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Genetic diversity and population structuring of *Schistosoma mansoni* in a Brazilian village

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Abstract

The digenean trematode *Schistosoma mansoni* is responsible for chronic schistosomiasis worldwide and in Brazil alone an estimated 35 million people are at risk. To evaluate epidemiological patterns among human definitive hosts, we assessed genetic diversity and population subdivision of *S. mansoni* infrapopulations in human hosts from the highly endemic village of Virgem das Graças in the state of Minas Gerais, Brazil. We believe this is the largest such survey to date. Genetic diversity of parasites, measured over eight polymorphic microsatellite loci, was relatively high and standard measures of inbreeding indicated that the population was panmictic. Furthermore, there were no significant isolation-by-distance of parasite infrapopulations and measures of population subdivision indicated significant but low to moderate levels of population differentiation. We conclude that patients within this village sample from a broad range of schistosome genetic diversity and effectively act as “genetic mixing bowls” for the parasites. These results contrast with those previously observed in the Brazilian village of Melquíades and thus provide the opportunity for comparisons of environmental and epidemiological differences that are likely to influence host-parasite coevolution and parasite transmission.

Keywords

Schistosoma mansoni; Population genetics; Microsatellite; Gene flow; Molecular epidemiology

1. Introduction

Schistosomiasis is a disease of significant medical impact, affecting an estimated 200 million people in 74 countries, with an additional 600 million living at risk of infection (Chitsulo et al., 2000). *Schistosoma mansoni*, one of the species responsible for intestinal schistosomiasis, is endemic throughout much of South America, the Caribbean, Africa and the Middle East. Although successful interventions have greatly reduced the burden of disease in several regions (e.g. the Caribbean islands), there are many areas where *S. mansoni* prevalence remains high and concerns such as drug resistance and resurgence necessitate continued investigation. In particular, elucidating the distribution of parasite genetic diversity is critical to understanding

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and predicting disease epidemiology. However, few investigations of schistosome genetic diversity have sampled extensively from parasite populations residing in the human host (but see Brouwer et al., 2001; Curtis et al., 2002), with researchers choosing instead to focus on more experimentally tractable sylvatic or domesticated definitive hosts (e.g. Barral et al., 1996; Sire et al., 1999, 2001a, 2001b; Prugnolle et al., 2002, 2005b; Théron et al., 2004; Shrivastava et al., 2005; Agola et al., 2006; Wang et al., 2006). Yet the extent to which epidemiologically relevant conclusions can be made based on investigations involving non-human definitive hosts is still unknown.

One of the primary reasons for studying parasite population genetics is to understand demographic parameters, such as gene flow and population size, which are not readily observable using conventional ecological methods. These insights allow inferences regarding the patterns of parasite transmission and recruitment within the environment. Generally, movement of the definitive host is expected to play the greatest role in parasite gene flow (due to greater host vagility). This has been shown in several studies of helminth population genetics (Blouin et al., 1995, 1999; McCoy et al., 2003). Yet while numerous studies of human water-contact behavior have assessed the myriad factors influencing risk of infection and transmission within human populations, the influence of these complex social and behavioral patterns on the gene flow of water-borne parasites is still uncertain. For instance, as Kloos et al. (1998) observed in the Brazilian village of Nova União, access to water sources may be restricted to a few people due to private ownership of land and water rights. This, combined with the observation that most human water contact occurred within 8–80 m of the household, should serve to reduce parasite gene flow and create isolated subpopulations of highly related parasites relative to the remainder of the population. Conversely, Kloos et al. (1998, 2006) also observed factors that would potentially increase parasite gene flow. Activities such as fishing led people to sites up to 350 m away from the household, and remote areas (e.g., agricultural-related) comprised a considerable proportion of water contacts, particularly among male hosts. Additionally, they noted that women often preferred to wash laundry in groups for the social interaction and that in areas with a dearth of suitable water sources, water contacts tended to cluster at preferred sites.

