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Letters

Molecular epidemiology pitfalls: some important clarifications

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Constantine presents the interest and danger in using molecular tools for epidemiological inferences, and provides useful advice on how to avoid pitfalls [1]. However, there are some important inaccuracies, which need to be addressed here.

The definition and use of the term homoplasy is not totally accurate in Constantine's *Opinion* [1]. Homoplasy does not refer to samples. The definition of homoplasy is the identity between two alleles that are not identical by descent, but by state. This occurs when an allele mutates into the same state as a previously existing allele, or when two different alleles cannot be distinguished by the technique used. According to Rousset [2], the effect of homoplasy on measures of population subdivision (i.e. F statistics) is weak and simply corresponds to the infinite allele model (where homoplasy never occurs because mutation always creates new alleles) with a higher mutation rate [i.e. $u' = ku/(k-1)$, where k is the number of possible allelic states and u is the mutation rate]. The effect of a limited number of possible alleles (homoplasy) is not null, but is weakly detectable on F_{st} only (measure of population differentiation) and only for very low k (number of possible allelic states). According to equations 3 and 6 of Ref. [2], the difference will never exceed 0.01 in most situations if $k > 2$ and the mutation rate $u \leq 10^{-4}$. Thus, two samples will rarely appear to be the same (if ever) just because of homoplasy. It seems that there is confusion, on one hand, between species and between populations and, on the other hand, between population genetics and phylogenetic analysis. Indeed, they do not deal with the same problems. Phylogenetic analyses are correct for studying species divergences and can also be applied to fully clonal species. Population genetics tools are more appropriate to study populations where recombination occurs.

The breeding system definition also appears to be inaccurate. Heterogamy (mating preferentially occurs between phenotypically divergent partners) is disregarded. Moreover, the Hardy–Weinberg equilibrium is not a breeding system, as suggested in the Glossary of Ref. [1], but is an expected genotypic distribution under a specific set of different assumptions and one of these assumptions involve the breeding system (i.e. random association of gametes). This particular genotypic distribution, known as $[p + (1-p)]^2$ in the di-allelic case, can be mimicked by partial clones [3] or with other special parameters sets that are in disagreement with the Hardy–Weinberg assumptions. Similarly, the definition of linkage equilibrium given confuses the Hardy–Weinberg assumptions with the genetic consequences expected in populations fitting such assumptions. A population that follows Hardy–Weinberg assumptions can maintain linkage disequilibrium between different loci for a very long time. Indeed, under Hardy–Weinberg assumptions, the rate of decrease in linkage between two genes with recombination rate r is proportional to $(1-r)^t$, where t equals the number of generations [4]. Because Hardy–Weinberg equilibrium is reached in a single generation [4], it is easy to imagine populations in Hardy–Weinberg equilibrium at each locus with a significant linkage between loci.

The sentence on the maximum gene flow that is sufficient to prevent differentiation is arbitrary and thus meaningless. An effective number of migrants of one is very low and would lead to $F_{st} = 0.2$, an amount of divergence that could be found between different species [5] and thus between significantly divergent samples. Any migration rate is sufficient to prevent divergence by drift alone and comments on the amount of gene flow, considering the significant expected variance of F_{st} estimates [6], is non-informative. In addition, Constantine's comment on the ideal populations on which population genetics analysis are said to be based appear to be unfair

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with regards to the theoretical work published in this field of research [2,3,5–11].

More generally, Constantine's article does not differentiate the different kinds of molecular data clearly, such as those obtained by sequencing which are appropriate for phylogenetic approaches (comparing species or clones), and those concerning co-dominant polymorphic data which are appropriate for population genetics approaches (within recombining species). Each set of data has its own properties and domain of application, therefore they are not equally efficient depending on the biological situation.

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Parasitic protozoa: thiol-based redox metabolism

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In a thoughtful and stimulating article, Sylke Müller *et al.* [1] describe the thiol-based redox metabolism of protozoan parasites and believe that the key enzymes involved in this metabolism have potential use as new drug targets. Additional information on this subject is provided here.

It has been proposed that, in the absence of glutathione (GSH) and the enzymes of GSH metabolism, catalase and superoxide dismutase (SOD, in *Giardia*), cysteine is the main component of antioxidant defense systems in *Entamoeba* and *Giardia* [2–4]. Moreover, both parasites possess alternative mechanisms for detoxification, similar to those known to exist in certain prokaryotes [3]. *Entamoeba histolytica* is capable of synthesizing cysteine *de novo* from sulfate. However, I am not aware of any data available on cysteine and/or cystine uptake by *E. histolytica*, but there is evidence that the parasite is equipped with at least two different transport systems for amino acids [5]. Pinocytotic uptake could be the major route for satisfying amoebic growth requirements [6] because vacuolar membrane proton-transporting ATPase and P-type Ca^{2+}-transporting ATPase have been reported in *E. histolytica* [7]. As indicated by Müller *et al.* [1], it would be interesting to determine whether a trans-sulfuration pathway also exists in *E. histolytica*. Certain enzymes of methionine metabolism have been described in *E. histolytica*, such as *S*-adenosylmethionine decarboxylase [8,9], a key enzyme of polyamine biosynthesis pathway, and methionine γ-lyase [10].

Lujan and Nash [11] studied the uptake and metabolism of cysteine by *Giardia lamblia* trophozoites, and concluded that L-cysteine is an essential growth factor because these trophozoites cannot take up L-cystine from the environment and cannot synthesize cysteine *de novo*. In addition, the analyses of L-cysteine uptake indicated the presence of at least two different transport systems in the parasite including: (i) a non-saturable system that probably represents passive diffusion; and (ii) a system that is specific for thiol-containing amino acids, which might represent facilitated diffusion. Cystathione γ-lyase and cystathione γ-synthase activities were not detected in trophozoite homogenates, suggesting that the trans-sulfuration pathway is not active in *G. lamblia*.

Nevertheless, *E. histolytica* needs a comparatively large amount of cysteine (0.1% w/v) for its axenic cultivation. The *de novo* synthesis of cysteine might play a significant role in supplying the amino acid during trophozoite invasion, when the parasites move from an anaerobic environment (caecum/colon) to highly oxygenated (aerobic) areas, mainly the liver. Bruchhaus and Tannich [12] studied the regulation of SOD expression in *E. histolytica* in the presence of an O_2^--generating system and found that there was an increase in SOD activity between 3.2-fold and 4.7-fold, in addition to an increase in Fe–SOD protein concentration between 2.7-fold and 5.5-fold. This suggests that regulation of SOD might contribute to protection from toxic oxygen metabolites

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