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Phylogeny of plasmodium falciparum

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has arisen in the past few years which maintains that myxosporeans have a heteroxenous cycle, using fish and oligochaetes alternately. The oligochaete-infecting stage produces spores that have hitherto been known as a group of parasites called actinosporeans. As proponents of this view, H. Yokohama, K. Ogawa and H. Wakabayashi (University of Tokyo, Japan) described a technique for collecting actinosporeans from oligochaetes and claim to have shown that the actinosporean Raobeia, from a tubificid, transforms into the myxosporean Myxobolus, in goldfish. In contrast, C. Szekely and K. Molnar (Hungarian Academy of Sciences, Budapest, Hungary) have been unsuccessful in their attempts to induce actinosporean infections in tubificids using 15 different myxosporean species, and attempts at direct infections of fish also failed. In his discussion of fish losses due to myxosporean disease, O.N. Yunchis (State Institute for Lake and River Fisheries, St Petersburg, Russia) made it clear that he was not convinced that all of these organisms have the indirect cycle described. The meeting was left to ponder on these incompatible views.

The impact on farmed fish, particularly salmonids, of the digenean Diplostomum has lead to an interest in the organism which was reflected in several papers. This helminth has a metacercarial stage in the fish eye and is a major helminth pathogen of freshwater fish. Most of the studies of Diplostomum have been undertaken in the Soviet Union, and were exemplified at this meeting by N.I. Yurpalova’s (Biological Institute Siberian Branch, Novosibirsk, Russia) description of the life cycle of D. chromatophorum. However, there has been an upsurgence of interest in other countries, notably in the UK. A common theme to all contributions was the difficulty of identification at the metacercarial stage by traditional methods. Techniques such as scanning electron microscopy (used by C.A. McKeown and S.W.B. Irwin, University of Ulster, Newtown Abbey, UK) and multivariate analyses (used by J.C. Chubb and M. Faulkner, University of Liverpool, UK) have enabled some morphological forms to be distinguished but our understanding of the status of these forms, and the many intermediates found, is still limited.

In all, 25 invited papers, 40 short papers and ten posters were presented. All the papers were given in English and therefore provided a useful opportunity for western workers to become acquainted with the wide range of activity in Soviet fish parasitology.

Because of the growing interest in this subject resulting from such factors as the increase in commercial aquaculture, the increasing appreciation of the pathological importance of parasites in food fishes and the use of parasites as biological indicators, it was decided at the closing session that an International Ichthyoparasitological Newsletter should be published biannually to allow for the informal dissemination of information and to serve as a point of contact for ichthyoparasitologists worldwide. The enterprise will be organized by K. Nagasawa (National Research Institute of Far Seas Fisheries, 5-7-1, Onido, Shizuoka, Shizuoka 424, Japan) and involve local co-ordinators in each country.

The symposium committee, chaired by D.I. Gibson (The Natural History Museum, London, UK), is in the process of arranging the Fourth Symposium, due to take place in Germany in 1995. It is hoped that, in a more accessible venue, the symposium will get the level of support that this growing discipline deserves.

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**Comment**

**Phylogeny of Plasmodium falciparum**

F.J. Ayala and W.M. Fitch

Waters et al. have compared the small subunit ribosomal RNA (rRNA) sequences of several Plasmodium species and concluded that *P. falciparum*, the agent of the most severe form of human malaria, is phylogenetically closer to two species (*P. gillainaceum* and *P. lophurae*) that are parasitic to birds than to other *Plasmodium* species that are parasitic to humans, simians or rodents. This is both interesting and unexpected but it does not support the notion that the origin of *P. falciparum* is from avian stock, nor that *infection* by *P. falciparum* is a recent acquisition of man and possibly coincident with the onset of an agriculture-based life style (Ref. 1). The matter is scientifically important and of considerable medical consequence, given that malaria is a major cause of human mortality. The conclusion that people probably caught the parasite from birds as an indirect consequence of agriculture has already reached the secondary literature.

Waters et al. show an average divergence between the rRNA sequences of *P. falciparum* and the two avian species of 8.96% (Fig. 2 of Ref. 1). The rate of evolution of rRNA sequences has been estimated as 1% divergence per 50 million years. Accordingly, the divergence of the *P. falciparum* and the avian lineages occurred approximately 448 million years ago. This date precedes the time of divergence of the mammal and bird lineages from their last common reptilian ancestor in the late Carboniferous, about 300 million years ago. *P. falciparum* may well be a recent human parasite, as has been argued but the evidence adduced by Waters et al. does not particularly favor the notion that the acquisition was by lateral transfer from an avian parasite. What their data show is that the genus *Plasmodium* is of ancient evolutionary origin and that the lineages leading to the species that parasitize various mammals diverged from one another several hundred million years before the mammal lineages diverged from each other, which happened less than 100 million years ago.

