numerous publications that came out from such studies have not halted the killing of wild elephants in Sri Lanka. Despite much discussion, argument and debate, and despite the efforts of both government and non-government organizations, the conflict between man and elephant shows no let up. While we deliberate, elephants die.

But not everything about elephant conservation in Sri Lanka is doom and gloom. There are some encouraging new developments. Three enterprising Sri Lankans are trying to reconcile elephant conservation with the welfare of the human communities. They have already demonstrated through their innovative, eco-friendly projects that there are indeed ways in which the rural people who suffer elephant depredations can derive tangible benefits from the presence of the elephant. Their projects are designed to utilize sustainably an end-product of the elephant that no one, so far, seems to have cared about – the dung.

An adult elephant on average can produce up to 200 kg of dung per day. Three innovative projects, all self-funded, plan to tap this resource to demonstrate that the elephant can indeed be an asset rather than a pest to farmers in conflict areas. One project, the brainchild of Thusitha Ranasinghe (Maximus Pvt Ltd) deals with the manufacture of paper from elephant dung. It was started in 1997 and has successfully produced and marketed what is known as 'pachyderm' paper. With the exception of toilet paper and chocolate wrappers, the 'pachyderm' paper can be used to manufacture a variety of items such as notebooks, cards, badges, boxes, albums, and other stationery which have proven extremely popular with both locals and foreign tourists who care about the environment. The second project concerns the transformation of 'pachyderm' paper by Ranjan Rajaratnam, CEO, Badu Pvt Ltd into such up-market, value-added products as lamp-shades and greeting cards. The 'pachyderm' paper used in the manufacture of lamp-shades, given its characteristic texture as a result of the fibrous nature of the elephant dung, provides a soft but bright light, ideal for homes. These items have become so popular that they are regularly exported to the West and therefore are an important source of foreign exchange. The third project, designed by S. Wijeyamohan, a wildlife biologist at the Vavuniya Campus, University of Jaffna, produces biogas from elephant dung for use as a cooking fuel by the rural people in conflict areas.

Biogas produced from elephant dung has a dual purpose: it can be used as a deterrent against crop-raiding elephants through night-time burning along the periphery of home gardens, given that wild elephants are known to avoid firelines set in the forest. While burning firewood as a deterrent will only promote further destruction of our already diminished forests by people gathering fuelwood, the use of biogas on the other hand, would obviate this need to collect firewood from the forest. Another by-product of the biogas project is the residue, which is a high-quality fertilizer for use in organic farming. Mushroom-growers are well aware of the value of horse dung as a substrate for their fungus cultures. The elephant being a non-ruminant, like the horse, its dung could be used to grow mushrooms. Wild mushrooms grow on elephant dung in the wild. Elephant dung has also proved effective against mosquitoes. Dried dung, if burnt, helps keep mosquitoes at bay.

It must be emphasized that elephant dung alone cannot be a panacea for the ongoing human–elephant conflict in Sri Lanka. Although the use of elephant dung for the benefit of the rural poor is unlikely to eradicate the conflict, it will certainly go some way towards changing the perceptions of the people and their hostility towards the elephant. Elephant dung can contribute towards conserving its producer – the island's largest land mammal. Conservation of elephant in Sri Lanka is inextricably linked to the welfare of the rural poor and the socially disadvantaged, who are struggling to survive in areas frequented by potentially dangerous wildlife. Elephant conservation is not only about the survival of Sri Lanka's terrestrial megaherbivore; it is also about dollars, diversity, and people's welfare too.

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RESEARCH NEWS

Sequencing of the malarial parasite genome reveals potential drug targets to combat malaria

Pratibha Mehta Luthra and Rajesh Luthra*

Malaria remains a serious endemic disease in more than 100 countries in Africa, Asia, Latin America and South America[1]. Almost forty per cent of the world population is at the risk of malarial infection. Each year, 100 million people experience malarial illness and there are reports of 1–2 million deaths per year. The mortality rate is between 20 and 50% in patients with severe and complicated disease.

Human malaria is caused by any of the four protozoan species of the genus *Plasmodium*, viz. *P. vivax*, *P. falciparum* *P. ovale* and *P. malariae*. *P. vivax* is responsible for the majority of malaria cases worldwide, and *P. falciparum*, which is susceptible to chloroquine, causes the majority of malaria-related fatalities[2]. The problem of choloroquine resistance in *P. falciparum* is increasing in intensity and has spread to almost all *P. falciparum*-infested areas.

The malarial parasite is required to alternate between its vertebrate and invertebrate hosts to complete its life cycle. Transmission of malarial parasite

from vertebrate host is mediated exclusively by mature gametocytes, the cells that are arrested in G phase of the cell cycle, until taken up in the blood meal of the engorging female mosquito[3]. Because of the complexity of intercellular regulatory pathways of microgametogenesis in *Plasmodium*, no universally applicable form of vector control is available. The most effective tool to deal with malaria has been antiparasitic drugs. The drugs currently being explored come from just three families: quinolines (quinine, chloroquine, mefloquine, primaquine), antifolates (sulphadoxine, pyrimethamine) and artemisinin derivatives[4]. Quinolines act within the food vacuole, in which the host haemoglobin is degraded as food stuff to haemozoin (Figure 1). Antifolates inhibit folic-acid biosynthesis by competing either with *p*-aminobenzoic acid for the active site of dihydrofolate synthetase or by inhibiting dihydrofolate reductase. Artemisinin, a sesquiterpene lactone peroxide (endoperoxide), exerts its effect by interfering with the plasmodial haemoglobin catabolic pathway.

