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The geography of malaria genetics in the Democratic Republic of Congo: A complex and fragmented landscape

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ABSTRACT

Understanding how malaria parasites move between populations is important, particularly given the potential for malaria to be reintroduced into areas where it was previously eliminated. We examine the distribution of malaria genetics across seven sites within the Democratic Republic of Congo (DRC) and two nearby countries, Ghana and Kenya, in order to understand how the relatedness of malaria parasites varies across space, and whether there are barriers to the flow of malaria parasites within the DRC or across borders. Parasite DNA was retrieved from dried blood spots from 7 Demographic and Health Survey sample clusters in the DRC. Malaria genetic characteristics of parasites from Ghana and Kenya were also obtained. For each of 9 geographic sites (7 DRC, 1 Ghana and 1 Kenya), a pair-wise R_{ST} statistic was calculated, indicating the genetic distance between malaria parasites found in those locations. Mapping genetics across the spatial extent of the study area indicates a complex genetic landscape, where relatedness between two proximal sites may be relatively high ($R_{ST} > 0.64$) or low ($R_{ST} < 0.05$), and where distal sites also exhibit both high and low genetic similarity. Mantel's tests suggest that malaria genetics differ as geographic distances increase. Principal Coordinate Analysis suggests that genetically related samples are not co-located. Barrier analysis reveals no significant barriers to gene flow between locations. Malaria genetics in the DRC have a complex and fragmented landscape. Limited exchange of genes across space is reflected in greater genetic distance between malaria parasites isolated at greater geographic distances. There is, however, evidence for close genetic ties between distally located sample locations, indicating that movement of malaria parasites and flow of genes is being driven by factors other than distance decay. This research demonstrates the contributions that spatial disease ecology and landscape genetics can make to understanding the evolutionary dynamics of infectious diseases.

1. Introduction

Malaria remains endemic in 104 countries, despite rollback and eradication efforts, and caused an estimated 207 million cases and 627,000 deaths in 2012, primarily in Africa (World Health Organization, 2013). The Democratic Republic of Congo (DRC) in particular suffers one of the highest burdens of malaria in sub-Saharan Africa: health surveys indicate that over a third of adults

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in the DRC have malaria parasites in their blood (Hay et al., 2010; Taylor et al., 2011). *Plasmodium falciparum*, one species of malaria parasite, causes nearly all infections within the DRC (Taylor et al., 2011). The success of malaria eradication efforts depends not only on local anti-malaria campaigns, but also on preventing re-introduction of malaria parasites from poorly-controlled areas into places newly free of the disease (Le Menach et al., 2011; Moonen et al., 2010). Given the potential for malaria re-introduction to undo progress in control efforts, an understanding of how malaria diffuses across a landscape, and how this diffusion can be limited or stopped, is essential. As yet, however, we have limited insight into how malaria spreads geographically (Lynch and Roper, 2011).

A landscape genetics approach holds promise for filling in this key knowledge gap. Indeed, because of limited movement of mosquitoes (a maximum of 10 km), the most likely means by which malaria spreads is through the movement of people carrying the parasites in their blood (Kaufmann and Briegel, 2004). Landscape genetics allows us to observe parasites in different locations and infer population movement from the distribution of the parasite's genetic markers, while also measuring geographic and landscape factors driving that distribution, either by promoting or by preventing dispersal (Biek and Real, 2010; Holderegger and Wagner, 2006; Manel et al., 2003; Storfer et al., 2010; Storfer et al., 2007). The flow of malaria genes across landscapes has been explored previously and such work has indicate a geographic structure to malaria genetics, that parasite populations can be fragmented depending on the connectivity (i.e. coastal or inland) of locations, that border regions can act as spaces of origin of drug resistance and that population movement threatens success of local eradication programs by re-introduction of malaria (Alam et al., 2011b; Griffing et al., 2011; Rebaudet et al., 2010; Schultz et al., 2010). Additionally, molecular epidemiology has been used to conclusively determine the source of a malaria outbreak among UN peacekeeping soldiers from Guatemala returning from the DRC, emphasizing the need for molecular surveillance as an integral part of malaria control and elimination program (Patel et al., 2014). As yet, however, few studies have focused on malaria genetics within DRC or connections to other sub-Saharan African countries, which, given the high burden of malaria in DRC and the focus of the global health community on malaria eradication in this region, indicates a need for greater research in this geographic and topical area. Medical geography in particular, with its emphasis on disease ecology and spatial statistics, can make valuable contributions to understanding how infectious diseases of humans are transmitted across spaces and places (Carrel and Emch, 2013).

