Mendel would cross many of these first generation hybrid plants. In this way, he obtained 7,324 second generation peas, of which 5,474 were round and 1,850 were wrinkled. The ratio of round to wrinkled was 2.96:1, or (within experimental error) 3:1. Experiments of this type were repeated with plants having other readily observable characteristics. In every case of two alternative parental characters, only one appeared in the first generation hybrids. Moreover, the character that vanished in the first generation hybrids always reappeared among one-fourth of the population of the crosses between the hybrids.

On the basis of these observations, Mendel reasoned that the characters responsible for the heredity of the form of the pea are carried and passed on to the next generation in discrete units. Each pea must possess a pair of such units, with one of the two producing the dominant characteristic, such as round peas (abbreviated R), and the other producing the recessive characteristic, such as wrinkled peas (abbreviated W). In the hybrids, both are present, but only the character of the dominant unit is expressed.
When two hybrids are crossed, four kinds of peas will be produced in statistically equal amounts, based on the alternative combinations of the individual heredity characters.

<table>
<thead>
<tr>
<th>Cross:</th>
<th>Hybrid 1 × Hybrid 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁/W₁</td>
<td>R₂/W₂</td>
</tr>
</tbody>
</table>

Peas: R₁/R₂  R₁/W₁  W₁/R₂  W₁/W₁

The wrinkled exterior of the recessive unit of heredity manifests itself in only the one-fourth of the population that is homozygous for W₁, the W₁/W₁ group. The other three-fourths would appear round, although they would be actually a mixture of homozygous forms (R₁/R₁) and heterozygous forms (R₁/W₁ and W₁/R₁).

The two types of wrinkled peas reveal that it is possible to distinguish between the composition of the genes, called “genotype,” and the physical appearance of the living organism, called “phenotype.” For the homozygous R₁/R₁ and W₁/W₁ plants, the genotype corresponds exactly to the phenotype. However, for the hybrid R₁/W₁ or W₁/R₁ plants, the phenotype appears the same as R₁/R₁ plants, although the genotype is heterozygous for the R and W genes. Thus, an important aspect of Mendel’s insights was distinguishing between the genotype and phenotype. The brilliance of Mendel’s deductions shines all the more brightly when compared with the impoverished ideas on the subject of inheritance advanced in the same period by Darwin. As noted by Stent and Calendar, “Indeed, Darwin’s ‘pangenesis’ concept of the mechanism of heredity, which envisages that each part of the adult organism produces ‘gemmules’ which are collected in the ‘seed’ for transmission to the offspring, was more or less the same as that propounded by Hippocrates some twenty-three centuries earlier.”

Although formulated with peas, the principles Mendel discovered apply to the genes for hemoglobin and its sickle variant. When the mutant form of hemoglobin associated with sickling cells was first detected in 1949 by Linus Pauling and his colleagues, they gave it the designation “hemoglobin S” (“S” for “sickle”). The individuals who are heterozygous for the sickle mutation, that is, the carriers of sickle trait, can be designated as “hybrids,” since they possess genes for both hemoglobin A and hemoglobin S. The children of two such hybrids (or carriers) will receive the chromosomes from each parent according to four possibilities of equal probability. The four possibilities are analogous to the four categories of genes for round and wrinkled peas. If we designate the genes of the father by adding f and those of the mother by adding m, the hemoglobin A and S genes will be distributed as follows:

Parents: Father × Mother

<table>
<thead>
<tr>
<th></th>
<th>Father</th>
<th></th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A₀/S₁</td>
<td></td>
<td>Aₘ/Sₘ</td>
</tr>
<tr>
<td>Child:</td>
<td>A₀/Aₘ</td>
<td>A₀/Sₘ</td>
<td>Aₘ/S₁</td>
</tr>
</tbody>
</table>

The sickle character is essentially “recessive,” since cells carrying an S gene do not normally sickle if the A gene is also present. On the average, the recessive character of sickled cells will be fully manifested in only the 25% of the children who possess the S₀/Sₘ genes. These children will have sickle cell anemia. The normal hemoglobin gene will be expressed in the other 75% of the children, who would be a mixture of individuals with only normal hemoglobin (A₀/Aₘ) and hybrid individuals with both hemoglobins (A₀/Sₘ and Aₘ/S₁). These latter individuals, while appearing normal because of the dominance of the A gene over the S, are carriers of sickle trait who can pass the gene on to their children. If two such second-generation carriers marry, the risk is again 1 in 4 that their children will have sickle cell anemia.³

We can abbreviate the scheme depicted above by denoting hemoglobin genes as simply A or S. Individuals can then be identified by their genotype and designated simply as AA, AS, and SS. According to the Mendelian scheme, then, for children of two carriers, the probability of the ratios of offspring are AA:AS:SS = 1:2:1.

