Parasite populations: **The puzzle of *Plasmodium***

Jody Hey

The issue of whether the malaria parasite *Plasmodium falciparum* is effectively clonal, as some argue, or undergoes outcrossing at a high rate, as many others believe, has been controversial. Recent data support the latter view, though no doubt the puzzle has not yet been laid to rest.

Address: Institute of Cell, Animal and Population Biology, University of Edinburgh, Ashworth Labs, King's Buildings, West Mains Road, Edinburgh EH9 3JT, Scotland, UK.
E-mail: jhey@mbcl.turgers.edu

Current Biology 1999, 9:R565–R567
http://biomednet.com/elecref/09609822009R0565

Surely somebody is wrong about *Plasmodium falciparum*, for current researchers investigating this deadly malaria parasite are of two very different minds about the recent evolutionary history of the species. The traditional view is that the species is diverse, as well as recombinogenic and outcrossing — like most eukaryotes — and that it has a long history of being a common human pathogen. In the face of this, it has recently been proposed that *P. falciparum* has a clonal population structure, with current populations having arisen from a very small and recent bottleneck.

The clonality argument has been espoused by Rich and colleagues, who reported [1,2] that the parasite has an effectively asexual population structure, with a strong population bottleneck within the last 50,000 years. It is the conclusion of clonality that is especially puzzling, as it does not seem to make sense in terms of the genetics and the life history of the parasite. The parasite carries 14 chromosomes and behaves genetically much like other eukaryotes, complex life cycle notwithstanding. Gametocytes are formed within the mammalian host and these can come together after they are taken up by an *Anopheles* mosquito during a blood meal. If the mammalian host has been multiply infected — a common occurrence in geographic areas of high infestation — then outcrossing can occur.

The fact that outcrossing does occur at least occasionally is demonstrated by the way *P. falciparum* has been genetically mapped. Genetic mapping requires the introduction of different strains into a single mammalian host [3], but it has been used to construct linkage maps and to find genes associated with drug resistance [4]. Of course, the possibility of outcrossing does not mean that it is common. Indeed, if the hypothesis of a population bottleneck is correct, then this could go a long way towards explaining the apparent clonality, as the now common ‘clone’ may have risen in frequency only recently, with little time or opportunity for recombination to have occurred.

The clonality/bottleneck hypothesis is based on two observations. The first is that *P. falciparum* has very low levels of a class of neutral (or nearly neutral) genetic variation. Indeed, within the circumsporozoite protein gene, *Csp*, not a single synonymous variant — a polymorphism that does not affect the amino-acid sequence because of genetic code redundancy — was observed [1]. The amount of this kind of variation is expected to be roughly proportional to the effective population size, and most organisms — including many viruses, bacteria, and eukaryotes — carry it in abundance.

Actually *P. falciparum* is moderately variable at the DNA sequence level, albeit in an unusual way. In the comparison among 25 GenBank *Csp* sequences, 18 polymorphisms were found, but all were of the amino-acid replacement variety [1]. This is an unusual observation — only rarely does replacement variation exceed synonymous variation — but it could have arisen by strong diversifying selection on *Csp*, the product of which is known to act as an antigen in malaria infections.

It was the presence of these polymorphisms at *Csp* that permitted the second argument for the effective clonality of *P. falciparum*, which was based on the apparent absence of historical recombination within the locus. If the species has had a large historical effective population size, with at least occasional recombination within genes, then some evidence of that recombination should be seen within the pattern of DNA sequence variation. A straightforward test of historical crossing over is to assess whether the linkage disequilibrium that is observed between polymorphic sites is lower for sites that are further apart. Crossing over reduces linkage disequilibrium and if it occurs to some extent then — on average, and all other things being equal — there should be more of it between sites farther apart. But for the *Csp* gene, the relationship between linkage disequilibrium and physical distance was flat, and thus revealed no evidence of crossing over [1].

If the clonal history story is correct, then the basic points made by Rich and colleagues [1,2] should be borne out with more data from more genes. But a recent report by Conway and colleagues [5] tells quite a different story of recombination. Their analysis of ten polymorphic sites of the merozoite surface protein 1 gene (*msp1*) revealed a marked pattern of declining linkage disequilibrium with distance between polymorphic sites. This is just what is expected if recombination has been occurring. Furthermore, the rate of

decline of disequilibrium with distance was least for the population that is known to experience the lowest rates of infection and so would be expected to have reduced levels of multiple infections (which are required for outcrossing to occur).

At present, the recombinational stories of *Csp* and *msp1* cannot be easily reconciled. Most of the *Csp* data were from a geographically restricted collection, and local populations can reveal clonal population structure following recent outbreaks of the parasite [6]. Another point is that the *Csp* collection carried several duplicated samples [7]. And finally, the *Csp* variation offers fewer opportunities to detect recombination, as all the longer distance contrasts involve one of just four 5′ polymorphisms — each of fairly low frequency in the sample — and the entire *Csp* region is only about one fourth of the length considered in the *msp1* study. Yet these considerations do not remove the basic expectation that if the *msp1* data are correct, then the *Csp* data should show the same pattern.

Might the lack of polymorphisms at the *Csp* locus be atypical for the genome, and perhaps reflect some aspect of *Csp* function? Rich and colleagues [2] have recently made a strongly negative response to this question. A GenBank survey of 10 *P. falciparum* loci revealed most to have some replacement polymorphisms, while not one revealed even a single synonymous polymorphism. The same pattern also emerged in another, only partly overlapping, survey of the literature. Escalante *et al.* [8] did find some evidence of synonymous polymorphism, but it was generally a good bit lower than the level of replacement polymorphism.

