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Are eukaryotic microorganisms clonal or sexual? A population genetics vantage

(parasitic protozoa/fungi/malaria/Leishmaniasis/*Toxoplasma*)

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ABSTRACT **We argue that the mode of reproduction of microorganisms in nature can only be decided by population genetic information. The evidence available indicates that many parasitic protozoa and unicellular fungi have clonal rather than sexual population structures, which has major consequences for medical research and practice. *Plasmodium falciparum*, the agent of malaria, is a special case: the scarce evidence available is contradictory, some suggesting that uniparental lineages may exist in nature. This is puzzling (because *P. falciparum* is known to have a sexual stage) and poses a challenge that can be readily settled by ascertaining the frequency distribution of genotypes in natural populations.**

Sexual reproduction is generally assumed to be a common mode of reproduction of eukaryotes. In the case of parasitic protozoa, the assumption of sexual reproduction relies largely on the presumption that these organisms are diploid, as well as on the occurrence of sexual recombination in the laboratory under appropriate circumstances (review in ref. 1), rather than on relevant evidence obtained from nature. Yet, whether or not sexual reproduction prevails in these organisms is of considerable medical and agronomic consequence as well as of scientific interest. These eukaryotic microorganisms include the agents of malaria, sleeping sickness, Chagas disease, and other parasitic diseases that affect more than 10% of the world population. The strategies for developing vaccines or curative drugs as well as for diagnosis and treatment are different for clonal and for sexual organisms.

That sexual reproduction may occur in laboratory cultures or even occasionally in nature does not by itself settle the issue, since that simply manifests that the potentiality for sexual reproduction has not been lost. What remains to be determined is the prevailing mode of reproduction of these organisms in natural circumstances. The evidence to settle the matter exists for some of these organisms and could be obtained for others without massive investment of resources or new scientific or medical advances. We herein advance a sustained argument to show that population genetic evidence and population genetic theory is all that is needed to ascertain the extent to which, if at all, these (or any other) organisms reproduce sexually in nature. We have already reviewed the evidence for *Trypanosoma cruzi*, the agent of Chagas disease (2), and some other protozoa (3). Here we develop further the argument and present the results of a survey of the available evidence for parasitic protozoa and unicellular fungi.

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CLONALITY IN MICROBIAL EUKARIOTES

The two genetic consequences of sexual reproduction are segregation and recombination. Population genetic methods make it possible to ascertain whether or not the distribution of genotypes in natural populations is consistent with the occurrence of segregation and recombination. The kind of evidence that is needed is the frequency distribution of genotypes rather than the direct observation of sexual or clonal reproduction, since direct observation could hardly settle the question of the generality of the process. A number of techniques are now in use for obtaining the frequencies of genotypes in populations: allozyme variation, immunological markers, restriction fragment length polymorphisms (RFLP's), and DNA sequencing are the most commonly used.

Whether or not reproduction is prevailingly clonal can be tested by the battery of population genetic criteria listed in Table 1, as we have pointed out (3). Some features deserving attention are the following.

Fixed heterozygosity (criterion *a*) is an obvious population genetic indicator of asexual reproduction: the persistence and/or overrepresentation of heterozygotes over the generations suggests absence of meiotic segregation. Fixed heterozygosity is incompatible not only with biparental reproduction but also with self-fertilization. Fixed heterozygosity becomes decisive evidence for clonality in situations where the evidence against sexual reproduction derived from other criteria might be attributable to samples containing mixtures of individuals from two populations or even two species (see below).

The presence of a particular multilocus genotype in great excess (criterion *d*) is often the most robust and significant evidence of clonal reproduction. Evidence of this sort is particularly telling when the same genotype reappears in excess in various, perhaps distant, localities or in samples taken years apart. This state of affairs indicates that the genotype is replicated as a unit, without the gene shuffling attributable to sexual recombination, and that this clonal mode of replication persists over time and space. When the genetic information derives from high-resolution methods, such as DNA sequencing, or RFLP patterns, repeated sampling of the identical genotype is a strong indication of absence of sexuality that does not require statistical tests. Statistical tests are, however, possible. The probability of the observed frequency of the excess genotype on the assump-

Abbreviation: RFLP, restriction fragment length polymorphism.