If the sickle mutation had arisen only once and had followed the 1:2:1 ratio with no additional factors, we would not be discussing it now, since it would never have reached the proportions that make it a major medical problem among individuals of African descent. However, the fact that the sickle mutation appears to have increased the resistance of AS individuals to malaria has led to the proliferation of carriers in the mosquito-infested African tropics. The eventual consequence has been that African children in increasing numbers have had two AS individuals as parents, and the unlucky 1 in 4 of these children has developed sickle cell
anemia. Any benefits these SS children might have in fighting malaria are heavily outweighed by the many deleterious consequences of sickling cells.

We can now formulate more quantitatively how the sickle mutation came to such abundance in Africa. We must assume that the AS individuals had some advantage over both AA individuals and SS individuals. The reproductive fitness of the SS individuals would have been diminished by the health problems associated with having sickling cells. The AA individuals would have been plagued somewhat more than AS individuals by the effects of malaria. It appears that the advantage of AS individuals over AA individuals in resisting malaria occurs especially in the early childhood years before children develop a degree of acquired immunity from their own antibody production. This view is consistent with the finding in Nigeria that fatal cases of malaria are almost invariably in infants, but precise estimates on the relative fitness of AA, AS, and SS individuals are difficult to obtain. Only limited data exists for the relative advantages of AS over AA individuals in resisting malaria; and, as we shall see, different studies have yielded conflicting results. Laboratory studies on the growth of the malaria parasite *Plasmodium falciparum* in red cells from AA, AS, and SS individuals have helped to reveal the molecular mechanism of the protection for AS individuals (see Chapter 6). However, studies at the cellular level do not provide information on the reproductive fitness of the individuals with these cells. Nevertheless, the weight of the evidence indicates that, in all probability, the effect of one S gene is relatively small; only on the order of 10% more AS than AA individuals survive to reproduce in every generation. The fitness handicap of SS individuals is also difficult to estimate, especially for historical times in Africa. Data from contemporary medical clinics and information available from field studies suggest that the presence of two S genes often has a devastating effect on Africans. The majority of these homozygous individuals succumb before becoming parents; and SS females who do survive to reproductive maturity are rarely able to surmount the strains of pregnancy.

The fragility and shortened life time of sickling red blood cells causes a state of weakness associated with anemia in general. However, the anemia per se is a relatively minor aspect of the overall problems associated with sickling cells. In fact, these other problems would probably be aggravated were the anemia lessened, since sickled cells cause blockages of circulation and more cells would worsen the obstructions. Other than the anemia associated with sickling cells, there are three severe types of problems faced by SS individuals: (1) greatly heightened susceptibility to bacterial infection, particularly in the early childhood years; (2) painful episodes (vaso-occlusive crises) associated with blockages in the circulatory system; and (3) progressive degeneration of various organs of the body caused by impaired circulation.

Concerning the risks to life in the early years, it has been shown that evidence of cell sickling can be found as early as 3 months. Prior to that age, the red cells contain appreciable amounts of the fetal form of hemoglobin which provide some protection against sickling. Once hemoglobin S predominates, high risks are encountered. From studies conducted in the United States we know that infants with sickle cell anemia have died suddenly due to infections, particularly pneumococcal sepsis and meningitis and to a lesser degree salmonella, shigella, and tuberculosis. Corresponding studies in Ghana indicate similar susceptibility to infections, although commonly with malaria and typhoid. The incidence of malaria infection indicates that in spite of whatever resistance to malaria AS individuals may have, young SS individuals suffer from an overall susceptibility to infection that overpowers any slight resistance to malaria that might have been associated directly with the S gene.

The childhood infections of SS infants arise mainly from loss of the functions of the spleen. Its extensive capillaries are among the first to be blocked by sickled cells; the result is usually a small, nonfunctional spleen by mid-childhood. Evidence of impaired spleen function can generally be detected in SS infants less than one year old. Progressive diminution in size of the spleen occurs, with SS children generally functionally asplenic by the age of 7.

Since the spleen plays a role in the defenses of the body to infection, particularly from bacterial sources, homozygous SS children become acutely susceptible to infection. Overturf and Powers cite data indicating an incidence of bacterial meningitis 25 times higher in children with sickle cell anemia than in normal children; the incidence of salmonellosis was found to be some 300 times higher. In a number of clinical centers, regular administration of prophylactic antibiotics has led to a marked improvement
in the health and survival of the population treated. Pneumococcal vaccines have also been used with modest success. It is now clear that young children carefully monitored and promptly treated at the first signs of infection will have a good chance of surviving this critical period. Of course, such care was out of the question in Africa historically, and a large part of the mortality associated with sickle cell anemia was undoubtedly due to early childhood infections. While intense health-care practices to carry SS children through the early critical years are still relatively rare in Africa outside certain clinical centers in large cities, such practices are expected to become more widespread, and the population of adolescent and adult sicklers will continue to rise.

As a result, the other consequences of the disease will become medical problems on an increasingly wide scale and will add to the sense of urgency to find a treatment for sickle cell anemia. These long-term consequences are responsible for significant further mortality in those SS individuals who reach adolescence.