Thus, numerous factors can act to either promote or restrict the gene flow of schistosome parasites among human hosts and the relevant influence of each factor in a particular population or village may vary as a result of environmental variables. Here we describe one of the most extensive population genetics studies of *S. mansoni* parasites collected from human hosts. Using parasites collected from the infrapopulations of 30 patients from an endemic area of Brazil, we assess the degree of population differentiation at the scale of a single village and compare our findings with those of similar studies of schistosome genetic distribution, focusing particular attention on a study of human schistosomes from a village in the same Brazilian state.

2. Materials and methods

2.1. Site and collection description

The geographical, socioeconomic and infection profiles of the study population, Virgem das Graças (VdG), have been described extensively elsewhere (Kloos et al., 2004, 2006; Gazzinelli et al., 2006a, 2006b). Briefly, it is a rural population located in the semi-arid Jequitinhonha Valley in the northeastern portion of the Brazilian state of Minas Gerais. The overall area of VdG consists of a central village (Taboca) and four hamlets (Suçuarana and Cardosos 1, 2, and 3) dispersed over a total area of approximately 60 km². In general, households are closely associated with the primary waterway, Cardoso Stream, and its several tributaries (Fig. 1a). The households participating in this study account for approximately half of the total area (32 km²) and are situated < 1 to 6,000 m in linear distance from one another (mean distance

2.03km). The overall prevalence of schistosome infection prior to sampling was relatively high (57%) (Kloos et al., 2004). At the time of this study, most residents had not received prior anthelmintic treatment because the National Schistosomiasis Control Program had not yet been implemented in VdG and the prevailing level of poverty, poor road conditions and distance to medical clinics precluded seeking treatment (Gazzinelli et al., 2006b). After patients were sampled, praziquantel treatment (50 mg/kg) was made available to all known infected persons.

In June 2001, fecal samples were collected from 30 patients representing 24 households in the area of VdG. Following the methods detailed in Sorensen et al. (2006), *S. mansoni* eggs from each patient were isolated and hatched to infect a patient-specific group of 15 laboratory-reared *Biomphalaria glabrata* with approximately 10 miracidia per snail. Cercariae from infected snails were pooled by patient and used to infect two to five BALB/c mice per patient isolate (100–120 cercariae/mouse). Mouse infections and subsequent collection of adult worms followed the procedures of Curtis et al. (2001a). Worms were enumerated and maintained separately according to worm sex, mouse and patient in water at -20°C .

2.2. Genotyping

Given the occurrence of clonal reproduction in the schistosome life cycle, genetic redundancy is expected and has been reported in numerous field studies (Curtis et al., 2002; Prugnolle et al., 2002, 2004; Théron et al., 2004). As such, extraction and genotyping of every individual collected would become economically demanding at the risk of little return in novel allele detection. Rather than choose an arbitrarily sized subset of individuals to genotype, the allelic diversity curve of Sorensen et al. (2006) was applied to determine the proportion of individuals beyond which the probability of detecting unique alleles would not compensate the cost of genotyping. It was determined that genotyping approximately 52% of the worms collected from each patient would yield the greatest return, an estimated 87% of the total allelic diversity. Thus, 52% of male and female worms were randomly selected from each infected mouse for genotyping. In the event that a mouse yielded fewer than 15 worms of a particular sex, all of those worms were genotyped.

Total genomic DNA was extracted from adult male and female worms following Sorensen et al. (1998). Eight schistosome microsatellite loci (L46951, SMD28 (Durand et al., 2000), C1, DA23, DO11 (Curtis et al., 2001b), SmBr1 (Rodrigues et al., 2002), SmBr12, and SmBr13 (Rodrigues et al., 2007)) were amplified in 15 μl multiplex reactions. Each reaction contained 1 X reaction buffer (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.1% Triton[®]X-100), 200 μM deoxyribonucleotide triphosphate (dNTP), 2 mM MgCl_2 , 167 nM of each fluorescently labeled primer (3–4 primer pairs/reaction), 0.6 U *Taq* DNA Polymerase (Promega), 0.8 mg BSA, and 50 ng of worm DNA. Thermal cycling was performed in either a MJ Research PTC-100 (Bio-Rad) or an Eppendorf Mastercycler gradient thermocycler under the following conditions: 4 min denaturation at 94°C ; 32 cycles of 30 s at 94°C , 30 s at 55°C and 1 min at 65°C ; and a final 3 min extension at 65°C . PCR products were electrophoresed on an ABI 377 sequencer, using GENESCAN[®]-500 [TAMRA] as an internal size standard in each lane and allele sizes were determined using GENESCAN v3.1 and GENOTYPER v2.5 software (PE Applied Biosystems). Individuals with failed amplifications or questionable peak patterns were genotyped twice.