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Table 1. Population genetic criteria of clonal reproduction

Label	Description
	Segregation (single-locus data)
a	Fixed heterozygosity
b	Absence of segregating genotypes
c	Deviation from Hardy–Weinberg equilibrium
	Recombination (multilocus data)
d	Overrepresented, widespread identical genotypes [statistical tests: combinatorial (*d*1), Monte Carlo (*d*2)]
e	Absence of recombinant genotypes
f	Linkage disequilibrium
g	Correlation between independent sets of genetic markers

Criteria *a* and *b* are qualitative; all others are based on statistical tests except *d*, which can be used qualitatively or statistically (*d*1 and *d*2).

tion of random association between loci can be tested by χ^2 or calculated by standard combinatorial methods as we have proposed (3). This last test is referred to as *d*1 in Table 1. Alternatively, the expected sampling frequency distributions can be generated by Monte Carlo methods and compared with the observed distributions (3), *d*2 (and *e*) in Table 1.

Linkage disequilibrium tests (criterion *f*) are the standard population genetic method of testing for nonrandom association between loci. When more than three loci are jointly considered, the theory and methods become cumbersome and Monte Carlo simulations become the choice method (4). We previously have used linkage disequilibrium tests in *T. cruzi* (4) and extend them here to other species.

We have reviewed the literature on protozoan and unicellular-fungi genetic markers that would be informative for our purposes—namely studies that give the frequency of single-locus and multilocus genotypes in populations. The results of the tests that we could make in each case are summarized in Table 2. Most organisms surveyed exhibit one or more strong indications of clonality, but the combined strength derived from the applicable clonality criteria varies from one to another organism. We have ranked the strength of the evidence by classifying each organism in one of the four following categories. (*i*) Organisms for which relevant information exists but does not support clonality; *Candida albicans* is the only organism in this category. (*ii*) Organisms for which the available information indicates a lack of gene shuffling, but the paucity of the data does not permit any definitive conclusions, so that clonality is only a working hypothesis; four fungi and several protozoa fall in this category. (*iii*) Organisms for which there is obvious evidence indicating a lack of gene shuffling, but the limited number of genetic markers surveyed makes probable that much additional variability exists within each genotype observed; several protozoan populations fall in this category. (*iv*) Organisms for which the available information supports clonality because the samples of genetic markers and of individuals are sufficiently large; *Leishmania* and most *Trypanosoma* fall in this category. We shall now discuss some particular cases.

Plasmodium falciparum. Whether reproduction is clonal or not cannot be resolved for several protozoa and fungi primarily because few individuals or few genetic markers have been sampled, but also in some cases because the data have not been published in an informative manner for the present purposes. This situation is particularly vexing in the case of *P. falciparum*, which has been the subject of numerous genetic investigations owing to its medical importance as the single largest agent of human mortality. It is commonly accepted that the agent of malaria requires a sexual stage in the mosquito host to complete the transmission cycle. Yet the possibility of an unknown uniparental cycle cannot be discarded. Such is the case in *Toxoplasma gondii*, which is taxonomically close to *Plasmodium*: our analysis suggests not only that a uniparental cycle exists in *T. gondii* (which was already known) but also that it is common and perhaps predominant. In the case of *P. falciparum*, the available evidence is contradictory. The data from a study of two isozyme loci in Africa are consistent with panmixia (39). But the only published multilocus allozyme study (21) manifests, on the basis of the allelic frequencies reported in ref. 40, a nonrandom association between loci. In addition, a study of six individuals by two-dimensional electrophoresis (22) yields significant linkage disequilibrium. Moreover, two additional studies show the widespread occurrence of identical genotypes. In one study, two stocks sampled in Thailand 280 km apart were identical according to high-resolution two-dimensional electrophoresis (23). In the other study, seven stocks sampled in various countries (from Africa, South America, and Southeast Asia) exhibited identical RFLP patterns when tested with a highly repetitive DNA probe (ref. 24; see Fig. 1).