In general, the children with sickle cell anemia who approach adolescence experience slower growth than their normal peers; a delay of several years in the arrival of puberty is commonly encountered. However, delayed puberty leads to a continuation of growth beyond the usual age range, so that the final height of SS individuals is on the average within the normal range. Serious consequences may then arise from problems of circulation. In virtually all cases, impaired circulation leads to episodes of intense pain which begin suddenly and may last for days. The chest is one of the frequent points of pain, along with the abdomen or joints of the arms or legs. When these painful crises occur in young children, the hands and feet are common targets. Depending on the individual, crises may recur frequently (at intervals of a few weeks) or, for the fortunate few, only rarely, if at all. Treatment at the present time is limited to sedation, analgesics, and fluids.

Apart from these intense episodes of pain, there are a series of inevitable changes that take their toll as well. The lungs are subject to progressive damage, which leads to poor oxygenation of sickled red cells that already have difficulty in binding oxygen (see Chapter 6). Decreased oxygenation of the blood places stress on the heart, which tries to compensate for the low oxygen supply by pumping more blood. As a result, enlarged, hyperactive hearts are found in SS individuals. Liver and kidney damage is also encountered. Various signs of circulatory problems can be readily detected in the eyes of SS individuals as they age; sometimes the result is a loss of vision in adults. Skin ulcers, often around the ankles, are one of the most aggravating problems for SS individuals. In Africa, scars from healed leg ulcers are one of the signs used for preliminary diagnosis of a homozygous SS individual awaiting hemoglobin testing. Bone deformities are also encountered, either as a result of infections or as a general symptom, such as the biconcave shape of vertebra (described pictorially as “fishmouth” vertebra) typical of SS individuals. Indeed, one of the rare SS individuals I met in Africa outside of big city clinics was a teenage boy in the village of Awgu, where I first encountered the ogbanye children. When I asked how he had been diagnosed, he showed me the x-ray that he had proudly saved which revealed the typical deformation of the vertebra. In certain clinics an x-ray is more readily obtained than a hemoglobin analysis. I eventually obtained a blood sample from this boy and found that he was indeed homozygous SS.

On the basis of the information we have about the health problems encountered by SS individuals, as well as information from other sources, we can now pursue the issue of the fitness of AA, AS, and SS individuals in the African tropics. In order to develop quantitative arguments concerning the evolution of the sickle mutation, we must define the “fractional reproductive fitness”—the fractions of the population of AA, AS, and SS individuals that live at least long enough to reproduce. If we assign the AS individuals an arbitrary fitness of one, then the fitness for AA individuals is less than one, owing to their somewhat greater susceptibility to malaria, and the fitness of SS individuals is substantially less than one because of the problems caused by sickling.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA</th>
<th>AS</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fitness</td>
<td>Fitness &lt; 1</td>
<td>Fitness = 1</td>
<td>Fitness &lt;&lt; 1</td>
</tr>
</tbody>
</table>

Since populations in Africa rarely exceed an incidence of AS individuals of about 30%, we can start our calculations by assuming that this is generally as high as the value can go. In other words, it has reached equilibrium. The final population of individuals with the sickle gene will thus be balanced by the advantages of malaria resistance versus the disadvantages of sickling. Relatively more AS individuals will be found when the AA mortality is specif-
cally increased by a malaria environment. However, a high SS
mortality will tend to limit the percentage of AS individuals by
reducing the number of S genes in the population. These factors
can be expressed mathematically using the equations of populations
genetics for what is called a "balanced polymorphism."
The incidence of the S gene found in the Igbo population (and
other comparable groups) is compatible with only a limited range
of values for the relative reproductive fitness of AA individuals,
even when the fitness of SS individuals varies widely, as sum-
marized in Table 3.1.

Case 1 represents the lowest possible fitness for SS (zero), which
corresponds to the possibility that SS individuals never repro-
duce. Under these circumstances a Fitness(AA) = 0.81 would be
required to achieve the percentage typical of the Igbo of 25% AS.
Therefore, 0.81 is the lower limit of Fitness(AA), since Fitness(SS)
is certainly greater than zero and higher values of Fitness(SS) lead
to higher estimates of Fitness(AA). As a result, Fitness(AA) must
fall within the range 0.80-1.0, but a Fitness(AA) as high as 0.95
(Case 5) would require a Fitness(SS) = 0.74, which is unrealisti-
cally high. Comparison of Case 1 and Case 5 illustrates that the
higher the Fitness(SS), the smaller the difference between Fit-
ness(AA) and Fitness(AS) required to achieve 25% AS for the
population. It is only when the Fitness(SS) is extremely poor, ap-
proaching zero, that the Fitness(AA) must be some 20% lower than
Fitness(AS) in order to favor AS individuals to a degree suf-
ficient to overcome the loss of S genes in the SS group.