Artemisinin is a promising class of naturally occurring antimalarials, isolated from the plant *Artemisia annua*[5]. The artemisinin derivatives, however, have short half-lives and short course treatment is associated with an unacceptably high (> 10%) rate of recrudescent infections. The rapid emergence of resistance of malarial strains to conventional drugs and the lack of a malaria vaccine have urged the scientific community to search for new targets and a new family of chemotherapeutic agents. A novel diterpene peroxide, isolated from *Amonium krevanh* Pierre, has shown potent activity against *P. falciparum*[6]. Taxol, a complex diterpene, isolated first from the plant *Taxus brevifolia*, which is normally used to treat a variety of human cancers, has been shown to be extremely effective against chloroquine and pyrimethamine-resistant malarial parasite[7]. The drug also exhibits considerable antiproliferative activity against other parasitic protozoa, including *Trypanosoma cruzi* and *Leishmania donovani*[8]. However, the life cycle of a malarial parasite, unlike other parasitic protozoa, is complex passing through morphologically and biochemically distinct developmental stages. During the life cycle of the unicellular malarial parasite, microtubules have been shown to be responsible for many essential functions, such as structural integrity, cell division, axonema structure, flagella integrity and exflagellation of male gametocytes. A natural limonoid azadirachtin, isolated from the neem plant *Azadirachta indica* has been reported to affect exflagellation[9]. Ultrastructure and biochemical studies have demonstrated that cellular cytoskeleton matrix consists of at least three different filaments. Each filament consists of heterodimers of α and β-tubulin. In *P. falciparum*, apart from the α and β-tubulin, γ-tubulin has also been identified[3]. This is a novel member of the tubulin super family and may be indispensible for nuclear division and microtubule assembly in the malarial parasite. Taxol has a unique mechanism of action; it promotes the assembly of stable microtubules and inhibits its disassembly. Selective inhibitors or drugs like taxol that interfere with the tubulin microtubules could lead to the possible development of directed chemotherapeutic prevention measures.

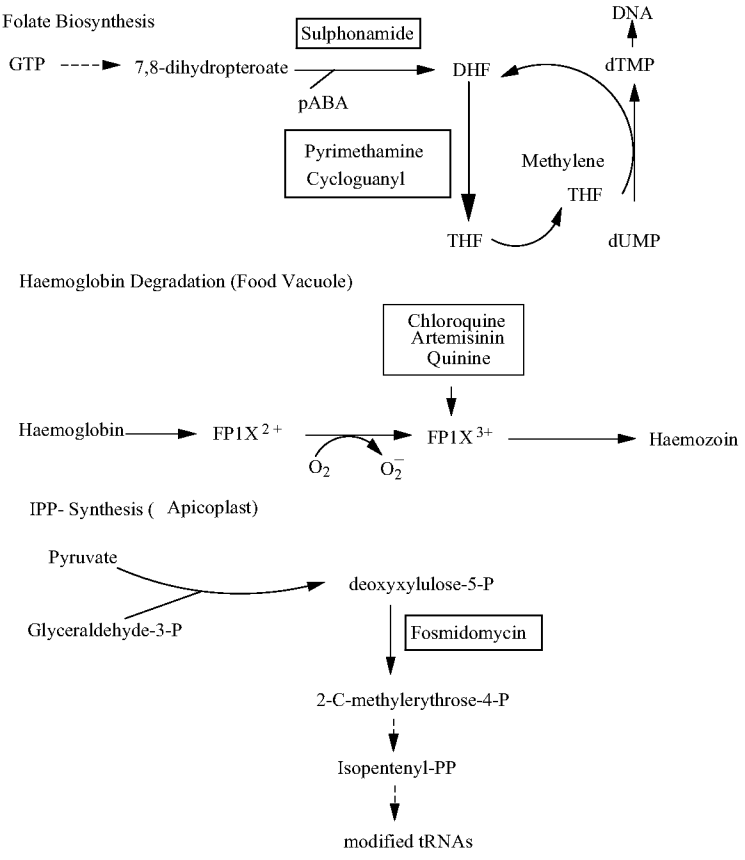

Figure 1. Metabolic steps inhibited by antimalarials in *Plasmodium falciparum*. GTP, Guanidine triphosphate; pABA, para-amino benzoic acid; DHF, Dihydrofolate; THF, Tetrahydrofolate; dUMP, Deoxy uridine monophosphate; dTMP, Deoxy thymidine monophosphate; IPP, Isopentenyl pyrophosphate; $FP1X^{2+}$ and $FP1X^{3+}$, Ferro and ferric protoporphyrin 1X.