The last two studies in particular, which rely upon methods of genetic labeling with high specificity, would suggest uniparental propagation for *P. falciparum* in some cases. Nevertheless, we have placed this protozoan in category *ii* because large population samples must be studied simultaneously for a number of genetic markers before the evidence for or against the existence or prevalence of a uniparental cycle becomes definitive. The possibility of accidentally aberrant results or of mixed-up cultures must be kept in mind when the informative data set is so small.

Unicellular Fungi. Only limited data sets exist of the kind needed to decide whether sexual or clonal reproduction prevails in unicellular fungi. Yet such evidence as exists favors clonal reproduction for all but one of the four species investigated.

Gene shuffling inhibition is apparent in the case of *Candida tropicalis/paratropicalis*, for which appropriate data are available (5). The evidence available for *Cryptococcus neoformans* (6) also supports clonality, although the possibility of accidentally aberrant sampling cannot be excluded owing to small samples. Our tests indicate significant nonrandom association between loci (criterion *f*) as well as absence of recombinant genotypes (*e*) and genotype overrepresentation (d*1* and d2), when the sample is treated as a whole; but some of these tests are also significant for the smaller samples resulting from separating the stocks according to mating type (serotypes A+C and B+D), suggesting gene shuffling inhibition within each of these pairs.

In the case of *Saccharomyces cerevisiae* (7), the test for linkage disequilibrium (*f*) is significantly positive, suggesting inhibition of gene shuffling, but the diversity is high (with as many isozyme patterns as stocks) so that it is impossible to establish that there are individual clones.

The case of *Candida albicans* is different (5). No sexual stage is known in this yeast (41) and a test for linkage disequilibrium is marginally significant (although it does not meet the 0.01 level of significance that we have set for this test). But the observed frequencies of multilocus genotypes are very close to the expected ones under the assumption of free recombination, so that the limited sample available (5) provides no evidence for clonality. Clearly, more extensive data are required (larger population samples and also more loci, since only four were assayed in ref. 5) to settle the issue.

GEOGRAPHIC POPULATION STRUCTURE

One potential source of error in interpreting the tests proposed in Table 1 is population subdivision; another is natural selection, which will be examined below. Consider two

Table 2. Organisms surveyed and rank—based on the strength of evidence for clonal reproduction