2.3. Data analysis

Only worms with complete genotypes were included in the analyses and clonal genotypes within each patient infrapopulation were reduced to one representative individual. To determine clonality, identical multilocus genotypes (MLGs) were first identified within patient isolates using the *matches* function in GenAlix version 6 (Peakall and Smouse, 2006). The

likelihood that these repeated MLGs occurred due to sexual reproduction was tested with the program MLGsim (Stenberg et al., 2003), where P_{sex} -values were calculated for each set of identical MLGs and compared with values generated by 1×10^7 simulations. All observed P_{sex} -values were highly significant ($P < 0.0001$) at $\alpha = 0.05$, falling well outside the distribution of simulated values and indicating that identical MLGs were indeed clonal.

Overall, a total of 585 adult worms (348 male and 237 female) were used for analysis, with all representative patient isolates consisting of a minimum 10 worms. To assess the genetic diversity of each patient's parasite infrapopulation, the number of alleles per locus and unbiased heterozygosity (H_s ; Nei, 1987) were calculated in FSTAT (Goudet, 1995) version 2.9.3.2 (freely available at: <http://www.unil.ch/izea/software/fstat.html>). The Weir and Cockerham (1984) estimator f of F_{IS} was calculated for each locus and across all loci to test for deviations from Hardy-Weinberg equilibrium (HWE). The hypothesis that the parasite population is not panmictic (i.e., $f \neq 0$) was tested in SPAGeDi version 1.2 (Hardy and Vekemans, 2002) via a two-tailed test of 20,000 permutations of alleles among individuals. Significance was set at $P < 0.05$. An exact test for linkage disequilibrium between pairs of loci was performed using the Markov chain method (10,000 dememorizations; 5,000 batches; and 10,000 iterations per batch) in GENEPOP web version 3.4 (Raymond and Rousset, 1995). To correct for multiple comparisons, the sequential Bonferroni correction (Rice, 1989) was applied to determine significance. Also, to ensure that genetic diversity measurements were not biased by worm sex, parasite infrapopulations were grouped by worm sex and a two-tailed test of 10,000 permutations of infrapopulations among the male and female groupings was performed in FSTAT.

Genetic differentiation of parasites was assessed with several measures of subdivision and over selected levels of a priori population groupings chosen based on demographic factors that might be expected to influence parasite gene flow among human hosts. Parasites groupings were by patient (i.e., infrapopulations), hamlet, patient sex, patient age (< 10, 11–20, 21–40 and 40+ years old) and patient infection intensity (< 100, 100–200, 201–300 and 400+ eggs per gram of feces). The standard estimator θ of F_{ST} (Weir and Cockerham, 1984) was calculated to assess the difference in allele frequencies among subpopulations. Significance of genetic differentiation was assessed with the G -based test (Goudet et al., 1996) following 50,000 permutations of parasite genotypes and 95% confidence intervals (CIs) were calculated by bootstrapping over loci in FSTAT. Because high levels of heterozygosity can lower the maximum observable F_{ST} considerably (Hedrick, 2005), even relatively low values of observed F_{ST} can indicate high levels of subdivision if they account for a large proportion of the possible maximum F_{ST} . Thus, we standardized all measures of F_{ST} to assess the proportion of F_{max} comprised by F_{obs} using the recoding method of Meirmans (2006) to calculate F'_{ST} . Both original and standardized (F'_{ST}) estimates of F_{ST} are reported.

