pauses in expression that allow for correct folding[18]. They may alter the efficency of DNA polymerases and repair enzymes, affect methylation, control transcription, transcription rates, mRNA stability[19]. As Hughes and Verra[10] point out, the rate of synonymous substitutions in organisms with highly biased genomes such as *P. falciparum* is less than expected. In this organism, the effect of selection against synonymous substitutions outlined above might be amplified.

To account for differences between the average synonymous substitution rate calculated from the divergence between *P. falciparum* and *P. reichenowi*[2], and the observed very low substitution rate in *P. falciparum*, I propose that a relatively high substitution rate occurred during speciation as the parasites adapted to the changed physical, biochemical and immunological environments in the new vertebrate and invertebrate hosts. However, once a stable host–parasite relationship was formed, there was strong selection against further substitution. Presumably this occurs at both synonymous and non-synonymous sites, but is modulated at non-synonymous sites by selection for antigenic diversity. Thus, the average synonymous substitution rate would agree with the observed divergence between different species, but the current substitution rates would be much lower.

If this is correct, then the lack of observed recombination between the 5' and 3' regions of the CS gene can be explained by sample bias, and not by genetic bottleneck.

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Allan Saul
Australian Centre for International and Tropical Health and Nutrition
The Queensland Institute of Medical Research
PO Royal Brisbane Hospital, Qld 4029, Australia

Reply

Allan Saul has presented a number of arguments in response to our recent publications on the evolution of *Plasmodium falciparum*.

In one paper[1], we analyzed polymorphisms among 25 circumsporozoite protein (*Csp*) gene sequences. Our primary stated objective in this study was to '… ascertain … the pattern and process by which variation arises in this important antigen'; and is reflected in a conclusion: 'linkage disequilibrium, patterns of recombination, and other evidence indicate that the genetic variation does not originate by sexual recombination'. Saul questions the generality of our conclusion, citing the fact that 18 of the *Csp* sequences are from Thailand – several from a single locality. However, the geographic sampling bias actually makes the data suitable for testing the extent of sexual recombination. If sexual recombination does occur, it would be more likely among isolates that are proximal, both temporally and spatially, as is the case with the Thai isolates. Our observation of high correlation of 5′ and 3′ NR (non-repeat), non-synonymous polymorphisms indicates that the extensive intragenic recombination within the central repeat (CR) is best explained by a mitotic mechanism, such as slipped-strand mismatch repair, rather than sexual recombination.

With respect to a second conclusion[1]: our observation 'that silent site polymorphism is virtually absent in the NRs of *Csp* (as well as in nine other *P. falciparum* genes), provides strong evidence that the world populations of *P. falciparum* strains have recent common ancestry'; Saul is correct in calling attention to the Thailand-bias of the *Csp* data set. Nevertheless, some strains come from Papua New Guinea (PNG), Brazil, Santa Lucia, The Netherlands and West Africa, and these also lack silent polymorphisms. However, our conclusion is not based solely on *Csp*, and is strongly supported by the findings detailed in our second paper[2], wherein we analyzed *Csp* and nine additional genes in 47 samples of *P. falciparum* and found complete absence of synonymous ('silent') nucleotide polymorphisms. The 47 samples in this more extensive investigation are from Central and South America (three countries), Africa (seven countries), Asia/Oceania (four countries) and Europe. The ten genes are located on at least six different chromosomes of *P. falciparum*. The total 30973 invariant silent codon sites yield 95% confidence boundary estimates of zero to 24511–57481 years for the last common ancestor ('cenancestor') of the *P. falciparum* populations. The range is a consequence of the variable estimates of mutation rates.

Our conclusion, based on coalescence theory and biological reasoning, is that, within the past several thousand years, the populations examined have derived from a single common ancestor. The consistency of results from different continents and different genes warrants the conclusion of recent origin for the *P. falciparum* populations. Indeed, additional data may change this conclusion, but it may well be that new sequences will help to narrow the time boundaries for the origin of the cenancestor. An independent study of ten, mostly antigenic, loci has shown a similar paucity of silent polymorphisms[3].

Saul cites work indicating that allelic variants of the merozoite surface antigen-1 (*Msa1*) gene, have an ancient origin pre-dating the split between *P. falciparum* and *P. reichenowi*. The separation of *Msa1* alleles into two distinct families, which are named after the strains from which they were originally derived (MAD20 and Wellcome or K1), was first reported by Tanabe *et al.*[4], who pointed out that this large locus (>5 kb) can be split into distinct regions based on the level of nucleotide polymorphism among the alleles. One highly polymorphic region contains amino acid repeats, as in the *Csp* CR region, which are subject to mitotic recombination and therefore are likely to mutate at rates associated with microsatellite loci, which are several orders of magnitude higher than the rate of point mutations[5]. For this reason, estimates of the allelic age of repeat regions – such as those found in *Csp*, *Msa1*, *Msa2*, *RESA* and *SERA* genes – cannot be determined by a point mutation model.

