Acta Tropica 128 (2013) 196–205

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Reductions in genetic diversity of *Schistosoma mansoni* populations under chemotherapeutic pressure: the effect of sampling approach and parasite population definition

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ARTICLE INFO

Article history:
Available online 15 March 2012

Keywords:
Schistosoma mansoni
Population genetics
Stochastic re-sampling
Tanzania
Sampling protocol
Preventive chemotherapy
Praziquantel
Monitoring and evaluation

ABSTRACT

Detecting potential changes in genetic diversity in schistosome populations following chemotherapy with praziquantel (PZQ) is crucial if we are to fully understand the impact of such chemotherapy with respect to the potential emergence of resistance and/or other evolutionary outcomes of interventions. Doing so by implementing effective, and cost-efficient sampling protocols will help to optimise time and financial resources, particularly relevant to a disease such as schistosomiasis currently reliant on a single available drug. Here we explore the effect on measures of parasite genetic diversity of applying various field sampling approaches, both in terms of the number of (human) hosts sampled and the number of transmission stages (miracidia) sampled per host for a *Schistosoma mansoni* population in Tanzania pre- and post-treatment with PZQ. In addition, we explore population structuring within and between hosts by comparing the estimates of genetic diversity obtained assuming a 'component population' approach with those using an 'infrapopulation' approach. We found that increasing the number of hosts sampled, rather than the number of miracidia per host, gives more robust estimates of genetic diversity. We also found statistically significant population structuring (using Wright's *F*-statistics) and significant differences in the measures of genetic diversity depending on the parasite population definition. The relative advantages, disadvantages and, hence, subsequent reliability of these metrics for parasites with complex life-cycles are discussed, both for the specific epidemiological and ecological scenario under study here and for their future application to other areas and schistosome species.

1. Introduction

In recent years there has been an increased focus on schistosomiasis control programmes, the main aim of which, to date, has been morbidity control. Preventive chemotherapy, that is the large-scale administration of drugs to at-risk populations, using praziquantel (PZQ) has become the global strategy for morbidity control (Hotez et al., 2007). However, the impact of such programmes on the genetic structure of the parasite populations is still largely unknown, although it is likely that prolonged selective pressure will be exerted. Thus far, there is little evidence for resistance to PZQ in schistosome samples derived from human hosts under field conditions (Doenhoff et al., 2002; Doenhoff and Pica-Mattoccia, 2006). However, heterogeneities in cure and egg reduction rates (Utzinger and Keiser, 2004), reports of individual treatment failures, resistance to almost all anthelminthics within the veterinary field (Coles et al., 2006), reduction in size of parasite *refugia* (Webster et al., 2008; Steinauer et al., 2010), and lack of any feasible alternatives to PZQ in the foreseeable future, dictate that vigilance should remain high (Cioli, 1998; Ismail et al., 1999; Fenwick and Webster, 2006; Doenhoff et al., 2008).

1.1. A need for sampling protocols

The design of effective monitoring and evaluation (M&E) strategies to enable identification and quantification of changes

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doi:10.1016/j.actatropica.2012.03.001

in parasite population genetic diversity following preventive chemotherapy will be crucial to this end, also allowing insights to be drawn into the life-history, transmission dynamics, and epidemiology of the infection. The recent development of multiplex microsatellite markers that are putatively neutral with regard to PZQ resistance (Shrivastava et al., 2005b; Gower et al., 2007; Golan et al., 2008; Steinauer et al., 2008) has permitted schistosome population structure to be characterised by typing miracidia directly, removing the need to passage worms through laboratory mammals, avoiding the potential biases of imposing artificial population bottlenecks, and allowing high throughput at affordable expense (Norton et al., 2010).

A recent study using such markers on the same dataset as used in the current report identified reductions in the genetic diversity of *Schistosoma mansoni* populations in Tanzania following just a single round of preventive chemotherapy with PZQ (Norton et al., 2010). The authors discussed a number of potential explanations for the observed reduction. For instance, they proposed that schistosome populations might be more highly structured than previously thought, and/or that not all parasites in *refugia* contribute to reinfection. A worrying potential explanation, however, would be that such a reduction reflects, at least in part, some form of selection of these parasites towards reduced PZQ efficacy (although these microsatellites are thought to be neutral markers (Gower et al., 2007; Norton et al., 2010)). This reduction could result from chemotherapy-induced selective immunity, whereby those parasite genotypes adapted to infect humans are differentially killed by the drug. An alternative hypothesis is that the lower genetic diversity observed may be a reflection of the shorter time available for reinfection (months versus years), although similar reductions in schistosome genetic diversity amongst those children newly entering the cohort each year, and hence not previously treated with PZQ, suggest this is not the case. Finally, these reductions could have been caused by secular changes, such as a decrease in the number of suitable habitats for intermediate host snails. Given the implications of these possible explanations with regard to the ecology, epidemiology, population genetics and control of schistosomiasis, it is important that monitoring of the genetic structure of parasite populations be included as part of control programmes from the outset and, indeed, the Schistosomiasis Control Initiative (SCI) has led the way as the first major control programme of human helminthiasis to do this.

