Loss of coral reef habitat is often associated with transitions to benthic communities dominated by noncalcareous benthos (4, 8). The results of Kayanne et al. (1) reveal metabolic measures to be sensitive indicators of reef health (9), but the findings more likely reflect the condition of the local sampling site than the normal behavior of healthy reef communities.

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Response: We are grateful for the comments we have received about our report (1). They bring up points that we did not fully discuss in the report.

Gattuso et al. stressed that our measured values of productions are remote from the common reef production rate. His reference of “standard performance” of $P_{\text{R}}/R = 1.0 \pm 0.1$ (2). The standard performance of coral reefs are a first-order estimation; it is not a precise indicator with regard to the CO$_2$ sink versus source problem. The threshold between CO$_2$ sink or source is very sensitive to precise organic and calcium carbonate production rates, and thus direct measurement of $P_{\text{CO}_2}$ changes provides better information. We should obtain more direct measurements of $P_{\text{CO}_2}$ changes to better address the reef sink versus source problem. Moreover, the idea that $P_{\text{R}}/R$ is close to 1 is partly based on the fact that the tropical ocean is depleted in nutrients for supporting net organic production. However, as we stated in our report, recent studies have revealed the importance of nitrogen fixation in coral reefs, which provides new nutrients to coral reefs.

As stated in our report, different reef zones might act differently as to CO$_2$ fluxes. The whole-reef production values in the reef—acts as a sink of CO$_2$. However, we cannot comment on the statement “export of DOC/POC from reef to ocean or export by downwelling sediment transport would not provide a credible mechanism for differential sequestration” without conducting actual measurement of these fluxes.

Gattuso et al. also question the representativeness of our study site. We agree that our report did not provide adequate explanation of which part of the reef community metabolism our measurements represented in relation to the flow regime. During high tides, our current measurements showed that water comes steadily from north-northeast with a current speed over 5 cm s$^{-1}$ [figure 2 in our report (1)]. This water comes from the outer ocean over the reef crest north of the study site and runs over coralline and living coral communi-ties [figure 1B in (1)]. During low tide and stagnant periods, we observed a slow current with an average of 3 to 4 cm s$^{-1}$, which mixed well the water around the study site surrounded by living coral patches. Therefore, our measurements likely show the metabolism of corals and algae on the reef flat at Shiraho. We are carrying out further measurements of currents at other points to clarify the flow regime.

Gattuso et al. also point out that errors of the mean daytime and nighttime $P_{\text{CO}_2}$ values overlapped with the offshore value. The purpose of this estimation was to convert the visually conspicuous relations of $P_{\text{CO}_2}$ and light intensity into a quantified discussion, but the data were too small to make a statistically rigorous conclusion; we agree that we should obtain more data in the future. However, we would like to point out that the light intensity in March, when we made the observations and estimations, is relatively low compared to other months in this island (mean light intensities are 552 and 864 μmol m$^{-2}$ s$^{-1}$ in March and in August, respectively). Productions depend primarily on light intensity, and we have obtained higher productions in August.

Gattuso et al. point out the discrepancy between our estimates of production and those of Nakamori et al. (9). Their estimates of organic production and calcium carbonate production, however, depend only on one daytime (4-hour) set and one nighttime (3.5-hour) set of measurements of pH and alkalinity changes. From these data, they estimated the whole day net production on the basis of the tentative assumption that the daytime length is 7.5 hours. After the publication of their study, we have accumulated numerous measurements on organic production and calcium carbonate production of Shiraho reef and have related them with actual change in light intensity. In addition to the conventional pH-alkalinity method to estimate the production, we computed them from more measured alkalinity and $P_{\text{CO}_2}$ changes, six times through $P_{\text{CO}_2}$ observation.
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 disequilibria” (linkage disequilibrium is the non-random association between genotypes scored at different loci). These two proposals taken separately are conceivably, 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 = 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 these 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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10. 30 October 1995; accepted 18 December 1995

Response: Tibayrenc and Lal highlight an important issue in genetic analyses of organisms in which sex is obligatory, especially with respect to species of medical importance such as P. falciparum. As stated by us and by Tibayrenc and Lal, a high degree of self-fertilization will have important medical consequences by favoring the maintenance of multi-locus phenotypes such as virulence and drug resistance. Assessing the mating structure of populations can be achieved indirectly by measurements of association between loci (linkage disequilibrium) and directly by measurements of heterozygosity. Our direct measurement of the degree of heterozygosity in the oocyst parasite population found that the mating structure was typified by a high degree of inbreeding which was in contrast to that previously found in a region of more intense malarial transmission, Tanzania (1). While such a high degree of inbreeding would be expected to result in linkage disequilibrium, in this study (2) we found no evidence for linkage, even when using sequence data only (GENEPOP Fisher exact, P > 0.1) (3). However, linkage analysis may produce misleading results (4) and as indeed pointed out by Tibayrenc and Lal, large sample sizes are required to detect linkage (5). In this study, linkage analysis was performed for a comparison with the heterozygosity data as malariologists had previously accepted the absence of linkage disequilibrium as evidence for a panmictic population structure (6). Our study highlighted the relative insensitivity of linkage analysis in assessing the extent of inbreeding.

A third point raised emphasizes the need to use selectively neutral loci to establish such mating patterns. In our report we used three loci, two of which were parasite surface antigens. The fact that all three loci produced the same inbreeding coefficient would suggest that the result found is real, although there is some evidence that regions of the merozoite surface proteins, other than those amplified, may be under selection (7).

The comments made by Tibayrenc and Lal are appreciated.
Editor's Summary

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