Determining the age of *Msa1* alleles based on divergence of non-repeat regions is also problematic. This 5 kb locus is characterized by extreme heterogeneity in the level of polymorphisms along its length. Amino acid similarities between the K1 and MAD20 allelic variants in each of the 17 distinct regions of the locus range from 10% to 97%. Hughes[6] has proposed that some of these regions may contain allelic variants as old as 35 my (million years), while others are probably only 5–10 my, and still others have diverged 'only very recently'. However, the virtual absence of synonymous polymorphisms among alleles of a given family is not in accord with the large number of accumulated silent substitutions expected in an ancient allelic family[3,7]. We concur that there is something unusual about levels of polymorphism in certain portions of the *Msa1* alleles, but wish to point out that

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these regions are not representative of the gene as a whole, let alone of the genome. Indeed, it is plausible that some of the highly dissimilar regions, eg. where amino acid identity is only 10%, may have resulted from recent heterologous chromosomal transfer, as has been associated with duplication and variation in other antigenic loci, such as *PfHRP*, *PfHRPII*, *RESA*, *Pf11-1* and *Pf332* (Refs 8–12). In all these cases, the loci have been duplicated, and the paralogous pairs have diverged from one another. While *Msa1* is known to be single copy, this does not preclude the possibility that a duplication occurred in the past and that one copy has been lost. There is also the possibility that partial internal duplications may have occurred within the gene and, consequently, that within the highly divergent regions, the alignment between the two allele families may be nonhomologous[7].

Saul proposes that the lack of silent polymorphisms at the various loci is the result of strong selection intensity for favorable codons. There is evidence that neither AT-richness nor codon bias can fully explain the paucity of synonymous polymorphism in *P. falciparum*[7]. He also hypothesizes that synonymous substitution rates are accelerated at the time of speciation and then drop off precipitously, so that between-species synonymous polymorphisms would be numerous (as indeed they are), while within-species synonymous polymorphisms would be negligible. If this unlikely, non-darwinian model of speciation were true, then we would expect to see a lack of within-species synonymous substitutions in all malaria species. However, such a pattern is not evident. On the contrary, intraspecific, synonymous polymorphisms are present in the non-falciparum *Plasmodium* species that infect humans and animals.

Saul refers to the *gnd* locus in *E. coli*, which has been shown to be under selection for favored codons. The selection intensity may be as high as one-third that of the selection against deleterious amino acids[13]. Despite this selection pressure, however, there is extensive synonymous site polymorphism in *gnd* sequences – among alleles from natural isolates, 143 of 367 synonymous sites are polymorphic – indicating that synonymous substitutions are not prohibited by selection[13]. Indeed, Guttman and Dykhuizen[14] have shown that a large portion (>32.5 kb) of the circular chromosome of 12 natural isolates of *E. coli* has undergone a selective sweep within the past 2400 years. Despite the extremely recent coalescence of two genes within this region, a number of synonymous sites are polymorphic (3.0% and 12.3% for *gapA* and *pabB*, respectively)[15]. We know of no evidence showing that codon bias can completely prevent silent site substitutions.

There is compelling evidence that the lineage of *P. falciparum* has been associated with the hominids for millions of years[16,17]. Expansions from restricted geographic distributions and population bottlenecks are common events in many species. Parasite populations are particularly subject to episodic population constrictions and expansions. We have suggested three possible scenarios in the case of *P. falciparum*[17]: (1) changes in human behavior, particularly the development of agricultural societies and urban centers that increased human population density; (2) genetic changes that increased the affinity within the host–vector–parasite system, including recent speciation of highly anthropophilic *Anopheles* vectors[18]; and (3) climatic changes that gradually increased ambient temperatures after the Würm glaciation, so that about 6000 years ago climatic conditions in the Mediterranean region and the Middle East allowed the spread of *P. falciparum* and its vectors beyond tropical Africa[18]. Sherman[19] has noted the late introduction and low incidence of falciparum malaria in the Mediterranean region, which post-dates historical times. Hippocrates (460–370 BC) described quartan and tertian fevers, but there is no mention of severe malignant tertian fevers, which suggests that *P. falciparum* infections did not occur in classical Greece, as recently as 2400 years ago.

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Stephen M. Rich
Department of Biology
University of Rochester
Rochester, NY 14627-0211, USA

Francisco J. Ayala
Department of Ecology and Evolutionary Biology
University of California
Irvine, CA 92697-2525, USA

Teaching of Parasitology to Medical Students in Japan

Pawlowski *et al.*[1] summarized the present status of the teaching of medical parasitology in Europe on the basis of a questionnaire distributed by D.R. Hart. The situation in European medical faculties was seen to be similar to that in Japan. In 1991, an important amendment to the 'standards for the establishment of universities' was enacted by the Ministry of Education, Science, Sports and Culture of Japan. The amendment led to extensive reform in most medical schools throughout Japan, and resulted in the establishment of a new curriculum where students are expected to meet the minimum requirements for parasitology (or medical zoology) within a very limited time. The curriculum reforms tended to compress the time allotted for basic sciences, and emphasized self-learning, problem solving ability, and prolongation of the clinical clerkship.

Therefore, in 1995, the Education Committee of the Japanese Society of Parasitology (chair: Y. Kaneda) surveyed the status of the teaching of parasitology in 80 Japanese medical schools. The findings of the survey, based on answers submitted by 67 responding institutions, indicated the following: 5–110 h (mean, 57 h) were spent on lectures and 0–60 h (mean, 21 h) on practical laboratory exercises in medical parasitology during the six years of medical school. Furthermore, medical parasitology as an obligatory course, was taught during the first, second or third year. In comparison with a previous survey in 1988, the time allotted for medical parasitology has radically decreased from a mean of 83 h and is now being taught more frequently during the first or second year. On the other hand, departments of parasitology are undergoing major revisions and cutbacks in many medical schools, because interest has waned as the number of clinical cases