Organism	Rank	Criteria supporting clonality (with refs.)
Fungi		
Candida albicans	*i*	None (5)
Candida tropicalis/paratropicalis	*ii*	*d*1, *d*2, *e*, *f* (5)
Cryptococcus neoformans B+C serotypes	*ii*	*e*, *f* (6)
Cryptococcus neoformans A+D serotypes	*ii*	*f* (6)
Cryptococcus neoformans all serotypes	*ii*	*d*1, *d*2, *e*, *f* (6)
Saccharomyces cerevisiae	*ii*	*f* (7)
Protozoa		
Entamoeba histolytica	*iii*	*d*1, *d*2, *e*, *f* (8, 9)
Giardia sp.	*iii*	*d*1, (10, 11); *d*2, *e*, *f*, (10); *g* (12)
Leishmania braziliensis guyanensis	*iv*	*d*1, *d*2, *e*, *f* (13)
Leishmania infantum	*iv*	*d*1, *d*2, *e*, *f* (14)
Leishmania tropica	*iv*	*a* (15); *d*1, *d*2, *e*, *f* (16)
Leishmania major	*iv*	*d*1, *d*2, *e*, *f* (17)
Leishmania Old World as a whole	*iv*	*d*1, *d*2, *e*, *f* (18)
Leishmania sp.	*iii*	*g* (19)
Naegleria australiensis	*ii*	*a*, *d* (20)
Naegleria fowleri	*ii*	*a* (20)
Naegleria gruberi	*ii*	*a* (20)
Plasmodium falciparum	*ii*	*d*1, *d*2, *e* (21); *f* (22), *d* (23, 24)
Toxoplasma gondii	*ii*	*d*1, *d*2, *f* (25, 26)
Trichomonas foetus	*ii*	*d* (27)
Trichomonas vaginalis	*ii*	*d* (27)
Trypanosoma brucei s. l.		
West Africa	*iv*	*d*1, *d*2, *e*, *f* (28)
East Africa	*iv*	*d*1, *d*2, *e*, *f* (28)
East Africa (wild)	*ii*	*e*, *f* (28)
Liberia	*iv*	*d*1, *d*2, *e*, *f* (28)
Busoga, Uganda	*iv*	*d*1, *d*2, *e*, *f* (29)
Lambwe Valley, Kenya	*iv*	*d*1, *d*2, *e*, *f* (30, 31)
Lambwe Valley (nonhuman stocks)	*iv*	*d*1, *d*2, *e*, *f*, (30, 31)
Ivory Coast	*iv*	*d*1, *e*, *f* (32)
Ivory Coast (nonhuman stocks)	*iv*	*d*1, *f* (32)
Trypanosoma brucei rhodesiense	*ii*	*a*, *d1*, *d2*, *e*, *f* (33)
Trypanosoma congolense	*iii*	*a*, *d1*, *d2*, *e*, *f* (34)
Trypanosoma cruzi	*iv*	*a*, *b*, *c*, *d* (2); *f* (4); *g* (35)
Trypanosoma vivax	*iv*	*d*1, *d*2, *e*, *f* (36, 37)

Ranks: *i*, the available data do not evidence clonality; *ii*, clonality is only a working hypothesis because the supporting evidence comes from small samples; *iii*, there is evidence for clonality but the limited number of markers prevents equating the strains with actual clones; *iv*, clonal population structure is well ascertained. The applicable criteria for clonality are listed with the references (in parentheses) for the source data. Criteria *a*, *b*, and *d* are qualitative; all others are based on statistical tests significant at the 0.05 level, or at the 0.01 level in the case of criterion *f*. The clonality tests are ours except for *g* in *Giardia* and *Leishmania* sp. obtained from refs. 12 and 19, respectively; the Lambwe Valley sample of *T. brucei* (30) also was found to meet criterion *e* in ref. 38.

geographically separate populations fixed, respectively, for alleles *A1* and *A2*. All individuals sampled would be homozygotes *A1/A1* or *A2/A2*, since there is not interbreeding between the populations. If samples from the two populations are unintentionally pooled and treated as one, erroneous conclusions could ensue, because tests of criterion *c* would be positive. Notice, however, that deviations from Hardy–Weinberg expectations would be due to a deficit of heterozygotes (the Wahlund effect in population genetics). On the contrary, if criterion *c* fails owing to excess heterozygotes, this favors clonality (or, possibly natural selection, see below) rather than geographic subdivision. Assume now that the two populations are fixed at another locus, respectively, for alleles *B1* and *B2*. Alleles *A1* and *B1* will be completely associated with each other, and the same is true for alleles *A2* and *B2* as well, so that criteria *e* and *f* could be strongly positive. Notice, however, that criterion *d* would fail: the probability of sampling the same multilocus genotype in both populations would be 0. In general, the more different the allelic frequencies between the populations in the sample, the more it will be the case that criteria *a* and *d* do not obtain: a consequence of geographic subdivision is that given genotypes (multilocus as well as unilocus) tend to be restricted to particular geographic areas. The association between criteria *d* and *a* (overrepresented multilocus genotypes that are widespread and exhibit fixed heterozygosity) is specially telling. This situation exists in *T. cruzi* (e.g., clones 19 and 39; ref. 2). Overrepresented, widespread multilocus genotypes without fixed heterozygosity appear in many other cases (e.g., zymodeme I of *T. gondii*, recorded in the United States and in France, ref. 25; additional examples are cited in ref. 3).