Since Case 1 and Case 5 provide unlikely extremes, more

<table>
<thead>
<tr>
<th>Case</th>
<th>Fitness(AA)</th>
<th>Fitness(AS)</th>
<th>Fitness(SS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.81</td>
<td>1.0</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.83</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>0.85</td>
<td>1.0</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>0.90</td>
<td>1.0</td>
<td>0.48</td>
</tr>
<tr>
<td>5</td>
<td>0.95</td>
<td>1.0</td>
<td>0.74</td>
</tr>
</tbody>
</table>

plausible parameters are presented in Case 2 with Fitness(AA) =
0.83 and Fitness(SS) = 0.10, Case 3 with Fitness(AA) = 0.85 and
Fitness(SS) = 0.22, and Case 4 with Fitness(AA) = 0.90 and Fit-
ness(SS) = 0.48. Although there is insufficient evidence to distin-
guish with certainty which of these three cases best represents the
past circumstances of the Igbo, the situation historically was
probably closer to Case 2 or Case 3, since Fitness(SS) approaching
0.50 is difficult to reconcile with the multiple consequences of SS
red cells. Therefore, we can tentatively conclude that for many
generations in the African tropics, only about 9 AA individuals on
the average for every 10 AS individuals survived to reproduce. At
the same time, the SS individuals were plagued by the various
childhood risks and the difficulties of adolescence, with young
women in particular likely to succumb during the added stress of
pregnancy, thereby contributing to an overall fitness of SS indi-
viduals of 0.10-0.50.

Since many African peoples have incidences of AS individuals
that are far lower than 25%, we can assume either that for these
people equilibrium has not yet been reached, or that if it has, then
the relative advantages of the AS individuals are reduced. It is
probably the latter case for certain populations, since the reduced
incidence of AS generally coincides with drier climates which
tend to be less supportive of the Anopheles mosquito that transmits
the malaria parasite. As a result, malaria is probably less severe a
problem in these areas and AA individuals are at less of a disad-
vantage compared with AS individuals than in wetter climates
which support Anopheles growth. Diet may also be a factor (see
below). Thus, the fitness of AA individuals (compared with AS at
1.0) may be considerably higher than 0.90 for population groups
with a lower incidence of the S gene. The impression may have
been created that AS individuals are almost always protected
against malaria, but the mathematical arguments make it clear
that the protection is only slight. Even in the critical childhood
period, the protection of AS individuals from malaria is not read-
ily measurable, as emphasized recently, for example, by Linda
Jackson on the basis of her field work in Liberia.

This current view of only limited protection against malaria
contrasts with early reports from A. C. Allison of a spectacular
protection against malaria in AS individuals. When volunteers
from the Luo ethnic group of East Africa were inoculated with
malaria parasites, Allison reported in 1954 that 14 of 15 AA individuals developed malaria, compared with only 2 of 15 AS individuals. Ethical considerations aside, the relatively small number of individuals involved in this study and other questions of procedure make it difficult to draw firm conclusions from these results. Overall, the protection against malaria in AS individuals now is accepted to be much more modest.\(^8\)

For our purposes, a principal reason for deducing a value of Fitness(AA) was to permit calculation of the microevolutionary time required for the S gene to reach its current incidence. Accepting the estimate of Fitness(AA) = 0.90, we can go on to the next step and consider the period of time during which the sickle gene was selected. We will then reconsider how sensitive the time period calculated is to the actual value for Fitness(AA).

In order to estimate the span of time required to account for the incidence of the sickle gene, we must make calculations that are similar to compounding interest on a bank account. As a first approximation, the value of Fitness(AA) = 0.90 means that AS individuals increased by roughly 10% in each generation until equilibrium was reached. We can ask how long it would have taken the AS individuals to double their share of the population. If money is placed in a bank account at 10% interest, it doubles in about 7 years. Similarly, the proportion of AS individuals would have doubled roughly every 7 generations. However, the estimates of the total time required to achieve current levels of the S gene are complicated by another factor, the size of the population affected.

Population groups which currently possess the sickle mutation to a significant degree comprise hundreds of millions of people. Nevertheless, if the sickle gene is at equilibrium, we can assume that it has been at equilibrium for some time, long enough that at an earlier time in history the affected group had a much smaller total population, say 10 million, and then grew with the same equilibrium values. If the sickle gene arose only once, within that population, it must have started at a time when the population was much smaller still, say 1 million.\(^9\) Starting at a value of one AS individual in a group with a population of 1 million, we may ask how many times the percentage must double to reach a value of 25% AS in the population. If you could persuade someone to give you one penny on the first day of the month, two pennies on

the second, four pennies on the third, eight pennies on the fourth, and so on, doubling the number of pennies every day to the end of the month, you would receive a billion pennies on the thirtieth day of the month. Clearly, doubling has a way of sneaking up on us. For our hypothetical case of the rise of AS individuals, to progress from one in a million to 25% of the population would take 17 doublings.