Using spatial methods to explore the genetic characteristics of malaria parasites found in humans across seven sites in the DRC, and a site each in Ghana and Kenya, we sought to understand whether genetic variation between sites is correlated with geographic space between those sites, how scaling up or down from national to sub-national scale changes the observed relationship between genetics and geography, and whether significant barriers to malaria gene flow exist. Given the complex ecology of malaria, dependent on mosquito vectors from the *Anopheles* genus, and the mobility of infected and susceptible populations across the DRC, we did not anticipate finding a strong correlation between genetic variation and geographic distances, as has been found for other infectious diseases such as H5N1 influenza and rabies (Carrel et al., 2010; Real et al., 2005). Instead, we hypothesized that a more fragmented genetic landscape would be found, reflecting variations in land cover, transportation routes, population movement, and conflict zones that are seen across the DRC.

2. Data and methods

In 2007 a Demographic and Health Survey (DHS) was completed on a spatially and statistically representative sample of individuals within the DRC, grouped within household clusters across the country. Survey participants had blood spots taken, from which we were able to retroactively detect malaria parasites via real-time polymerase chain reaction (real-time PCR), as has been previously described (Taylor et al., 2011). This research received institutional review board approval at the Kinshasa School of Public Health, the University of North Carolina–Chapel Hill and the University of Iowa.

To explore how genetic characteristics of malaria parasites vary across the DRC, we selected seven DHS clusters, each with ten or more asymptomatic *P. falciparum*-positive individuals, for analysis. The clusters were selected based on the hypothesis that the Congo River, a principal route of human transportation, would facilitate diffusion of *plasmodium* parasites. Three clusters (81, 88 and 183) were chosen because they were located on or near the Congo River, a principal route of human transportation (Herderschee et al., 2011); two clusters (164 and 211) were chosen because they were not on the river but were approximately the same distance apart from the three river sites as the river sites were from one another; and two final clusters (29 and 203) were chosen because they were far away (>1000 km on average) from the other clusters (Fig. 1). The median distance between clusters was 709 km (IQR = 883 km). This geographic diversity allowed us to explore whether sites linked via the river, sites the same distance from one another but not river linked and sites geographically distant had differing patterns of genetic relatedness.

These seven clusters contained 82 individuals infected with *P. falciparum*. In addition to the samples from the DRC, 72 and 62 infected individuals previously reported from Ghana and Kenya respectively were included for comparative analyses of parasite microsatellites across political borders (Alam et al., 2011a; McCollum et al., 2012).

Five neutral, meaning not under selective pressure, microsatellite markers on two different chromosomes (C2M33, C2M34, C2M29, C2M27 on chromosome 2; and C3M40 on chromosome 3) were chosen to assess the population structure of parasites. Microsatellites are short repeating sequences of DNA base pairs, typically with high heterozygosity, that can be used to determine genetic relatedness of individuals. All specimens were amplified either through a single-round or hemi-nested Polymerase Chain Reaction as described previously; (Anderson et al., 2000, 1999; Roper et al., 2003). PCR products were then separated by capillary electrophoresis using an ABI 3130xl genetic analyzer. The alleles were scored using GeneMapper software, version 3.7 (Applied Biosystems, Foster City, CA). Alleles were binned, depending on the size of the repeat unit, to the nearest 2 or 3 nucleotides in length.

Since there is a possibility that individuals could be infected with multiple variants of *P. falciparum*, it would be difficult to accurately assign microsatellite allele sizes to particular variants within each individual using capillary electrophoresis. However, because the genetic relatedness index above is calculated at microsatellite marker, the linkage between each marker (i.e. that they were inherited together as an intact unit) is not necessary to ascertain. Therefore, we included all individuals even if they were infected by mixtures of *P. falciparum* variants, as evidenced by mixtures at one or more microsatellite markers. Because the data format for GenAlEx and SPAGeDi requires that each individual have unique microsatellite marker profile, we created “virtual” unique variants for individuals that were infected with multiple variants to ensure we include all possible variants in circulation in our study populations. If two or more alleles were detected on one