The biases introduced by lumping separate populations must be minimized in any case by independently testing samples from different locations whenever possible. This we have done in Table 2 for the extensive data available separately from East and West African *T. brucei* (28) as well as for different countries: Liberia (28), Busoga in Uganda (29), Kenya (the "subspecies" *T. brucei rhodesiense*, ref. 33; the Lambwe Valley, refs. 30 and 31), and Ivory Coast (32). Whenever feasible, we have analyzed the data also after

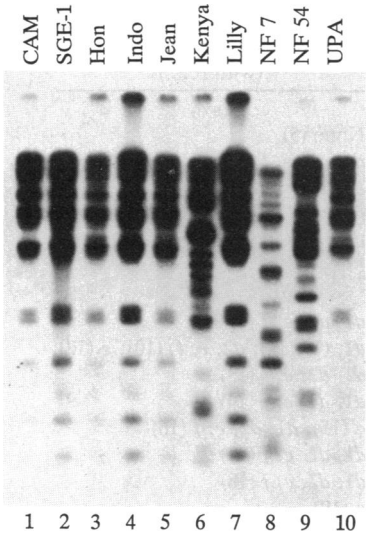

FIG. 1. RFLP patterns of *Plasmodium falciparum* stocks hybridized with a highly repetitive DNA probe (24). Lanes: 1–5, 7, and 10, identical patterns although samples derive from widespread regions: Africa (Senegal, Gambia, Uganda, and Central Africa), Central America (Honduras), and Southeast Asia (Vietnam); 6, sample is from Kenya; 8, sample is from Ghana; 9, sample is from an African individual sampled in Amsterdam's airport.

excluding the human stocks to test the clonality of *T. brucei* in the nonhuman reservoir.

We have used a set of human population samples to evaluate the potential bias introduced by lumping data from allopatric populations. The samples consist of nearly 1600 individuals assayed for four polymorphic gene loci located in four different chromosomes (J.A., unpublished data). The four loci are highly polymorphic with the average frequency of the second most common allele ranging from 0.135 to 0.348. The individuals originate from Madagascar, Mali, French Polynesia, Yugoslavia, France, and French Guiana (Indian people). Gene flow between these populations is negligible. We have pooled the data and generated random samples of comparable size to those available for the organisms in Table 2. With one exception, none of the criteria listed in Table 1 results in statistically or otherwise significant results. For example, the observed and expected frequencies of the most common genotypes in the samples were compared by χ^2 tests in each of four randomized samples of 150 individuals each; none of the tests is statistically significant ($P > 0.20$). The $d1$ test also gives statistically insignificant results in all four tests ($P > 0.25$). The exceptional case concerns tests for linkage disequilibrium (criterion f), which are sometimes (although scarcely) significant for random samples from the pooled data. In a test of 100 samples of 200 individuals each, significance disequilibrium was obtained at the $P < 0.05$ level in 7 cases, which is 2 more than would be expected by chance. At the $P < 0.01$ level, 2 samples were significant, 1 more than expected by chance. As a consequence of these tests, we decided to use the $P < 0.01$ level of significance in all tests of linkage disequilibrium reported in Table 2 to minimize the likelihood that significance might appear by chance or as a consequence of commingling samples from distinct geographic populations.