By various calculations, we have now assembled all the information necessary to estimate the time of hemoglobin S microevolution. If the incidence of AS individuals doubled every 7 generations and 17 doublings would bring us to current levels, we have a total of $7 \times 17 = 119$ generations. At about 20 years per generation, our estimate is that the evolution of sickling has been going on some 24 centuries, or 2,400 years. For many reasons, this is a rough estimate and could be off by many centuries. If SS individuals had even poorer reproductive fitness, which may well have been the case, with a value of Fitness(SS) approaching 0.20 or less, then Fitness(AA) would be reduced to 0.85 and the time estimates for the rise of AS individuals could be cut by hundreds of years. Since African women traditionally marry soon after puberty, the generation time may have been several years less than 20 years. Furthermore, we have neglected effects of migrations, which are known to have been major factors in African history.\(^10\)

Migrations almost certainly played a role in the spread of the sickle gene, especially through central and southern Africa. While the diversity of languages in West Africa suggests independent origins for these languages several thousands of years ago, the Bantu languages of central and southern Africa are more closely related. Historical evidence indicates that this distribution reflects migrations of peoples who originated in the Benue River region in eastern Nigeria, where related languages are still spoken. The migrations are believed to have occurred in the first millennium B.C. and thus in the time period estimated for the origin of the sickle mutation. For reasons to be presented in Chapter 7, it would appear that the sickle gene arose independently among the Bantu peoples at an early stage of their migrations. Other migrations from the region of Nigeria toward the west are likely also to have contributed to the spread of the sickle gene.

Multiple origins of the sickle gene and migrations would change our estimates of population sizes somewhat, and would
decrease our estimates of the time during which microevolution occurred. In contrast, when population densities were lower, malaria may have been less prevalent and the selective advantage of AS individuals compared with AA individuals would have been lower. Thus, there are a number of competing factors that are difficult to analyze, but the general trend of the calculations is probably valid, indicating origins for the sickle mutation in the time span of the first millennium B.C. to the first millennium A.D.

The influence of migrations of entire populations on the spread of the sickle gene would have been a relatively slow process, compared with the influence of traders, who transported large numbers of slaves over great distances. Four old men from Kano in northern Nigeria, who had at one time participated actively in slave trade, related to M. J. Herskovits the route they traveled with their caravans—a journey of some 1,800 miles into what is now Ghana. There is evidence to suggest that such travels were made far back in antiquity, so that slave trade as well as migrations could have significantly influenced the rise of the sickle gene.11

Another factor that we have neglected, but which may have contributed to the rise of the sickle gene, is polygamy. As noted by a Ghanian specialist in sickle cell anemia, F. I. D. Konotey-Ahulu,

Population geneticists in developed countries ... completely ignore the role of polygamy in sickle cell disease by looking merely at gene frequency rather than at the total number of diseased children produced. Gene frequency might remain the same if polygamy were similarly practiced by individuals with the [sickle] trait and by normal homozygous [AA] individuals. However, the disease problem ... increases many fold if [individuals with sickle] trait take many wives. Indeed, the argument that the abnormal gene frequency goes up with polygamy cannot be entirely dismissed, because one of the reasons that Africans acquire more wives is the death of children born to the first wife. Such deaths occur more commonly in children of men with trait genes who often continue to acquire more wives until they find one whose children do not die from chwechwechwe.12

*Chwechwechwechwe* is the name for a set of symptoms in the Ga language of Ghana that Konotey-Ahulu claims is the indigenous identification of sickle cell disease. Konotey-Ahulu suggests that the onomatopoetic quality of the word *chwechwechwe* reflects the "relentless, repetitive, gnawing" of the pain associated with sickle cell anemia.

While on the decline in many regions, polygamy is nevertheless still practiced widely in parts of tropical Africa. For example, it is not hard to find Igbo men today with two wives, but they appear to be in the minority and polygamy is far below the excesses of some of the famous chiefs such as Onyeama, who claimed 53 wives at the time of his death in 1933. While polygamy may have given the sickle gene an extra boost, it could not be the primary factor. Polygamy alone does not explain the appearance of enough AS individuals to produce the SS children whose deaths could have provoked men to take more wives. Therefore, we conclude that the influence of malaria must be the most important factor, as deduced from the striking geographical coincidence of regions where the sickle gene is prevalent and where malaria is endemic. Added to this is the convincing laboratory demonstration that the malaria parasite does not multiply as well in AS cells, particularly at low levels of oxygenation, as it does in AA cells (see Chapter 6).13

The predominant factor in the spread of the sickle gene to the Western Hemisphere was, of course, slave trade, which ruptured African societies and whose consequences still weigh heavily on black Americans. In the United States currently about 10% of the black community are carriers of sickle trait. Therefore, on the average, 1% (10% × 10%) of all black couples are at risk of having a homozygous SS child. For these heterozygous couples, on the average 1 child out of 4 will be afflicted with sickle cell anemia, that is, about 1 out of every 400 black children born in the United States. This incidence, which is far higher than for other known genetic diseases, has led to routine testing of newborns for sickle cell anemia in several states. One consequence of the relatively high levels of the sickle gene in the United States was that sickle cell anemia was recognized as a disease earlier than would otherwise have been the case. In the long run, efforts to develop a treatment for American blacks, currently led by the Sickle Cell Branch of the National Institutes of Health, will also benefit Africans with sickle cell disease.