How to effectively and efficiently achieve the incorporation of such population genetic M&E strategy into large-scale preventive chemotherapy control programmes is, however, not necessarily straightforward. In the past, the choice of sampling protocols has often been driven by practicalities and intuition as opposed to evidence-based decision making, and subsequently this is one of the most under-represented areas in empirical studies of parasite populations (Jarne and Theron, 2001). The collection, processing, and analysis of biological field samples are both time-consuming and costly processes in a disease area that is already poorly supported. As a rule of thumb, one study recommended a sample size of 20 parasites per individual host, five hosts per site, and 10 sites (Jarne and Theron, 2001). However, the specific approach will necessarily be dictated by the biological questions being addressed in each study, and the particular epidemiological and ecological situation at each location. Just as performing power and sample size calculations are now regarded as standard steps in epidemiological study design, devising and justifying sampling strategies in order to most effectively utilise scarce time and resources, and address appropriately the questions under investigation, should be considered in any molecular ecology and genetics study and particularly in human parasitology with regard to control programmes (Silva et al., 2006; Churcher et al., 2008; Churcher and Basáñez, 2009).

1.2. Metrics of genetic diversity

Metrics of genetic diversity, such as allelic richness (*AR*), and observed and expected heterozygosity (respectively H_O and H_E), were originally developed for sexual, free-living organisms undergoing direct life-cycles. How these measures perform in parasitic species is still poorly understood (Blouin et al., 1999; Curtis and Minchella, 2000). Whilst a profound understanding of the genetic structure of parasite populations, combined with their epidemiology, can lead to insights on important biological processes such as the underlying level of transmission, gene flow, size of reproductive units, breeding strategy, and emergence of anthelminthic resistance, amongst others (Nadler, 1995; Fisher and Viney, 1998; Paterson and Viney, 2000; Prugnolle et al., 2005a), the ability of current tools to achieve this is uncertain, given how surprisingly little is known about the population genetic structure of most species of parasitic helminths (Prugnolle et al., 2005a).

Schistosomes undergo a complex life-cycle involving separate sexes and the alternation of generations (with a sexual phase in the definitive mammalian host and an asexual phase in the intermediate snail host), which potentially can lead to population structuring between 'infrapopulations' (those parasite populations within individual human hosts) and 'component populations' (the pool of parasite populations within a collection of hosts) (Margolis et al., 1982; Bush et al., 1997). However, the distinction between the infrapopulation and component population is not clear-cut in schistosomes, as the parasite's obligate asexual phase in the snail can and does lead to genetic exchange between infrapopulations; parasites released from one infrapopulation can eventually be taken up by another, after passing through the intermediate host (Criscione et al., 2005). The amount of mixing of offspring will determine how distinct parasite infrapopulations are and influence the outcome and interpretation of measures of genetic structure using currently accepted metrics. If mixing levels are low (if offspring infect their original host, or are transmitted in a 'clumped' fashion) the infrapopulations will tend towards truly separate populations (Criscione et al., 2005). However, if offspring are very well mixed then the transmission process will only separate adult schistosomes into infrapopulations each generation but will not result in recurrently isolated generations within individual infrapopulations (Criscione et al., 2005). Although there will likely be some exchange of genetic information within and between parasite populations (Rollinson and Simpson, 1987), the condition of panmixia, upon which much of the field of population genetics is based, will not always be fulfilled, leading, under these circumstances, to questioning the applicability of some of the standard measures of genetic diversity (*AR*, H_O and H_E for example). Further, during the transmission process, each host is unlikely to acquire worms randomly from the whole population (Blouin et al., 1999; Curtis and Minchella, 2000), and the asexual stage of the life-cycle will likely produce large numbers of genetically related clones (Sire et al., 2001; Yin et al., 2008; Lu et al., 2010). This variance in clonal reproductive success, combined with synchronous release of cercariae and rare infection events can lead to hosts being infected with parasites heavily biased towards a few genotypes (Waples, 1998; Sire et al., 1999; Prugnolle et al., 2005a; Lu et al., 2010), as well as to the possibility of heterozygote deficiency, selfing, and mating within clones (Prugnolle et al., 2005a). A recent study carried out in China, focussing on *Schistosoma japonicum* (a species with multiple potential definitive hosts), found values of the Wright F_{ST} statistic (a measure of the correlation between genes at different levels of a (hierarchically) subdivided population) that indicated low gene flow among hosts (both intermediate and definitive) (Lu et al., 2010).

A further potential confounder is that it is currently not possible to sample the worms directly (as they are not expelled upon