NATURAL SELECTION

Multilocus criteria are particularly helpful in cases where single-locus criteria might be positive as a consequence of natural selection. Consider a case where homozygotes *A1A1* and heterozygotes *A1A2* are both present, but homozygotes *A2A2* are absent. This could happen in a sexual organism if homozygotes *A2A2* are lethal. Natural selection would, of course, tend to eliminate the allele *A2* from the population, but the polymorphism could be transitional, or the fitness of the *A1A2* heterozygotes might be greater than that of the *A1A1* homozygotes, in which case both alleles *A1* and *A2* would persist in the population. Natural selection explanations might be carried out from one to another locus, although the likelihood that several such ad hoc explanations are the case, rather than clonality, decreases as the number of loci increases. Indeed, ad hoc natural selection explanations soon become farfetched when multiple loci are considered because the number of explanations required to account for the missing genotypes increases geometrically with the number of loci (because the number of possible genotypes at n loci is the product of the number of genotypes at each locus). Thus, whereas natural selection may (and probably does) account for some of the peculiarities of the genotypic distributions analyzed, it could hardly account for all of them. The overrepresentation of a few multilocus genotypes and the absence of most others even from large samples become particularly difficult to explain by natural selection against most of the segregating genotypes when the number of polymorphic loci is large.

CONCLUSIONS

The issue of clonal reproduction (12, 42), or at least separate evolution of distinct parasite lineages (38), has been raised before. To our knowledge, however, we originated the proposal that the matter can be resolved by population genetic considerations and only by them and have extended such considerations to numerous unicellular eukaryotes. The results are unexpected. Except for *Candida albicans*, all organisms for which relevant evidence is available give indication that gene shuffling is inhibited and that there exist persistent uniparental lineages. In a number of cases the evidence is sufficiently strong to warrant the conclusion that clonal propagation is the predominant mode of reproduction. In cases where limited data are available, cryptic speciation and other explanations may account for the data, although the practical consequences would be similar as for clonality.

A clonal model of population structure does not imply that sexuality is totally absent but rather that it is not common enough to prevent the appearance and propagation of uniparental lineages that are stable in time and space. Thus, the model we propose is compatible with successful recombination experiments in the laboratory, as have been achieved in *T. brucei*, *P. falciparum*, and *E. histolytica* (review in ref. 1). Moreover, our model does not imply that the clones identified by a limited number of genetic markers are genetically homogeneous. A more extensive genetic analysis is bound to reveal additional variability among the individuals classified as members of the same clone.

The agronomic and medical implications of the model that we are proposing deserve immediate attention (3). Even if it turns out that uniparental lineages coexist with biparental ones (a possible explanation for the contradictory results observed in the case of *Plasmodium falciparum*), the model herein endorsed markedly departs from the potentially interbreeding population structure often favored in parasitology (1). In quasi-panmictic models, individuals in a population share in a common gene pool that is reshuffled every generation. In the clonal model, individuals do not share in a common gene pool but consist of independently propagating clonal lineages. These clonal lineages, rather than species as wholes, are the useful taxonomic units. Moreover, the properties that are of medical or agronomic importance need to be investigated separately for distinct clones, since these properties may be quite different from one to another clone when

the genetic chasm between these is large. In a panmictic population instead, the conclusions derived from a relatively few randomly selected individuals may be generally applicable to all members of the population. Where widespread clones ("major clones," ref. 43) exist, these should become the subject of preferent and sustained research, particularly when they are known to cause serious pathological effects. In a panmictic population, the individual is genetically ephemeral, and it is the population as a whole that needs to be characterized. In a clonal model, it is the clone that persists genetically, and the population made of all existing clones is just a collection or class with few if any operational consequences.

The considerations just advanced bear on taxonomic practice. If clonal reproduction prevails in eukaryotic microorganisms, there is little justification for naming a new species or subspecies each time an individual is found to be genetically quite different from others previously assigned to an existing species—clones of ancient divergence are likely to be all genetically quite different from one another. It is more parsimonious and helpful simply to recognize natural clones for what they are and to identify them as such within the umbrella provided by a preexisting species name.

