Self-Fertilization, Linkage Disequilibrium, and Strain in Plasmodium falciparum

We are impressed by the elegant data presented by R. E. L. Paul et al. (1) on the high rate of self-fertilization in Plasmodium falciparum, the agent of the most malignant form of malaria. This study contributes significantly to our knowledge on this pathogen's basic biology. Nevertheless, we find that there is a major logical gap in the conclusions arrived at by Paul et al. They appropriately argue that frequent selfing in P. falciparum is a medically relevant feature, for it should favor the maintenance of “multi-locus phenotype associations,” in particular those governing virulence, drug resistance, or variant surface antigen polymorphism. This is quite logical: Self-fertilization, by inhibiting genetic recombination, leads to a situation of actual clonality (2) (offspring genotypes that are identical to the parental cells), which should help stabilize those multi-locus associations that are elsewhere favored by natural selection. Then they state that “there was sufficient outbreeding to disrupt any linkage disequilibrium” (linkage disequilibrium is the non-random association between genotypes scored at different loci). These two proposals taken separately are conceivable, but they are incompatible to each other.

If self-fertilization, as evidenced by studying the three loci MSP-2, MSP-1, and GLURP, was unable to maintain any multi-locus association between these loci (as shown by lack of linkage disequilibrium at the three loci), it is not tenable that it could significantly help in stabilizing any other multi-locus combination. Two possibilities can be entertained. First, selfing can play in itself a significant role in maintaining multi-locus association, and this should be observed with the MSP-2, MSP-1, and GLURP loci. Second, the natural selection has the dominant role in stabilizing those multi-locus phenotypes associated with virulence, drug resistance, or “immunologically sensitive” variant antigens (a statement that is consistent with the observation of linkage equilibrium at other loci). In the latter case of variants mainly maintained by natural selection, the role of self-fertilization would appear consequently limited.

Another concern in the approach used by Paul et al. lies in the difficulty of evidencing any linkage disequilibrium with their data. Each of the three loci under study exhibits considerable allelic variation. The expected frequency of each possible multi-locus combination is therefore low, which proportionally lowers any possibility of evidencing linkage, even with exact statistical tests. This situation leads to a large type II error risk (to see no significant linkage while linkage does exist). If a conservative model is taken, in which five equiprobable alleles (much less than actually recorded in these data) are segregating at each locus, the probability of any multi-locus combination does not exceed 0.23 as = 0.008. This renders difficult to evidence any significant linkage, unless considerable sample sizes are used, which is not the case in this study.

Although the discovery of high-rate self-fertilization in P. falciparum is a major breakthrough in our knowledge of the agent of malaria, its actual impact on this parasite's population structure in humans still has to be clarified by classical population genetic means that depend on linkage disequilibrium analysis. The notion of strain in microbiology relies on the existence of stable multi-locus associations (especially, of course, those combinations dealing with medically relevant characters), and if no such multi-locus associations are found in P. falciparum, the notion of strain has to be held in abeyance for this parasite. Should this be verified, any efforts for individualizing multi-locus genotype (that is, strain characterization) in P. falciparum may not be successful, for those genotypes will appear as most unstable. The only approach that remains possible in this case is the typing of individual genes.
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