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REVIEWS

Letter

Premature Rejection of Plasticity in Conversion

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The thesis presented by Koepfli and Yan [1] – that human malaria parasites in natural infections do not adjust conversion rates in an epidemiologically relevant manner – is not supported by the data they present.

What is conversion rate? It is the proportion of asexual stages that produce merozoites destined to differentiate into gametocytes versus merozoites committed to further asexual replication. This simple definition disguises immense difficulties in measuring conversion, as recognised by Koepfli and Yan [1].

It is often incorrectly assumed that the following metrics make good proxies for conversion rates (Box 1): (i) gametocyte prevalence (the proportion of patients that have gametocytes); (ii) gametocyte density (the concentration of gametocytes in the blood); (iii) gametocytaemia (the proportion of red blood cells infected with gametocytes); (iv) the proportion/ratio of gametocytes (number of gametocytes relative to asexuals). Intuitively, of course, the higher the conversion rate, the more gametocytes should be detected. But given the dynamic nature of asexual parasite densities, the same conversion rate can produce profoundly different gametocyte densities. Instead, any count of gametocytes must be related back to the density of asexuals in the cycle that produced those gametocytes. It cannot be assumed that asexual density measured on a particular day is a good proxy for the density a week (or more) in the past. Another problem – well known in evolutionary ecology – is using cross-sectional population data to infer characteristics of individuals [2]. In the case of malaria parasites, variation between patients means that changes in conversion rates within individual infections can only meaningfully be detected from high-resolution longitudinal monitoring of individual infections. Sequestration and a long life-span of gametocytes in some species adds to the complexity.

Thus, we agree with Koepfli and Yan [1] that no study of natural human malaria infections has been able to accurately measure conversion. But this means it has not been possible to test whether parasites in natural infections modulate conversion rates – and a hypothesis should not be rejected without a fair test. Koepfli and Yan [1] assume that conversion is constant and so only knowledge of asexual density is required to predict gametocyte density. But, correlation is not causation: a positive correlation between asexual and gametocyte densities does not exclude other explanations. Of course, asexual densities correlate positively with gametocyte densities: organisms with more resources will, in general, invest more in reproduction [3]. This trend is not linear and is very variable [4,5], as demonstrated in Koepfli and Yan's Figure 2 [1], this variation could be explained by changing conversion rates.

Intuitively, 'no plasticity in conversion' appears a parsimonious explanation but it does not fulfil Occam's razor. To reject the hypothesis that parasites in natural human infections modulate their conversion rates requires three onerous conditions be met. First, provision of alternative explanations for a wealth of data from natural infections (e.g., [6–9]) and neurosyphilis malariatherapy patients [10]. Second, demonstrating that observations of conversion being adjusted in animal models and human parasites in culture – in the manners predicted to maximise parasite fitness (reviewed in [11]) – are incorrect. Third, explaining why human-infecting parasites are exempt or unable to follow the same strategies (i.e., reproductive restraint and terminal investment) adopted by insects, birds, mammals, plants, and parasites in laboratory studies [11]. Notably, Koepfli and Yan [1] do not offer any such explanations. Our thesis is that more extensive laboratory studies are needed to establish proof-of-principle for how and why parasites adjust conversion in response to the diversity of circumstances they encounter during infections. Such environmental factors rarely change in isolation, and so, exploring their relative influences on conversion rates is most efficiently achieved

Box 1. Frequently Used Proxies for Conversion Rates

Conversion rate is calculated most simply by dividing gametocyte density by parasite density at the time of commitment [11].

Gametocyte prevalence, density, and gametocytaemia provide estimates of transmission potential but preclude inference of conversion rates without information on asexual densities. For example, 10^2 gametocytes/ml could originate from 10^6 asexuals/ml with 0.01% conversion, or from 10^3 asexuals/ml with 10% conversion, a difference of three orders of magnitude. An exception is made when asexual densities are equal at the time of commitment, because variation in the resulting gametocyte densities can reflect different conversion rates.