The origin of sex is a central evolutionary question that has received extensive attention of late and conflicting accounts (e.g., refs. 44 and 45). Two alternative hypotheses that can be tested are that (*i*) clonality in eukaryotic microorganisms is a remnant of the primitive condition that persists in bacteria (46); or (*ii*) it is a secondary adaptation to the parasitic condition. (The second hypothesis can be more narrowly formulated as adaptation to human parasitism, since the rapid spread of mankind throughout the world presents a peculiar ecological challenge to any parasite.) These hypotheses can be tested simply by finding out whether nonparasitic eukaryotic microorganisms (such as free protozoa, saprophytic fungi, or unicellular algae) reproduce sexually or clonally. If their reproduction is clonal, the first hypothesis is favored and the second rejected, whereas the opposite will follow if it turns out that free-living eukaryotic microorganisms reproduce sexually.

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1. Baker, J. (1989) *Int. J. Parasitol.* **19,** 465–472.
2. Tibayrenc, M. & Ayala, F. J. (1988) *Evolution* **42,** 277–292.
3. Tibayrenc, M., Kjellberg, F. & Ayala, F. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2414–2418.
4. Zhang, Q., Tibayrenc, M. & Ayala, F. J. (1988) *J. Protozool.* **35,** 81–85.
5. Lehmann, P. F., Kemker, B. J., Hsiao, C. B. & Dev, S. J. (1989) *Clin. Microbiol.* **27,** 2514–2521.
6. Safrin, R. E., Lancaster, L. A., Davis, C. E. & Braude, A. I. (1986) *Am. J. Clin. Pathol.* **86,** 204–208.
7. Subden, R. E. & Irwin, D. (1982) *Can. J. Microbiol.* **28,** 1047–1050.
8. Proctor, E. M., Wong, Q., Yang, J. & Keystone, J. S. (1987) *Am. J. Trop. Med. Hyg.* **37,** 296–301.
9. Sargeaunt, P. G., Williams, J. E., Jackson, T. F. H. G. & Simjee, A. E. (1982) *Trans. R. Soc. Trop. Med. Hyg.* **76,** 401–402.
10. Meloni, B. P., Lymbery, A. J. & Thompson, R. C. A. (1988) *Am. J. Trop. Med. Hyg.* **38,** 65–73.
11. Nash, T. E., McCutchan, T., Keister, D., Dame, J. B., Conrad, J. D. & Gillin, F. D. (1985) *J. Infect. Dis.* **152,** 64–73.
12. Meloni, B. P., Lymbery, A. J. & Thompson, R. C. A. (1989) *Am. J. Trop. Med. Hyg.* **40,** 629–637.
13. Desjeux, P. & Dedet, J. P. (1989) *Trans. R. Soc. Trop. Med. Hyg.* **83,** 610–612.
14. Moreno, G., Rioux, J. A., Lanotte, G., Pratlong, F. & Serres, E. (1986) *Leishmania: Taxonomie et Phylogénèse: Applications Eco-Epidémiologiques*, Colloques Internationaux de Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale (CNRS/INSERM, Paris), pp. 105–119.
15. Le Blancq, S. M. & Peters, W. (1986) *Trans. R. Soc. Trop. Med. Hyg.* **80,** 113–119.
16. Pratlong, F., Lanotte, G., Ashford, R. W. & Rioux, J. A. (1986) in *Leishmania: Taxonomie et Phylogénèse: Applications Eco-Epidémiologiques*, Colloques Internationaux de Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale (CNRS/INSERM, Paris), pp. 129–139.