International bodies have long recognized the extent of human suffering caused by malaria, and many initiatives have been taken over the years to eradicate malaria. In 1996, an international consortium of scientists from more than a dozen institutions set out to determine the sequence of the malarial parasite genome with the hope that it might lead to the development of effective drugs and vaccines against this devastating disease. The end of 2002 marked the successful completion of sequencing of the *P. falciparum* genome[10].

The *P. falciparum* genome comprises 14 chromosomes, 23 million base pairs and an estimated 5279 genes. The strategy for determining the *P. falciparum* genome sequence[10] involved physical separation at first of its 14 chromosomes by pulsed gel electrophoresis; the genome was then sequenced by a whole chromosome shot-gun strategy as that applied for sequencing the human, mouse and

fruit fly genomes. Random fragments of genome DNA were first cloned in bacteria and sequenced, and the overlapping clones were then assembled into contiguous sequences. The biggest hurdle to sequencing of the *P. falciparum* genome was its extremely biased base composition. More than 80% of the bases are either As or Ts. In fact, regions of the genome that do not code for genes average more than 85% As or Ts. The high AT bias meant that several experimental and computational hurdles had to be overcome to accurately sequence and assemble the genome. Sequence accuracy was verified by optical restriction mapping. In this technique, a purified chromosome is cut into segments with an enzyme known to cut DNA at particular sequences, and the segments are separated according to size by gel electrophoresis, producing the optical map, which is then compared with predicted patterns derived from the assembled sequence. The function of 60% of the postulated 5279 genes is at present unknown, because these genes match no other sequence in existing gene data banks. Authentic peptides corresponding to 2391 of the genes, however, have been identified using mass spectroscopy, including many of those for which functions have not yet been found.

The genome sequence of *P. falciparum*[10] and comparison of its genome with that of a rodent malarial parasite *Plasmodium yoelii yoelli*[11], and two proteomic studies of the proteins expressed at different stages in the life cycle of the parasite[12,13] have thrown some light on parasite biology and possible drug targets[14]. One notable feature of the parasite genome is the apparent absence of genes for proteins that produce ATP. There are no predicted genes for protein components of two key mitochondrial enzymes, ATP synthase and NADH dehydrogenase. Perhaps, *P. falciparum* generates and stores energy by using novel proteins or mechanisms, which may act as potential drug targets. About 10% of the predicted genes encode for proteins associated with apicoplasts which are known to be involved in the biosynthesis of fatty acids, isoprenoids, membrane proteins and for iron metabolism. Analysis of these genes may reveal other functions and possibly new drug targets. Proteomic studies have indicated that the regulation of protein levels in the parasite is controlled through mRNA processing and translocation rather than by gene transcription. This is perhaps a general feature of the parasite and another potential drug target.

Much before the completion of the malarial parasite genome-sequencing project, its fruits are being realized. A mevalonate-independent pathway of isoprenoid biosynthesis, present in *P. falciparum*, was shown to represent an effective target for chemotherapy in malaria[15]. The non-mevalonate pathway involves a free or phosphorylated intermediate of 1-deoxy-D-xylulose, resulting from a condensation of pyruvate with glyceraldehyde 3-phosphate, and has recently been described in eubacteria, algae and higher plants[16]. Jomaa *et al.*[15] identified two genes that encode two key enzymes in the DOXP pathway – DOXP-synthase and DOXP-reductoisomerase – from sequence data provided by the malaria genome project. DOXP pathway has been found to be functionally active during growth of the malarial parasite inside red blood cells and the pathway, in particular DOXP-reductoisomerase activity is essential for parasite growth. Two phosphonate compounds, fosmidomycin and FR 900 098, when given orally were found to inhibit DOXP-reductoisomerase, and were able to inhibit the growth of *P. falciparum* in culture, and to cure mice infected with the related malarial parasite *P. vinckei*.

Studies on the parasite genome are being carried out to reveal how *P. falciparum* interacts with its host and carrier, and about genes involved in parasite regulation by the human immune system. Much of the genes for variant surface proteins are found at the ends of the chromosomes where they undergo higher rates of genetic exchange. This process generates greater diversity, allowing the parasite to evade the host defences. Proteomic analysis[12] revealed that peptides derived from many of the *var* genes occur in sporozoites, which are produced in mosquitoes and invade the human liver during the initial infection. Research on *P. falciparum var* genes has focused on their role in enabling infected red blood cells to stick to small blood vessels in the brain. This feature is associated with the fatal form of the disease, cerebral malaria[14]. Thus the sequence of the genes tells us something about its functions, but the position of genes on the chromosome can also contribute to the biology of the parasite.

The genome of the malarial parasite vector *Anopheles gambiae* – the mosquito, has also been simultaneously sequenced[17]. Information about the parasite and mosquito genome together with draft sequences of the human genome can lead to better understanding of the interactions among these species that have long been evolving together. Comparison of the parasite genome with the human genome might further reveal some pathways that are specific to the pathogen, which might help in the development of specific drugs with lesser side effects.

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