The proportion of parasites that are gametocytes is also an inadequate proxy. Between commitment and detection of mature gametocytes, *Plasmodium falciparum* will have completed at least five asexual cycles. Imagine two infections with the same asexual density 'a' and conversion rate 'c' (which could vary over time) whose asexuals (i) double or (ii) halve in each subsequent cycle. The proportion of gametocytes at the time of sampling would be (i) $\frac{a*c}{(1-c)^5*a*2^5}$ or (ii) $\frac{a*c}{(1-c)^5*a/2^5}$. The proportion of gametocytes (i) underestimates or (ii) overestimates conversion by 32-fold. This difference of three orders of magnitude could explain Koepfli and Yan's [1] subdivision of Figure 2B and adds to noise generated by gametocyte developmental times and differential mortality.

with experiments. Subsequently, more realistic predictions can be made for, and tested in, natural infections.

We hope that Koepfli and Yan [1] stimulate efforts to improve the collection of relevant data from more intense sampling of patients. If gametocyte development time is known, detecting gametocytes at any specific age enables conversion to be inferred. We also suggest the following. First, detection problems are exacerbated when smaller blood volumes are used to measure gametocytes than asexuals, so larger sample volumes should be used for gametocytes. Second, in addition to quantifying gametocytes and the asexual parasites they originated from, measures of environmental factors (e.g., multiplicity of infection, anaemia, immune responses) at the time of commitment should be made. Third, to differentiate signal from noise (i.e., avoid type 2 statistical errors), considerably more patients than most cohort studies contain are required. Fourth, measurements of sequestration rates and variable stage-specific mortality rates are ideal but not necessary because modern algorithms to calculate conversion [12] can estimate and account for these factors from data on parasite and red blood cell densities alone.

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Letter

Complex Determination of the Gametocyte Conversion Rate

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Malaria transmission is a fascinating field of study at the intersection of evolutionary biology and infectious disease control, with many questions awaiting answers. Do parasites alter their investment into transmission in response to factors in the human host, such as immune response? Do they sense transmission intensity, for example the frequency of anopheline bites, and if so, how do they respond to it? What is the impact of the genetic background of the parasite, human host, and vector? In a recent article we have discussed how population-based studies can inform this research [1]. In their response, Reece and Schneider have misinterpreted key points of our article.

In order to study if and when malaria parasites adjust the gametocyte conversion rate in response to external factors, *in vitro* parasite culture, animal models, and controlled human infection trials have been used, historic malariatherapy data reviewed, and mathematical modeling applied.

It remains challenging to confirm processes observed in controlled systems in epidemiological field studies. Epidemiological data are inherently noisy, with densities of asexual parasites and gametocytes in many asymptomatic infections around the technical limit of detection. Developing *Plasmodium falciparum* gametocytes sequester in inner organs for 10 days, and sampling in cohorts is usually not sufficiently frequent to compare densities of mature gametocytes to asexual densities at the time of gametocyte conversion, further complicating studies on triggers of gametocyte commitment. Few studies have assessed the conversion rate directly [2]; parameters frequently gathered, such as the proportion of all infections carrying detectable gametocytes, or densities of mature gametocytes, are only indirect measures.

In our recent article we have focused on these difficulties when interpreting data from epidemiological studies [1]. We have shown that, in many cases, differences in the proportion of gametocyte-positive infections might be explained by different mean asexual parasite densities, for example when children with little acquired immunity and high parasite densities are compared to adults with high levels of acquired immunity and low parasite densities [3]. In other situations, an adjustment to the gametocyte conversion rate seems plausible, but has not been proven in field studies, for example in the case of altered gametocyte densities in mixed-species infections [4]. We have made suggestions for further research, such as the use of molecular markers for early gametocytes [5], or field studies to assess whether *P. falciparum* gametocyte densities follow