17. Maazoun, R., Pratlong, F., Lanotte, G. & Rioux, J. A. (1986) in *Leishmania: Taxonomie et Phylogénèse: Applications Eco-Epidémiologiques*, Colloques Internationaux de Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale (CNRS/INSERM, Paris), pp. 119–129.
18. Lanotte, G., Rioux, J. A. & Serres, E. (1986) in *Leishmania: Taxonomie et Phylogénèse: Applications Eco-Epidémiologiques*, Colloques Internationaux de Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale (CNRS/INSERM, Paris), pp. 27–40.
19. Beverley, S. M., Ismach, R. B. & Pratt, D. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 484–488.
20. Cariou, M. L. & Pernin, P. (1987) *Genetics* **115,** 265–270.
21. Sanderson, A., Walliker, D. & Molez, J. F. (1981) *Trans. R. Soc. Trop. Med. Hyg.* **75,** 263–276.
22. Tait, A. (1981) *Mol. Biochem. Parasitol.* **2,** 205–218.
23. Fenton, B., Walker, A. & Walliker, D. (1985) *Mol. Biochem. Parasitol.* **16,** 173–183.
24. Oury, B. (1989) Ph.D. thesis (Univ. of Grenoble, France).
25. Dardé, M. L., Bouteille, B. & Pestre-Alexandre, M. (1988) *Am. J. Trop. Med. Hyg.* **39,** 551–558.
26. Dardé, M. L., Bouteille, B. & Pestre-Alexandre, M. (1990) *Bull. Soc. Fr. Parasitol.* **8,** 238 (abstr.).
27. Nadler, S. A. & Honigberg, B. M. (1988) *J. Parasitol.* **74,** 797–801.
28. Gibson, W. C., Marshall, T. F. deC. & Godfrey, D. G. (1980) *Adv. Parasitol.* **18,** 175–246.
29. Gibson, W. C. & Gashumba, J. K. (1983) *Trans. R. Soc. Trop. Med. Hyg.* **77,** 114–118.
30. Gibson, W. C. & Wellde, B. T. (1985) *Trans. R. Soc. Trop. Med. Hyg.* **79,** 671–676.
31. Mihok, S., Otieno, L. H. & Darji, N. (1990) *Parasitology* **100,** 219–233.
32. Mehlitz, D., Zillmann, U., Scott, C. M. & Godfrey, D. G. (1982) *Tropenmed. Parasitol.* **33,** 113–118.
33. Tait, A., Barry, J. D., Wink, R., Sanderson, A. & Crowe, S. J. (1985) *Parasitology* **90,** 89–100.
34. Gashumba, J. K., Baker, R. D. & Godfrey, D. G. (1988) *Parasitology* **96,** 475–486.
35. Tibayrenc, M. & Ayala, F. J. (1987) *C.R. Hebd. Séances Acad. Sci. Sér. C* **304,** 89–92.
36. Allsopp, B. A. & Newton, S. D. (1985) *Int. J. Parasitol.* **15,** 265–270.
37. Kilgour, V. & Godfrey, D. G. (1975) *Ann. Trop. Med. Parasitol.* **69,** 329–335.
38. Cibulskis, R. E. (1988) *Parasitology* **96,** 303–322.
39. Carter, R. & Voller, A. (1975) *Trans. R. Soc. Trop. Med. Hyg.* **69,** 371–376.
40. Walliker, D. (1985) *Ann. Soc. Belge Méd. Trop. Parasitol. Mycol. Hum. Anim.* **65,** 69–77.
41. Scherer, S. & Magee, P. T. (1990) *Microbiol. Rev.* **54,** 226–241.
42. Weber, J. L. (1988) *Exp. Parasitol.* **66,** 143–171.
43. Tibayrenc, M. & Brenière, S. F. (1988) *Mem. Inst. Oswaldo Cruz* **83,** 249–255.
44. Michod, R. E. (1990) *Mol. Evol.* **122,** 135–144.
45. Stearns, S., ed. (1987) *The Evolution of Sex* (Birkhaeuser, Basel).
46. Selander, R. K., Musser, J. M., Caugant, D. A., Gilmour, M. N. & Whittam, T. S. (1987) *Microb. Pathog.* **3,** 1–7.