Letters

Heterogeneity of Host-related DNA Sequences in Schistosomes

Simpson and Pena have recently commented[1] on our paper entitled 'Existence of host-related DNA sequences in the schistosome genome'[2].

In a previous report, Simpson and colleagues[3] investigated the possibility that host antigens detected on the parasite surface were due to the presence and expression of the respective genes in the schistosome genome. However, they found that the H-2 histocompatibility antigens expressed on the surface of lung-stage parasites are not encoded by the parasite genome and that the antigens are acquired as intact glycoproteins from the host. In our work, the DNA fingerprints of schistosomes using the mo-2 sequence[4], a mouse-specific mini-satellite, resemble those of the ICR mouse, the experimental final host. In addition, the DNA fingerprints of *Schistosoma japonicum* recovered from AKR mice resemble those of the AKR mice themselves. Clearly, the DNA fingerprints of schistosomes using the mo-2 sequence also distinguish their final hosts. These observations suggest that the mo-2 sequence is incorporated into the schistosomes within one generation, rather than during a long period of their evolution.

The sequences detected in the DNA isolated from eggs of *S. japonicum* and *S. mansoni*, and adult worms of *S. japonicum* include the mouse type 1 and type 2 Alu sequences, the mo-2 sequence, the type A retrovirus sequence and endogenous type C retrovirus (A-1) sequence, which is almost completely absent from the *env* region[5]. The sequences homologous to the *env* gene of mouse ecotropic and xenotropic retroviruses were apparently detected in the DNA of male *S. mansoni* adults by blot hybridization[6]. Thus a heterogeneity of host-related DNA sequences can be observed among schistosome genomes.

According to their article, Pena had shown hybridization of *S. mansoni* genomic DNA with Jeffreys' 33.15 probe, yielding DNA fingerprints (unpublished). The core sequence used in our study, obtained from Jeffreys *et al.*[7], differs from the 33.15 probe and could not be detected in the DNAs of *S. japonicum* and *S. mansoni* under the conditions we describe[2].

If host-related sequences in the schistosomes can be incorporated into the schistosomes within one generation, as the mo-2 repetitive sequence apparently is, both incorporation and elimination might occur during their life cycle. Therefore, it is important to know what kinds of host-related DNA sequences can be detected in different developmental stages of the parasite.

Simpson and Pena referred to earlier work[8], which showed differentiation of schistosomes by species, strain and sex, and they claimed that the repetitive sequences at the different stages of the parasite life cycle do not show stage-specific variation. However, they had not extensively observed stage-specific DNA alteration of the schistosomes in their work. Further studies on DNA rearrangement during the development of schistosomes will be necessary to clarify how this happens.

Generally the different bands obtained with fingerprinting probes are due to multiple mini-satellite loci, which are scattered throughout the genome. However, the mo-2 mini-satellite is located on one or more restricted chromosomes in the mouse (R. Kominami, unpublished) and this may indicate that this sequence is preferentially transferred to the schistosome genome, although its mechanism is still unknown. This evidence has led to the hypothesis that unequal incorporation of the host gene occurs in the schistosome genomes. The phenomenon of genetic exchange between parasite and host is not without precedence: *Drosophila melanogaster* is thought to have acquired transposable P elements from a parasitic mite[9]. Progress in this field of research must be expected.

References

1 Simpson, A.J.G. and Pena, S.D.J. (1991) *Parasitology Today* 7, 266
2 Iwamura, Y. *et al.* (1991) *Parasitology* 102, 397–403
3 Simpson, A.J.G. *et al.* (1983) *J. Immunol.* 131, 962–965
4 Kominami, R. *et al.* (1989) *Mouse Newsl.* 85, 85
5 Khan, A.S. *et al.* (1982) *J. Virol.* 44, 625–636
6 Tanaka, M. *et al.* (1989) *Parasitology* 99, 31–38
7 Jeffreys, A.J. *et al.* (1985) *Nature* 316, 76–79
8 McCutchan, T.F. *et al.* (1984) *Proc. Natl Acad. Sci. USA* 81, 889–893
9 Marx, J. (1991) *Science* 253, 1092–1093

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Hohlzylinders

Recent speculation as to the nature of the so-called spherical bodies of *Plasmodium* species and whether they may be the source of the extrachromosomal 35 kilobase (kb) DNA, was of interest to our laboratory, as we have found similar structures in abundance in the developmental stages of a species of *Haemogregarina* (Fig. 1). Contrary to Kilejian's suggestion[1], these bodies were not first described from malaria parasites. It appears that they were first recognized by Scholtyseck and Piekarski[2] as a 'grosse Vakuole mit Kräftinger Wandung' in *Eimeria* and have since been known by various names: 'Lamellärer Körper', 'Hohlzylinder' and 'Golgi adjunct' in *Toxoplasma*[3,4], 'dickwandiger Vakuole' and 'dickwandiger Vesikel' in *Frenkelia*[5], 'unknown structure' in *Besnoitia*[6] and 'vésicule plurimembranaire' in *Toxoplasma, Dehornia* and others[7]. As was guessed by Wilson[8], these structures are, in fact, recognizable in other apicomplexans, including the gregarine *Selenidium*[9], the piroplasm *Anthemosoma*[10] and some poorly known coccidia such as *Coelotropha*[11] and *Aggregata*[12].

