When are parasites clonal?

SIR—Keymer, May and Harvey drew attention in News and Views[1] to a paper by Tibayrenc and colleagues[2] who claimed that infections of most human parasitic protozoa are 'clonal', that is, derived from the reproduction of a few highly successful genotypes. This idea of clonality, with its implication that sexual reproduction is irrelevant in nature, is a stimulating one, and may be correct for some of these organisms. But we think it is wrong with respect to the most important agent of human malaria, *Plasmodium falciparum*.

First, sexual reproduction is an obligatory step in the parasite cycle in nature, and not a laboratory artefact. Apart from the rare cases of infection by blood transfusion, humans are infected with malaria by mosquito bites. The mosquitoes inoculate sporozoites, which are the haploid meiotic products of zygotes derived from mating of gametes in their stomach.

Second, genetic recombination has been directly demonstrated by infection of mosquitoes with two appropriately marked cloned lines, and by subsequent analysis of blood stage progeny[3,4]. All the expected recombinants have been observed.

Third, there is extensive genetic diversity in *P. falciparum*. Creasey *et al.*[5] studied 20 isolates from each of three countries, Thailand, Zimbabwe and Brazil, for 20 genetic loci determining isoenzymes, proteins detected by two-dimensional electrophoresis, antigens and drug-sensitivity, and found that every isolate had a unique combination of alleles at these loci.

Fourth, the blood of malaria-infected patients frequently contains two or more parasite genotypes[6]. When an individual mosquito takes in a mixture of clones, recombination between parasite genes is probably inevitable, and the enormous diversity of genotypes of *P. falciparum* seen in the wild is a consequence of this process.

Finally, Keymer *et al.*[1] and Tibayrenc *et al.*[2] drew attention to early work in our laboratory, in which we found that the genotypes GPI-1, ADA-1, LDH-1 and PEP-1 occurred in 10 out of 17 parasite samples[7], implying that this is more than would be expected on a basis of free recombination between the loci for these enzymes. But the recurrent finding of the same genotypes in independent parasite isolates can be used as evidence of a 'clonal' origin only if the respective allele frequencies in each locality and the number of loci are taken into account. ADA-1, LDH-1 and PEP-1 are by far the most common alleles of these enzymes in most African and South East Asian countries, from which these isolates originated. To say that parasites with this combination of alleles are clonal would be tantamount to saying that human beings who are blood group O and have haemoglobin A are 'clonal' (sexual reproduction in humans is not in dispute).

Thus the general model discussed by Keymer *et al.* cannot apply to *P. falciparum*. Infections of the South American parasite *Trypanosoma cruzi* may well exhibit clonal infection patterns[8], but surely one group of parasitic protozoa cannot be generalized to others having very different types of reproductive cycle. In fact, genetic recombination in *P. falciparum* has important practical consequences, because parasites resistant to two different drugs can arise through this mechanism from singly resistant parasite clones. Thus, *P. falciparum* seems, unfortunately to our detriment, to reconcile the biological advantages of sexual reproduction in the mosquito with a devastatingly efficient aptitude for asexual reproduction in the liver and blood, which causes disease and death in the human host.

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SIR—We would like to offer a different perspective from that of Keymer *et al.*[1] on the recent paper by Tibayrenc and colleagues[2]. With the exception of the malaria parasite *Plasmodium*, clonality is the conventional wisdom for parasitic protozoa, not a novel working hypothesis. Much of the recent work on recombination has focused on the African trypanosomes[9] in which sexual reproduction clearly occurs, but is not obligatory and may not be common in nature. For *Leishmania*, *Entamoeba* and New World *Trypanosoma*, recombinants have been produced in laboratory experiments, but infrequently. Those who work with these genera have believed, and still believe, that reproduction can be assumed clonal until more substantial proof is offered to the contrary.

The assertion that the most important species of human malaria parasite is clonal deserves a closer look, for sexual reproduction in *P. falciparum* is obligatory. However, Tibayrenc *et al.* tested for a significant deficit of recombinants by adopting the null hypothesis that *P. falciparum* exist in a globally panmictic population. In finding such a deficit among just 17 samples (from 15 isolates) from at least 8 African and Asian countries[7], they may simply be demonstrating that parasites do not interbreed with sufficient frequency to counteract local selection, bottlenecks and drift. The lack of free recombination across continents is hardly surprising. Incidentally, Tibayrenc *et al.* listed as separate criteria for clonality over-represented multilocus genotypes and the absence of recombinants — surely two sides of the same coin. Another of their criteria is that correlations should exist between independent sets of genetic markers; in fact, such correlations seem hard to find within taxa[10,11].

Results of greater significance for *P. falciparum*, bearing on the management of drug resistance and the use of vaccines, will be obtained from more carefully formulated null hypotheses. Taking, say, an African village as a transmission unit, theory will need to take into account the frequency of mixed infections in individual people and mosquitoes, immunity, interactions between clones, linkage and crossing-over at meiosis. Carter and Voller[12] made a start some years ago, showing that enzyme genotype frequencies in two sets of isolates from The Gambia and Tanzania were consistent with independent assortment, showing no sign of the linkage disequilibrium expected in clonal lineages.

Although we do not offer here an ideal set of testable hypotheses, we suggest that future analyses of protozoan clonality, particularly of *Plasmodium*, will yield more convincing results if carried out with more data on restricted geographical and taxonomic scales — not by making intercontinental comparisons with just a handful of isolates.

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Scientific Correspondence

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