The dimensions of the slavery holocaust are difficult to grasp. According to estimates summarized in the *Cambridge Encyclopedia*...
of Africa, in the period between 1701 and 1810 over 7 million slaves were exported from Africa to the Americas. According to firsthand accounts from the later years of the eighteenth century, approximately 16,000 Igbo alone were sold into slavery each year. The practice of slavery tore at the fabric of Igbo society, as the threats of kidnapping rose dramatically and prisoners taken in warfare were also increasingly likely to be sold into slavery. By the first half of the nineteenth century transatlantic slave trade had declined, and by 1830 the ports of the Niger delta were ceasing to export slaves, as Great Britain and then other nations took active measures to eliminate slavery. Nevertheless, the corrupting effects of slave commerce remained with the Igbos for some time. One of the most unfortunate consequences was the disregard for human life bred by the slave trade, as evidenced by human sacrifices during burial ceremonies for important persons. While the practices probably predate transatlantic slave trade, the rituals reached incredible proportions, particularly in the period of "surplus" when the external markets for slaves were disappearing. According to E. Isichei, 40 slaves were killed at the burial of the leader Obi Ossah in 1845, with the surplus of slaves reaching such levels that by 1880 the rate of exchange was one horse for four to six adult slaves. Overall, slavery was a practice that decimated the population and diverted energy from more durable economic activities. According to some observers, the retarded economic development of equatorial Africa that has persisted into the twentieth century can be laid at the feet of the slave trade. The particular horrors associated with the transatlantic passage have also been thoroughly documented.14

Once the Igbo slaves reached the Americas, most traces of Igbo ethnic identity appear to have been lost in the caldron of slave commerce. This disappearance contrasts with other ethnic groups, such as the Yoruba, who have left a clearer legacy that includes relatively intact communities, notably in Cuba. Nevertheless, a 1794 history of the British colonies in the Americas by B. Edwards includes a description of slaves belonging to a group called the "Eboes." In addition to the stereotypical and deprecating remarks typical of the period, the author states that "if their confidence be once obtained, they manifest as great fidelity, affection and gratitude as can reasonably be expected from men in a state of slavery." In support of the identification of these "Eboes"

as the Igbos of Nigeria is Edwards' observation that these people "universally practice circumcision"—a practice prevalent to this day among the Igbos. Melville J. Herskovits has also summarized some of the particular characteristics reported for Igbo slaves in the New World, including a relatively high degree of suicide which, he observed, reflected the "sensitive and independent spirit" of these people.15

Few additional clues are available to link any slaves to their Igbo roots. Early lists of American slaves assembled by Newbell Puckett include a small number of given names, some of which coincide with entries in the Dictionary of Igbo Names compiled by John Njoko. These include:

<table>
<thead>
<tr>
<th>Name</th>
<th>Gender</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aba</td>
<td>Female</td>
<td>Branch</td>
</tr>
<tr>
<td>Acha</td>
<td>Male</td>
<td>Best</td>
</tr>
<tr>
<td>Ada</td>
<td>Female</td>
<td>First daughter of family</td>
</tr>
<tr>
<td>Chima</td>
<td>Male</td>
<td>God knows</td>
</tr>
<tr>
<td>Lolo</td>
<td>Female</td>
<td>Woman priest</td>
</tr>
<tr>
<td>Onah</td>
<td>Male</td>
<td>Homeward</td>
</tr>
</tbody>
</table>

Slave lists in America indicate a marked decline in African names during the nineteenth century. According to Newbell Puckett, a quarter of the male slaves in the eighteenth century had names that were recognizably African, but "by the mid-nineteenth century less than one-half of one percent of all names collected suggest the possibility of African origin."16

Even in Igbo territory, the traditional names are being replaced in large measure by Christian names. Among the ogbanje children at the Awgu school, nearly half had names commonly found in any American elementary school: Caroline, Elizabeth, Felix, Vincent. The remainder had Igbo names. Some I am not able to translate, but the following occur in Njoko's dictionary:

<table>
<thead>
<tr>
<th>Name</th>
<th>Gender</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chibue</td>
<td>Male</td>
<td>God is king</td>
</tr>
<tr>
<td>Onouchkukwu</td>
<td>Male</td>
<td>Spokesman of God</td>
</tr>
<tr>
<td>Ngosika</td>
<td>Female</td>
<td>Blessing is greater</td>
</tr>
</tbody>
</table>

The influence of Christian missionaries, reflected in the partial abandoning of traditional names, may have also served to mask traditional responses to sickle cell disease, but—as will be discussed in the following chapter—not entirely.
In summary, we may conclude that the sickle mutation occurred a few thousand years ago, and rose in incidence owing to natural selection assisted by other factors, including voluntary and involuntary migration. At the time that the sickle mutation began to spread, the transition from hunting and gathering to sedentary farming societies was occurring on a widening scale. The introduction of root crops such as yams led to the slash-and-burn cultivation that provides breeding sites for the Anopheles mosquito which transmits malaria. The spread of this disease ultimately led to the selection of sickle cell trait. The replacement of a single amino acid would have remained innocuous—existing almost exclusively in the heterozygous state in a small fraction of the population, as happens for most of the hundreds of other hemoglobin mutations discovered—were it not for malaria. By regarding growth of the malaria parasite, the sickle gene provided its possessors with a slight advantage over noncarriers, such that about 10% more survived to pass on their genes to the next generation. This advantage of AS individuals over AA individuals was almost certainly too small to be noticed by the Africans in whom this slow statistical game was being played.