To evaluate the proportion of variance explained by each parasite grouping, analysis of molecular variance (AMOVA) was implemented with ARLEQUIN version 3.01 (Excoffier et al., 2005). Furthermore, because water contact behavior in rural areas and, thus, transmission of *S. mansoni* is expected to be spatially clustered (Cairncross et al., 1996; Watts et al., 1998; Bethony et al., 2004; Brooker et al., 2006), we investigated patterns of isolation-by-distance. Using the method of Rousset (1997), we assessed the correlation between genetic distance (measured as $\theta/(1-\theta)$) and the logarithm of linear geographic distance at three levels: among households, among hamlets, and patient sex. Significance was tested via a Mantel test with 15,000 permutations in FSTAT.

The Bayesian clustering analysis of STRUCTURE version 2 (Pritchard et al., 2000; Falush et al., 2003) was also utilized to test for subdivision of parasites into distinct genetic clusters. The maximum number of clusters (K) was set at 30 to simulate the maximum number of populations

expected if patient infrapopulations were acting as demes. Under the assumptions of admixture and allele frequency correlation, each K -value was replicated 10 times with burn-in and Markov chain Monte Carlo lengths of 25,000 and 50,000, respectively. Estimation of the true value of K followed the procedure of Evanno et al. (2005).

2.4. Village comparison

Previously published investigations of parasite genetic differentiation within another Brazilian village (Melquíades: Curtis et al., 2002) indicated that different parasite genetic patterns existed in VdG. Only measures of F_{ST} were reported in the Melquíades study; thus, to assess these differences more fully, allelic data obtained from the Melquíades data (Curtis et al., 2002) were analyzed using the same parameters as VdG with the exception that missing data were allowed for a maximum of two loci per individual worm and only male worms were genotyped across the seven microsatellite loci (four of which were also utilized for VdG).

As described more extensively elsewhere (Bethony et al., 2001, 2004; Gazzinelli et al., 2001; Kloos et al., 2001), the village of Melquíades consists of households distributed into 14 hamlets in nine valleys, each with at least one independent waterway, over a total area of approximately 100 km². Seventeen patients of mixed ancestry and sex, ranging in age from seven to 70 years, were sampled from 10 households across seven of the hamlets (about 40km²; Fig. 1b). Mean distance among households studied in Melquíades is similar to that of VdG (2.13 km). At the time of the study, overall *S. mansoni* infection prevalence in Melquíades was approximately 78% (Curtis et al., 2002). Patients included in the study had not received praziquantel treatment during the 2 years prior to this study and all infected individuals were presented with treatment following sampling.

Lastly, because both patient and parasite sample sizes were substantially greater for VdG than Melquíades, all VdG genetic diversity calculations were re-run with subsamples to ensure that observed differences were not an artifact of sampling effort. To create an equivalent sample of patients between VdG and Melquíades, a mean of approximately 2.4 patients per hamlet were randomly subsampled from the VdG data set. Likewise, a random subsample of five and 10 parasites per VdG patient infrapopulation was taken to represent Melquíades parasite samples. For each reduced data set, genetic diversity measures were calculated and compared with those of the full VdG data set. Each of the three subsamplings and recalculations was repeated a minimum of five times.

3. Results

3.1. Virgem das Graças

3.1.1. Genetic diversity—We detected no significant effects of worm sex on measures of genetic diversity (all $P > 0.66$); thus, sexes were pooled within patient infrapopulations. Additionally, the greater sampling effort in VdG had no influence on observed genetic diversity outcomes. No recalculated diversity measures at any permutation of the three subsamplings differed significantly from the results obtained using the full data set (all two-way t tests $P > 0.4$).