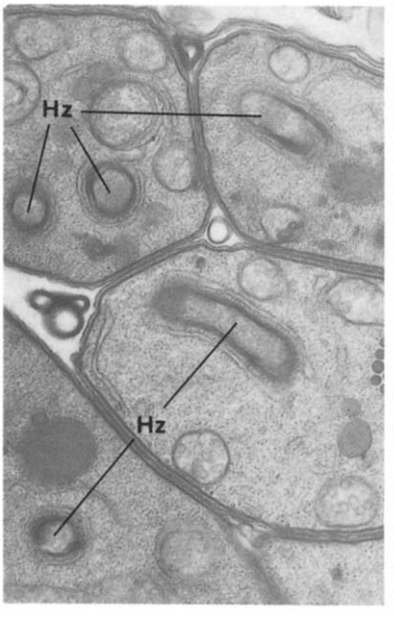

Fig. 1. Sporogonic stages of a haemogregarine demonstrating the multimembraned organelles or hohlzylinders (Hz) in various planes of section.

The relative abundance with which we have found these multimembraned bodies in a haemogregarine (Fig. 1), as have Vivier *et al.*[13] in *Hepatozoon domerguei*, suggests that the Adeleorina may provide an ideal model for determining whether or not they harbour the 35 kb DNA, or are remnants of a plastid present in dinoflagellate-like ancestors. In relation to this last question, it is of note that these structures are not apparent in the apicomplexan-like dinoflagellate *Spiromonas*[14] nor in *Perkinsus atlanticus*[15]. What is evident from this discussion is that we presently lack a common term to describe these enigmatic organelles. Scholtyseck and Piekarski's original term is obviously too cumbersome, as are others. 'Spherical body' is potentially misleading, as in most cases they are cylindrical in three dimensions (Fig. 1). Furthermore, this term could be confused with the spherical dense bodies of apicomplexans. Similarly, 'Golgi adjunct' is potentially dubious since an association with the Golgi apparatus has not been satisfactorily demonstrated. As such, Van der Zypen and Piekarski's 'hohlzylinder' appears to be both appropriately descriptive and concise.

References
1 Kilejian, A. (1991) *Parasitology Today* 7, 309
2 Scholtyseck, E. and Piekarski, G. (1965) *Z. Parasitenkd.* 26, 93–115
3 Van der Zypen, E. and Piekarski, G. (1967) *Z. Bakt. Parasit. Infekt. Hyg.* 203, 495–517
4 Sheffield, H.G. and Melton, M.L. (1968) *J. Parasitol.* 54, 209–226
5 Kepka, O. and Scholtyseck, E. (1970) *Protistologica* 6, 249–266
6 Sheffield, H.G. (1966) *J. Parasitol.* 52, 583–594
7 Porchet-Hennere, E. (1972) *Protistologica* 8, 245–256
8 Wilson, I. (1991) *Parasitology Today* 7, 309
9 Schrevel, J. (1971) *Protistologica* 7, 101–130
10 Vivier, E. and Petitprez, A. (1969) *Protistologica* 5, 363–380
11 Vivier, E. and Hennere, E. (1965) *Protistologica* 1, 89–104
12 Porchet-Hennere, E. and Richard, A. (1970) *Z. Zellforsch.* 103, 179–191
13 Vivier, E. *et al.* (1972) *Protistologica* 8, 315–333
14 Brugerolle, G. and Mignot, J.P. (1979) *Protistologica* 15, 183–196
15 Azevedo, C. (1989) *J. Parasitol.* 75, 627–635

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Plasmodium falciparum: Birds to Humans

Ayala and Fitch present a theory about the evolution of parasites with which we disagree, as we feel that it may not take into consideration the peculiarities of the ribosomal (r) RNA system of *Plasmodium*, or be consistent with the literature concerning biological comparisons of the members of the genus. Although their comments are well founded, we would like to present some of the considerations that lead us away from this novel theory. This will give also give us an opportunity to appraise the reader of other criticisms of our recent manuscript. All criticism is welcome and some is to the point.