While the analysis of the rise of AS individuals presented here is consistent with information from several sources, it does leave one perplexing question. Why did the sickle mutation appear in large numbers of individuals in Africa, but not in other parts of the world where malaria is endemic? The latest evidence (to be presented in Chapter 7) indicates that the sickle gene arose at least three times in Africa, whereas in the Mediterranean basin, different mutations involving the loss of the beta chain of hemoglobin arose and reached appreciable frequencies as a response, presumably, to malaria. When beta chain deficiencies occur in the homozygous state, a disease called beta-thalassemia is produced which often has more severe consequences than sickle cell anemia. Why sickle cell anemia should have predominated in Africa and beta-thalassemia in the Mediterranean basin is an intriguing mystery.

One possible explanation for the unique appearance of the sickle mutation in Africa concerns the role of diet. Linda Jackson has called attention to cassava, or manioc (Manihot esculenta, Crantz), which is one of the main staples for many African peoples and contains chemicals that can spontaneously liberate cyanate.

As discussed more fully in a later chapter, cyanate was one of the first compounds investigated as an antisickling agent for use in treating individuals with sickle cell anemia. Jackson's studies in Liberia have revealed that although malaria is encountered uniformly in different regions of the country, the incidence of the sickle gene is uneven, with lower values found in areas where cassava is regularly consumed. Although migrations could be responsible, an effect of diet may also be involved.

Cyanate from dietary sources could have influence on either sickling or malaria (and perhaps both). According to laboratory studies, cyanate inhibits growth of the malaria parasite, Plasmodium falciparum. Earlier work had established that cyanate inhibits sickling by reacting directly with hemoglobin. On the basis of these results, Jackson has proposed two ways that cassava consumption may have influenced the distribution of the sickle mutation. On the one hand, where cassava is consumed regularly in large quantities, the antimalarial effects of the cyanate may minimize the advantage of the sickle mutation. If the malaria incidence is partially reduced by diet, less of an advantage for survival with the hemoglobin S mutation would be expected for AS individuals as compared with AA individuals. This effect would explain the lower incidence of AS individuals among cassava consumers. On the other hand, sporadic consumption may lead to a modification of the hemoglobin S by cyanate that counteracts sickling, thereby increasing the fitness of the homozygous SS individuals. While this effect would not explain why lower levels of the sickle gene are found in regions with high cassava consumption, it might, according to Jackson, explain why the sickle mutation arose only in Africa, if a dietary factor is responsible.17

Unfortunately for this argument, cassava was introduced from South America by traders only a few hundred years ago, well after the sickle mutation was widespread, so it is probably not the dietary factor responsible for the spread of the sickle gene. The yam, on the other hand, a traditional foodstuff of many West Africans and a plant that has probably been cultivated for thousands of years in the region, is a better candidate. In an analysis of factors that might influence the incidence of the sickle mutation in many different ethnic groups, William Durham found a strong correlation between high S levels and yam consumption once the
data were adjusted for average rainfall. The rainfall favors growth of the Anopheles mosquito that transmits the malaria parasite, and therefore the selective advantage of the S mutation is greater in regions of heavy rainfall. In general, rainfall is limited to a short wet season, during which a burst of mosquitoes appears, since they require ponds for reproduction. In some regions there are two closely spaced rainy periods, but a peak of mosquito growth occurs only in the first. While in general the incidence of the S gene is greater in regions of more abundant rainfall, for comparable levels of rainfall the incidence of the S gene is higher for yam-consuming peoples (generally ethnic groups with languages in the Kwa class of Niger-Congo languages), as compared with rice-consuming peoples.

One possible explanation for the higher frequency of the sickle mutation among yam eaters is the effect of cyanates or other chemicals from yam that interact with hemoglobin. If the severity of sickling among SS individuals were reduced in this way, the Fitness(SS) would be raised and the percentage of AS individuals would consequently increase. One difficulty with this argument is that the same effect could diminish the properties of AS cells that help them resist malaria. Thus, even though the numbers of AS individuals born would increase, their reproductive fitness would decrease. Nevertheless, African populations could have the best of both effects, if they abstained from eating yams during the peak weeks of mosquito infestation. The ability of AS cells to resist malaria would not be diminished during the most critical period, and for the rest of the year yam consumption could improve the condition of SS individuals. Perhaps African societies have achieved this ideal, since as Durham states, “I find it provocative, therefore, to note that the consumption of new yams [the first of each year’s crop] is sternly prohibited among virtually all the Kwa yam growers until after the first of the annual rainfall peaks, and the only malaria peak, has passed.” Indeed, one of the major social events of the year for many African societies is the yam festival celebrating the moment when the new yams may be consumed.