From five to 27 alleles were observed per locus in the 585 worms genotyped, with a mean of 14.1 alleles per locus (Table 1). Mean unbiased heterozygosity (H_S) ranged from 0.17 to 0.84 (mean 0.63) and did not differ significantly among parasite infrapopulations (Kruskal-Wallis: $\chi^2 = 5.13$, d.f. = 29, $P = 1.0$). The global estimate of F_{IS} indicated a significant excess of heterozygotes ($f = -0.019$; 95% CI -0.04, 0.001; $P = 0.02$), and the multilocus estimates of F_{IS} for six infrapopulations exhibited significant deviation from Hardy-Weinberg expectations (Table 1). Four infrapopulations (51, 314, 406, 261) were significantly less than zero, indicating

heterozygote excess, while two (267, 456) were significantly greater than zero, indicating heterozygote deficiency. Of the remaining 24 infrapopulations, the majority (75%) of the multilocus f values were slightly negative, suggesting a relatively outbred parasite population. Following sequential Bonferroni correction, approximately 104 out of 840 locus-pair tests were found to exhibit significant linkage disequilibrium.

3.1.2. Genetic differentiation—Parasite population subdivision was investigated over five levels of a priori grouping (Table 2). In all cases, G-based tests indicated that F_{ST} was significantly greater than zero. Patient infrapopulations treated as subpopulations exhibited a highly significant F_{ST} of 0.058 (95% CI 0.047, 0.066; $P < 10^{-4}$), and when grouped by hamlet had a lower, but still significant, value of 0.016 (95% CI 0.011, 0.019; $P < 10^{-4}$). When standardized, we found that the observed values of F_{ST} accounted for 15% and 4.7% of the potential maximum differentiation among infrapopulations and hamlets, respectively, indicating moderate subdivision among infrapopulations and little differentiation among hamlets (as designated by Wright, 1978). Grouping infrapopulations according to patient sex, age or infection intensity also revealed significant but very low subdivision, with standardized values of F_{ST} never higher than 0.04 (Table 2).

AMOVA also suggested a lack of substantial population subdivision and indicated no grouping of parasites. In all cases, variation within infrapopulations accounted for the majority of the variance (94.15–94.31%; $P < 0.001$; Table 2). Among-infrapopulation variation described a significant ($P < 0.001$) but low percentage of the variance at all levels of subdivision (5.32–5.83%). When parasites were grouped by hamlet and infection intensity, variation among groups explained a significant ($P = 0.024$ and 0.021 , respectively) but very low proportion of the variance (0.53 and 0.46%, respectively). Furthermore, there was no significant correlation between genetic distance and geographic distance (log-transformed) at any level (all $r^2 < 0.11$ and $P > 0.19$), indicating no isolation-by-distance.

Assignment of individuals to populations via Bayesian clustering resulted in estimated ΔK values of $K = 2, 13$ and 15 . Only at the level of $K = 2$ were a majority of individuals assigned with greater than 70% probability to any particular cluster. However, clustering was random and not associated with either patient or hamlet. At all other levels of K , most individuals were assigned to clusters with equal probability, indicating no definitive group assignment. This lack of clear assignment supports the absence of genetic structuring exhibited by the previous measures.

3.2. Melquíades

3.2.1. Genetic diversity—Overall genetic diversity was slightly higher in Melquíades than in VdG (Table 1). A total of 14 to 24 alleles were observed per locus, with a mean of 18.1 alleles per locus. Mean unbiased heterozygosity (H_S) was slightly higher than that of VdG over all infrapopulations (0.67), but not significantly so (Mann-Whitney $U = 14,485$, $P = 0.122$). However, the global estimate of F_{IS} was significantly greater than zero ($f = 0.204$; 95% CI 0.118, 0.286; $P < 10^{-4}$) and indicative of a highly inbred population. Multilocus estimates of F_{IS} for infrapopulations were also generally positive, with 11 out of 17 being significantly greater than zero. Following Bonferroni correction, we found no significant linkage disequilibrium.