Table I shows the percent divergence (or the average difference) between *Plasmodium* species (or sets of species), calculated from the data displayed in Fig. 2 of Waters et al. (and assuming the phylogenetic relationships inferred by these authors). Table I also gives the estimated time since the divergence of the species compared, based on the rate of evolution of rRNA sequences. The two most closely related *Plasmodium* species, according to the data, are *P. vivax* and *P. fragile*, which have human and simian hosts, respectively. The estimated time of their divergence...
is 177 million years ago, much before the divergence between marsupial and placental mammals, which happened in the Cretaceous, which began 144 million years ago. The divergence between the two avian parasites *P. gallinaceum* and *P. lothoarae* is estimated to have occurred approximately 218 million years ago, much before the divergence of the oldest living bird lineages, which shared their last common ancestor also in the Cretaceous. The last common ancestor of *P. falciparum* and the two avian Plasmodium species is estimated to have lived 448 million years ago, in the Ordovician, before the evolution of the first terrestrial vertebrates.

The divergence dates given in Table 1 are subject to various sources of error, most notably the rate of evolution of rRNA. However, it seems unlikely that the error would be as great as an order of magnitude. If we assume a rate of rRNA divergence that is ten times faster than that estimated by Wilson et al., the divergence of *P. falciparum* from the two avian Plasmodium species would have occurred 45 million years ago, whereas the last common ancestor of humans and apes lived less than ten million years ago.

One important result of Waters et al. is that the Plasmodium phylogeny depicted in their Fig. 2 is incongruent with the phylogeny of the host species. If both phylogenies are correct, this implies that host switching must have occurred. But it does not tell us either the host from which humans acquired *P. falciparum* or the time when this happened. If humans acquired *P. falciparum* from some other vertebrate as recently as the beginning of agriculture, then the rRNA of the Plasmodium that parasitizes this vertebrate should be barely distinguishable (if at all) from *P. falciparum*. A survey of other mammals and birds, using techniques such as PCR-amplified DNA or immunology, may be the best way to explore how widespread such close relatives of *P. falciparum* are.

References


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**Leishmanial Protein Kinases Phosphorylate Components of the Complement System**


As Leishmania promastigotes develop in the sandfly vector, the parasites change from noninfective logarithmic forms to virulent metacyclic forms which pre-adapt to life in the mammalian (human) host. In *L. major*, changes in two surface antigens (promastigote surface protease and lipophosphoglycan), both involved in the invasion of host macrophages, have been observed. Also during this change to metacyclics, protein kinase activity increases. Prior to macrophage phagocytosis, *Leishmania* promastigotes are exposed in the blood to the human complement system. As this protein network is responsible for killing many of the pathogens of the human host, the evasion of the complement cascade is an integral part of the parasite life cycle. It has been previously reported that phosphorylation of specific amino acids on proteins can modulate the kinetics of protein proteolytic cleavage in biological processes. Using the externally oriented protein kinase-I (LPK-I) from *L. major*, these colleagues from the Weizmann Institute have succeeded in phosphorylating purified C3, C5 and C9. Concentrating on C3, they have identified Ser71 of C3a (equivalent to Ser720 in C3) as the potential phosphorylation site of LPK-I. Treatment of C3 with either methylamine or freeze-thawing C3 prevented phosphorylation, suggesting a role for substrate conformation in the recognition of LPK-I. As both C3 and C3b are phosphorylated by viable *L. major* promastigotes, more than one surface protein kinase is implicated. Extracellular protein phosphorylation may play a role in the interaction of the parasite with the host’s immune system and in the survival of *Leishmania*.

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**Large-scale Eradication of Rabies Using Recombinant Vaccinia—Rabies Vaccine**


From laboratories in France and Belgium comes a 'tour de force' in a pilot field application of an infectious agent. The major European disease vector of rabies is the red fox, Vulpes vulpes, and the only appropriate method of vaccination is the distribution of live vaccine bait. The use of attenuated rabies virus strains is unsuitable because they retain pathogenicity for rodents, and are pathogenic (in North America) for the striped skunk and ineffective in the racoon. A recombinant vaccinia virus, VVTGgRB, expressing the surface glycoprotein of rabies virus, has been constructed and used in vaccine bait which also contains a tetracycline biomarker. A 2200 km² region of southern Belgium was approved by WHO for full-scale vacci-