While the role of yams in the diet could help explain the incidence of the S gene in a population, it does not directly explain why the S mutation was selected in Africa because of its antimalarial effects, whereas beta-thalassemia arose in the malarious Mediterranean basin. Moreover, the latest results with cyanate indicate that it is a very weak antisickling agent in vivo. Therefore, if a specific factor is present in yams, chemicals of an entirely different class may be involved.

An additional set of clues as to the nature of these chemicals comes from another deficiency of red blood cells, but one that does not involve hemoglobin. Certain populations around the world show a deficiency of the enzyme glucose-6-phosphate dehydrogenase, but their distribution is less clearly related to malaria than is the sickle mutation. In 1976 Huheey and Martin proposed that a deficiency of this enzyme does provide resistance to malaria, but only when fava beans are part of the diet. Ingestion of fava beans (Vicia faba) can cause red cell hemolysis, a condition known as favism which is particularly common in Mediterranean and Middle East countries. Apparently fava contains strong oxidizing agents that are normally neutralized in a series of reactions involving the enzyme glucose-6-phosphate dehydrogenase. Moreover, the Plasmodium parasites responsible for malaria are particularly sensitive to oxidizing agents. Even normal oxygen levels inhibit their growth, and a key to successful laboratory cultivation of the parasites in red cells is to diminish oxygen levels. Therefore, for individuals deficient in glucose-6-phosphate dehydrogenase, the active agents in fava, while causing favism in extreme cases, may provide a natural protection against malaria by releasing oxidizing agents. This theory has received strong confirmation recently by Golenser and co-workers, who have demonstrated a direct inhibition of Plasmodium falciparum development by the agent from fava, isouramil, but only when the parasites are grown in cells deficient in glucose-6-phosphate. 19

The connections among favism, glucose-6-phosphate, and malaria suggest that any changes which create a more oxidizing environment in the red cell may impede development of the malaria parasite. Indeed, an explanation along these lines is suspected for beta-thalassemia, involving oxidation of iron in the excess hemoglobin alpha chains, accompanied by production of highly reactive oxygen radicals and superoxides. In the case of sickle cell hemoglobin, a direct effect of this type is less obvious, but various disruptions of cellular equilibria caused by membrane deformations and damage associated with sickling could have similar effects. In this case as well, dietary factors may play a decisive role.
in determining which type of mutation is likely to be selected for its antimalarial effects. The extensive analysis by B. Ames of strong oxidizing compounds found in food illustrates that there is no shortage of candidates for possible agents that could influence red cell oxidation, in conjunction with red cell enzyme or hemoglobin deficiencies.\textsuperscript{20}

Overall, we cannot say with certainty whether or not diet played a specific role in the evolution of sickling cells. A great deal more information will be necessary to settle this matter. What we do know is that, with or without a dietary mechanism, eventually the AS individuals in tropical Africa reached large enough percentages of their respective populations that marriages between two AS individuals became increasingly common and resulted in the birth of some SS children. The sickle gene can be thought of as a natural form of chemotherapy against malaria which, although modestly successful, has a latent side effect that appears only in subsequent generations. In this case, the side effect is the appearance of disease in homozygous SS children. That they were born in large numbers, we can be sure. Since 25\% of the Igbo are carriers, 6\% (25\% \times 25\%) of all couples consist of parents who are both carriers, and one in four of their children, or 1.5\% of all Igbo children born, will be homozygous SS. At the same time, we know that children throughout history died in large numbers from many causes, with the pattern of high infant mortality being reversed only in this century and only in more medically advanced societies. Therefore, the question is now squarely framed: Were Africans generally aware of sickle cell anemia before its discovery in the United States, and if so, how did they respond?

\textbf{CHAPTER 4}

\textbf{AFRICAN REPEATER CHILDREN}

From our glimpse into the past, we have reconstructed several stages in the history of human evolution and sickle cell anemia. A remaining issue to be considered is the extent to which Africans perceived and specifically identified the consequences of sickling cells in their bloodstream. Were the \textit{ogbanje} children in the Awgu school the end result of a chain of events that was initiated with a single mutation in the DNA, one that caused red cells to sickle and to impinge on a segment of the human population which, in turn, explained these experiences largely on the basis of animistic concepts? These possible linkages were continually on my mind as I crossed the soft rolling hills between the lodgings in Enugu and the Awgu school. There were scores of walkers along the road, mainly women with enormous loads carried on their heads—a bundle of firewood, a large water jug, or sometimes a tub of produce on the way to market—women who had perhaps suffered the repeated deaths of their children from sickle cell disease.

One of the first goals of this study was to obtain blood samples from the \textit{ogbanje} children and their parents in order to test for hemoglobin S. The efforts to arrange for blood samples required several visits; with each one, more information and deeper insights into the \textit{ogbanje} tradition were obtained. We discovered that these children, with one exception, were not born missing a portion of their finger (as was "one of the stubborn ones" described by