Ayala and Fitch suggest that times of divergence between species can be accurately estimated by calculating the percentage of molecular drift as 1% equalling approximately 50 million years and the dates they have calculated consequently run counter to our speculations on the phylogeny of *P. falciparum*. One possible reason not to attach dates to percentage drift in this particular analysis would be that it contains some sequence not considered to be core sequence. Further, n contrast to other organisms, the maintenance of sequence diversity among rRNA units seems to be a necessary part of the biology of this parasite. Let us first give an example of how this affects the phylogenists' view of *Plasmodium*. At least two different rRNA small subunit genes occur in *P. falciparum* that are both expressed and yet vary in sequence by over 15% (Ref. 3). If the two sequences were given to a molecular cladist without further information, analysis would indicate that the species which contained each of these genes diverged 750 million years ago. The effects of concerted evolution are documented and beautifully explained in *Fundamentals of Molecular Evolution* by Li and Graur[4]. Briefly, tandemly repeated, multicopy genes, like rRNA genes, undergo concerted evolution, which means that they do not evolve independently of one another. Mechanisms like unequal crossing over and gene conversion maintain homogeneity between members and have the effect of erasing evolutionary history. However, rRNA genes in *Plasmodium* exist in isolation instead of tandem arrays, accumulate mutations rapidly and drift from each other in the absence of commonly used correction mechanisms. Hence correction mechanisms that alter the rate of accumulation of random mutations in the rRNA genes do not seem to exist in malaria parasites. Although no one can say the extent to which the rate of evolution of these genes is affected, the apparent absence of common correction mechanisms would vastly accelerate the rate of sequence change. This factor so separates these organisms from others that to make even rough determinations of time is difficult. This does not discredit the molecular cladists' approach, it simply emphasizes *Plasmodium* spp as exceptional and the fact that one cannot simply apply general theories to an organism without understanding its peculiarities. Two questions arise: why can ribosomal drift not be even roughly depended on to determine branching times for *Plasmodium* and why would we want to make any statements if it was undependable? One likely answer to the former is easy. Since time scales derive from the studies of genes undergoing concerted evolution (eg. the rRNA genes of most organisms), such scales cannot be used to approximate divergence times of genes not undergoing concerted evolution (eg. the rRNA genes of *Plasmodium*). Even putting a time scale to the comparison of rRNA sequences that are all undergoing concerted evolution can vary by as much as an order of magnitude, as noted by Ayala and Fitch. Putting a time scale to the comparison of gene sequences undergoing concerted evolution with those that are not should be questioned.

How, then, can we make sense of the data on *Plasmodium* rRNA sequences? We simply measured relationships on the basis of nucleotide change per position. We compared allelic forms of the asexually expressed genes and calculated relative distance rather than time. With this information we were able to say that rodent parasites were more phylogenetically related to other species of rodent parasites than to any other species of nonrodent malaria parasite. Likewise, avian and primate species could be grouped. The exception was *P. falciparum* (and possibly *P. malariae*), which was clearly much more significantly related to the two examples of avian parasites than to anything else in the analysis. More traditional means of evolutionary enquiry (anatomical, physiological and morphological comparisons) concur with the notion that *P. falciparum* would be better grouped with avian parasites than with other species of primate malaria[5–7]. It is important that different approaches provide complementary data and we know of none that support the Ayala and Fitch viewpoint.

If one is to accept the theory put forward by Ayala and Fitch, then one must accept it all because it is based on the one calculation, 1% drift = ~50 million years. The ramifications for the acceptors are many. This would mean that the evolutionary trees developed by Garnham and others[5,6], based on thorough studies of the anatomy and biology of parasites, are totally incorrect. Malaria parasites would have had to evolve into species long before the hosts in which they now live had evolved. Farenholz's rule would also have to be totally incorrect[8].

It should also be mentioned that we were incompletely quoted by Ayala and Fitch as having said, '*P. falciparum* is a recent acquisition of man possibly coincident with the onset of agricultural life style'. That particular phrase was used to describe Boyd's theory[9], not our results. What we really said was: 'This study would be consistent with the commonly held view that infection by *P. falciparum* is a recent acquisition of man possibly coincident with an agricultural life style'. Given that we had some relative view of the relationship between the species, we turned in the discussion of the data to suggest possible reasons for the anomalous relative position of *P. falciparum*. We felt that the data were inconsistent with regard to Farenholz's rule and the phylogenetic position of *P. falciparum* but found no inconsistency with Boyd. They also criticized us for providing a faulty time frame when no time frame at all was provided in our manuscript for the reasons given above. The only time frame that we have seen reported is in the secondary literature[10] and this should not be considered a reliable source to base criticism on. We also pointed out several other biological features that relate avian parasites and *P. falciparum*, which Ayala and Fitch fail to note.

Now on to other criticisms. Brooks and McLennan join us in pointing to the fact that directionality of transfer cannot be absolutely assessed with our data. They feel that any transfer that occurred was from human to bird, while we favour, as was discussed, the bird-to-human scenario. They are also of the opinion that we need to establish that *P. falciparum* is nested among species infective to birds to prove the bird-to-human hypothesis. In other words, the relative phylogenetic position of *P. falciparum* should group within the avian cluster, thereby eliminating the human-to-bird transfer.

Bill Collins of the Centers for Disease Control has also questioned two of our statements. We said that data of this type