To the proponents of the clonality hypothesis, the simplest explanation for these polymorphism frequencies is a combination of a strong recent population bottleneck, which would have reduced the opportunity for neutral mutations to come to high frequency, and a strong dose of natural selection, which would have raised the frequencies of the many amino-acid replacement variants [2]. But it must be noted that this explanation is itself quite complicated. The genes that show an overabundance of replacement variation are a functionally heterogeneous mix; and if natural selection is invoked to explain the presence of this variation, then there must necessarily be a different story for each gene.

It is fair to inquire whether there is something unusual about the *P. falciparum* genome — as opposed to the recent demographic history of the species — that would permit replacement polymorphism, and yet tend to preclude synonymous variation. A look at those genes that have been sequenced does quickly reveal one oddity, and that is the high proportion of A and T bases in the genome. Within protein-coding genes, the average A/T content is 70%, and this rises to 85% at the third position of codons. But while these patterns do reduce the opportunity for synonymous polymorphisms, they do not explain why observed levels are so much lower than seen for replacement polymorphisms [9].

An extensive survey of codon bias, A/T content and the relative rates of different types of polymorphism has provided some clues to where the resolution of the puzzle may lie [8]. One analysis was of the level of divergence at synonymous and replacement sites between *P. falciparum* and the related chimpanzee parasite *P. reichenowi*, particularly whether this is consistent with the level of polymorphism within *P. falciparum*. From five genes compared between the species, the average ratio of 'per site' replacement substitutions to synonymous substitutions — each type of substitution being measured as a proportion of the number of available sites for that type of substitution — was 0.703. This remarkably high ratio means that a replacement mutation is 70% as likely to fix as is a synonymous mutation. By way of contrast, the ratio among 32 genes from *Drosophila* is 0.122 [10]. Given that, in the highly A/T-biased genome of *P. falciparum*, only about 20% of all random mutations are expected to be synonymous, the high replacement/synonymous ratio means that the overall rate of replacement substitutions is considerably higher than of synonymous substitutions. In short, this unusual pattern of substitution resembles that for polymorphisms and may go a long way towards explaining the unusual patterns of polymorphism observed by Rich and colleagues [1,2].

The paper by Escalante *et al.* [8] also contributes directly to the debate on clonality, as five of the twelve genes included in their study showed evidence of intragenic recombination. In this case, the evidence came not from an analysis of linkage disequilibrium, but rather by a method that focuses more directly on the swapping of portions of DNA sequence among different copies of a gene [11].

The idea that *P. falciparum* populations were subject to a historical bottleneck and are effectively clonal may thus be in trouble. The recent evidence for intragenic recombination [5,8] casts doubt on the hypothesis of clonality, and the surprisingly high ratio of replacement to synonymous substitutions between *Plasmodium* species [8] casts doubt on the significance of the low level of synonymous polymorphism within *P. falciparum*. But if the puzzle is not yet fully resolved, then at least researchers can look forward to the demise of the debate. The two global views of the history of this parasite are so far removed from one another, and the basic distinctions between them so accessible to test by fairly straightforward population genetics, that at least one view must not stand for long.

Acknowledgements
I thank David Walliker for comments on this manuscript.

References

1. Rich SM, Hudson RR, Ayala FJ: ***Plasmodium falciparum*** antigenic diversity: evidence of clonal population structure. *Proc Natl Acad Sci USA* 1997, 94:13040-13045.
2. Rich SM, Licht MC, Hudson RR, Ayala FJ: Malaria's Eve: evidence of a recent population bottleneck throughout the world populations of ***Plasmodium falciparum***. *Proc Natl Acad Sci USA* 1998, 95:4425-4430.
3. Walliker D, Quakyi IA, Wellems TE, McCutchan TF, Szarfman A, London WT, Corcoran LM, Burkot TR, Carter R: Genetic analysis of the human malaria ***Plasmodium falciparum***. *Science* 1987, 236:1661-1666.
4. Wellems TE, Walker-Jonah A, Panton LJ: Genetic mapping of the chloroquine-resistance locus on ***Plasmodium falciparum*** chromosome 7. *Proc Natl Acad Sci USA* 1991, 88:3382-3386.
5. Conway DJ, Roper C, Oduola AM, Arnot DE, Kremsner PG, Grobusch MP, Curtis CF, Greenwood BM: High recombination rate in natural populations of ***Plasmodium***. *Proc Natl Acad Sci USA* 1999, 96:4506-4511.
6. Arez AP, Snounou G, Pinto J, Sousa CA, Modiano D, Ribeiro H, Franco AS, Alves J, do Rosario VE: A clonal ***Plasmodium falciparum*** population in an isolated outbreak of malaria in the Republic of Cabo Verde. *Parasitology* 1999, 118:347-355.
7. Saul A: Circumsporozoite polymorphisms, silent mutations and the evolution of ***Plasmodium falciparum***. *Parasitology Today* 1999, 15:38-39.
8. Escalante AA, Lal AA, Ayala FJ: Genetic polymorphism and natural selection in the malaria parasite ***Plasmodium falciparum***. *Genetics* 1998, 149:189-202.
9. Hughes MK, Hughes AL: Natural selection on ***Plasmodium*** surface proteins. *Mol Biochem Parasitol* 1995, 71:99-113.
10. Li WH: *Molecular Evolution*. Sunderland, Massachusetts: Sinauer; 1997.
11. Sawyer S: Statistical tests for detecting gene conversion. *Mol Biol Evol* 1989, 6:526-538.