

**A hat trick - *Plasmodium*, *Anopheles* and *Homo***

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**Abstract**


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The genomes of the malaria parasite, its vector and its host are now sequenced. This has been a tremendous scientific achievement. But will it offer hope to the millions who die from malaria each year? Yes, but only if combined with political will and social change.

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On August 29th every year, just before the onset of the rains, the devout Orthodox of Greece would celebrate the feast of Ayios Ioannis o Rigologos - St John the Malariologist. History does not record whether or not this devotion had any greater effect than the remedy of the more pragmatic Victorian Cambridgeshire Fenmen, who, on market day, "would stand in a druggist's shop ... and lay down their pence for a small packet of opium" [1]. The origin of malaria, the ague of the Fenman, was obvious to all - bad air, a "miasmatic emanation in the air" [2]. We may snigger at these hypotheses today, but malaria was then, as today, predominantly a disease of the poor. They did not command the attention of scientists and doctors then, any more than today they command the priorities of the major pharmaceutical companies, or, until the last decade or so, of many scientists. The idea that an insect could transmit disease was preposterous until Patrick Manson's demonstration, in the 1870s, that mosquitoes were the vector of filarial worms, the causative agent of elephantiasis. It was to fall to Ronald Ross, working in India, and Giovanni Grassi, in Italy, to show - in the late 1890s - that anopheline mosquitoes transmitted the malaria parasite (itself discovered only in 1880 by Alphonse Laveran).

We might be congratulated on how far we have come in the last century or so in our understanding of malaria. We might - were it not for the fact that the burden of malaria remains heavy, not in the Cambridgeshire Fens or the Pontine Marshes, but in much of sub-Saharan Africa, in Asia and in South America. The impact of malaria in Africa is horrific: it kills over one million children under five every year. Wen

Kilama, now Chair of the African Malaria Vaccine Testing Network, puts this in perspective: "It is like loading up seven Boeing 747 airliners each day, then deliberately crashing them into Mount Kilimanjaro" (quoted in [3]). The vast majority of those who die are among the poorest of society [4]. The impact of malaria, and other vector-borne diseases, to families and economies is simply appalling and cannot be ignored.

Ignored, of course, it has not been. Environmental engineers have drained marshes, entomologists have sprayed insecticides from the Cape to Cairo, immunologists have spent millions in the elusive search for a vaccine, pharmacologists have developed (a few) new drugs to kill the parasite, hoping against hope that they can outwit natural selection [5]. Hundreds of dedicated malariologists have devoted their careers to combating malaria. Yet, as Robert Desowitz so graphically tells us, the situation is now getting worse, not better [6]. In the 1960s, for example, malaria had been all but eradicated in India and Sri Lanka; today there are some 20,000 deaths a year due to malaria in India alone [7]. In the latter part of this period the proportion of monies the United States Agency for International Development (USAID) spent on malaria fell from 11% in 1985 (\$49 million in 1992 money) to 1.8% in 1994 (\$9.7 million) [8]. Thankfully, the tide may now be turning, with several high-profile initiatives to control malaria [9,10].

In the heady days of molecular biology few were attracted to study tropical diseases; neither species of *Plasmodium* nor mosquitoes were models for anything but themselves. It is

only a dozen years ago that both individual scientists and some funding agencies began to argue that the application of molecular techniques to the study of both parasites and their vectors might eventually have an impact on malaria. I rarely praise administrators, but we must give great credit to those in the MacArthur Foundation, World Health Organization (WHO) and the Wellcome Trust who backed a small band of scientists who were willing to pioneer the use of modern biological techniques to study malaria. Credit, because they were very strongly criticized for doing so, for diverting monies from more practical studies of malaria control, for example of the efficacy of insecticide-impregnated bednets (see, for example, [11]). The criticism was understandable: few of the scientists involved had any first-hand experience of malaria, all were using technologies beyond the wildest dreams of scientists in the countries where malaria is endemic and none could promise any practical benefit within decades. Only a few seriously engaged with scientists from the countries where malaria is endemic.

The publication of the “complete” genomic sequences of *Anopheles gambiae*, the most important of the vectors of malaria [12], and of two species of *Plasmodium* including *Plasmodium falciparum*, the most dangerous of the parasites of man [13] and *P. yoelii*, one of the best model parasites [14], together with that of human, is the end of the beginning of the efforts initiated in the early 1990s to study the fundamental biology of these organisms. Their publication was accompanied by the self-congratulatory hyperbole that is now customary on such occasions, spiced by rivalries between journals and between funding agencies and by the extraordinary profusion of honorary authors on these papers. These honorary authors should be ashamed of themselves - they devalue the enormous efforts of those who did the work.

But let us put all of that aside. Mosquitoes are extraordinarily abundant and versatile organisms. Most, though not all, are blood-feeders and thus wonderfully adapted to transmit blood-borne parasites, be they viruses, protozoa or nematode worms. Indeed the US even considered *Aedes aegypti* as a vector of biological warfare agents [15]. (I am not giving any credence to the malicious and politically motivated claims that they did so in India, thereby derailing what promised to be very informative trials of mosquito control by the sterile insect technique [16-18].) One approach to disease control is to eliminate - or emasculate - these vectors. Indeed that was almost achieved in many places, by insecticides, by the early 1960s. Then nature kicked back with insecticide-resistant mosquitoes, and the institutions of the WHO and other agencies were simply too hide-bound to cope (see [19] for an insider's account of the failure of the WHO's eradication campaign). Today she is kicking back again - developing resistance to the pyrethroids used, to such good effect, to impregnate bednets, extensively used for control in Vietnam and in some parts of Africa.

## The parasites

*Plasmodium* is the first of the Protoctista to be sequenced, the first of an extraordinarily diverse kingdom of some 30 phyla. Sequencing the genome of *Plasmodium falciparum* is a major technical achievement - not least because its assembly was so tough, because the genome is 80.6% A+T. The hope is that this sequence will offer new targets for both antimalarial drugs and vaccines. This hope is far from a day-dream: antimalarials in clinical use target only three aspects of the metabolism of *Plasmodium*, heme breakdown (targeted, for example, by chloroquine and artemisinins), folate biosynthesis (by sulphonamides) and electron transport (by atovaquone, for example); Gardner and colleagues identify, from their analysis of the metabolism of *P. falciparum* [13], at least 10 other classes of target, including some in the apicoplast, the extraordinary organelle derived from a plastid used by the parasite for both fatty acid biosynthesis and for the mevalonate-independent biosynthesis of isoprenoids [20].

The search for a vaccine against malaria has been long and arduous. Some thirty or so parasite proteins are now target antigens for vaccines and the genome offers many more. There are some 236 genes, in three families, encoding proteins that are expressed on the surface of infected erythrocytes [13]. These genes are mostly sub-telomeric in location and these regions, which may be up to 70 kilobases in length, promiscuously recombine between chromosomes - presumably in an adaptation to generate variation so as to evade the host's immune response to infection. Gardner *et al.* [13] predict that the sequence offers “hundreds” of potential novel targets for vaccines; now the trick will be to mine the proteome of *Plasmodium* for the identification of those targets that are not hypervariable.

None of these efforts, to develop new antimalarials or effective vaccines, will impact malaria unless they are available in the countries where the disease is endemic. There is no doubt that they will impact tourist malaria, but that is relatively trivial. The political challenge is not only to encourage research but also to ensure that its products are not simply used to bolster the share prices of multinational pharmaceutical companies, as we have already seen with agents active against human immunodeficiency virus (HIV). The Agreement on Trade Related Aspects of Intellectual Property (TRIPS) is a major barrier to the provision of affordable drugs to the world's poor; and the US government continues to block the implementation of the Doha Declaration, which states that public health has precedence over the patent rules of the World Trade Organization [21].

## The vectors

As a *Drosophila* geneticist I rejoice in the genomic sequence of another fly, albeit one with whom *Drosophila* has not enjoyed a common ancestor for 250 million years or so.

There is a rich mine of information here for the biologist, not least the rather surprisingly low fraction of predicted proteins in *A. gambiae* that have clear orthologs in *Drosophila melanogaster* (47%), despite very considerable conservation of synteny between the two species [22]. There has been considerable interest in engineering mosquitoes to be, for example, resistant to parasites, and then attempting to drive the responsible transgene into natural populations (see [23-25]). For this even to be a starter knowledge of the population structure of the vectors is vital. Many mosquito 'species' have very complex populations, and *A. gambiae* is no exception. These populations are speciating in front of our eyes; not only are there seven sibling species that differ in ecology and vectorial capacity, but there are species *in statu nascendi*, to use Dobzhansky's felicitous phrase. In Mali, for example, *A. gambiae sensu strictu* exists in three reproductively isolated chromosomal forms, with clear evidence of differential adaptation [26-28] and two characterized molecular types (based on rDNA), and these classifications overlap [27]. The strain of *A. gambiae* sequenced was, fortuitously, one in which the genomes of two of these forms had been mixed by laboratory crossing. This proved to give the sequence assemblers some headaches [12], but has provided researchers with a wealth of single-nucleotide polymorphisms (SNPs) for population studies. There can be no question that the availability of these markers will be a stimulus to studies of the population genetics and evolutionary processes in this species complex, studies that will be of far more than academic interest should either the sterile insect technique or the release of genetically transformed flies become a feasible control measure.

### The host, and his responsibilities

Was it worth it? As a biologist interested in fundamental issues, one must, enthusiastically, answer 'Yes'; for a malarialogist working in the field in a chronically underfunded lab in Ouagadougou, I suspect the answer will be 'No', as she would rather have a dissecting microscope or a Shop-Vac (a hand-held vacuum cleaner for collecting resting mosquitoes). I have a strong hunch that malaria will not be defeated by science, or at least, not by science alone, although I accept the arguments of Carlos Morel, Director of Tropical Diseases Research for WHO, that basic research is for the long, not the short, term [29].

Yet, whatever the advances of science, the defeat of malaria, as with so many diseases that primarily affect the poor and disenfranchised, will depend upon political will, and on economic and social reforms. It is no coincidence that malaria, and many other vector-borne diseases, have been all but eliminated from Europe and North America yet remain scourges in the Third World; no coincidence that on the Texan side of the Rio Grande there were 64 cases of mosquito-transmitted dengue fever in two decades (1980-1999) but over 60,000 cases in the Mexican states immediately to

the south of the Rio Grande [30]. It would cost about \$470 million per year to provide free insecticide-treated bednets to all in rural tropical Africa; this is about half of the amount spent (\$860 million) on the control of cat fleas in the USA (C. Curtis, personal communication; the cat flea control figure is based on data from MK Rust, University of California Riverside). Bednets will not eliminate malaria - but they will have an immediate and dramatic effect on morbidity and mortality. But I must not end on so pessimistic a note. The greatest achievement of the last decade, an achievement crowned by the publication and analyses of the genomes of parasite, vector and host, has been to bring malaria to the centre stage of modern biology: to paraphrase the words of Sam Weller, we now "know wot's wot, we does" [31]. And that must be good; it must offer hope.

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