3.2.2. Genetic differentiation—As reported in Curtis et al. (2002), measures of F_{ST} at both patient and hamlet levels of subdivision were significantly greater than zero (patient: $\theta = 0.111$; 95% CI 0.092, 0.127; $P < 10^{-4}$ and hamlet: $\theta = 0.055$; 95% CI 0.042, 0.072; $P < 10^{-4}$). Standardized values of F_{ST} indicated that observed levels of genetic partitioning in Melquíades accounted for 35.4 and 20.4% of the maximum possible differentiation among patient

infrapopulations and hamlets, respectively (Table 2). These values of F'_{ST} indicate very high levels of differentiation among both infrapopulations and hamlets (Wright, 1978). To ensure that these levels of differentiation were not driven by a particularly distinct hamlet (see below), F_{ST} and F'_{ST} were recalculated in the absence of that hamlet (Hamlet V). Levels of differentiation dropped somewhat but still remained relatively high and indicative of very limited gene flow (0.30 and 0.12 for infrapopulations and hamlets, respectively). Grouping populations according to patient sex, age and infection intensity also revealed significant inbreeding and subdivision, but subdivision was not any greater than that among patient infrapopulations alone. AMOVA indicated that, as with VdG, variation within infrapopulations still accounted for the majority of molecular variance in Melquíades for all potential groupings of parasites (89.02–89.33%; $P < 0.001$; Table 2). Likewise, the percentage of variance described by variation among infrapopulations within groups was significant ($P < 0.001$) and almost double that of VdG, but still remained relatively low (10.15–11.01%). Regardless of the level of subdivision, variation among groups did not account for a significant percentage of the variance (all $P > 0.17$).

Unlike VdG, however, trends toward isolation-by-distance of parasite infrapopulations, while not always significant, tended to be stronger in Melquíades. Genetic distance between parasites grouped by household did not show significant correlation with distance among households ($r^2 = 0.02$, $P = 0.32$). The correlation increased when parasites were grouped by hamlet, but remained non-significant ($r^2 = 0.15$, $P = 0.08$). When considered by patient sex, we found that genetic distance between parasites from male patients in a hamlet was significantly correlated with the distance between hamlets ($r^2 = 0.456$, $P = 0.027$), while no significant correlation was observed for females ($r^2 = 0.08$, $P = 0.33$).

Bayesian clustering of individuals in Melquíades indicated that K values of 4 and 5 were the most probable, with 4 having the highest modal value of ΔK . At this level, approximately 75% of the individuals were assigned to a cluster with greater than 70% probability. Individuals from Hamlet V were consistently assigned to their own distinct cluster, regardless of the level of K applied. Parasites from the single patient in Hamlet IV also clustered very strongly together regardless of K , although the grouping was not completely exclusive, as two other infrapopulations (one each from Hamlets I and II) also shared the assignment. The other seven infrapopulations from Hamlets I and II tended to be assigned by patient into the remaining two clusters, but designations were not associated with the hamlet of origin. The parasites from Hamlet III were also assigned to the same two clusters as Hamlets I and II, but assignments were mixed within the two representative patients. Downstream in Hamlets VI and VII, however, very few worms were strongly assigned to any cluster, and what little assignment existed was indiscriminate.

4. Discussion

To the best of our knowledge, this is the largest study of schistosome population genetics to assess the degree of *S. mansoni* genetic subdivision within a human host population. Results shown here indicate that human hosts in Virgem das Graças are sampling a broad range of parasite genotypes and effectively serving as “genetic mixing bowls” for the parasite population, regardless of host household location, sex, age or infection intensity. While it is not surprising that definitive hosts are a significant factor in parasite gene flow (Blouin et al., 1995; 1999; Brouwer et al., 2001; Sire et al., 2001a; McCoy et al., 2003; Prugnolle et al., 2005a; Wang et al., 2006), the degree to which this has been assessed in human hosts is limited (Brouwer et al., 2001; Curtis et al., 2002). Both Brouwer et al. (2001) and Curtis et al. (2002) assessed the genetic variation of schistosomes among humans, but the study that is more directly comparable to our work is that of Curtis and colleagues, due to similarity of study scale, host demographics and molecular markers. Levels of *S. mansoni* genetic variation were

assessed across seven microsatellite loci in 17 patients of mixed sex and age in the Brazilian village of Melquíades. Located in the same state and approximately 230 km southwest of VdG, Melquíades had a similar household distribution to VdG, both in distance to waterways and overall distance among houses. However, results of the genetic analyses revealed several notable differences. Although genetic diversity and heterozygosity of the VdG and Melquíades schistosome populations were relatively high in both populations and did not differ significantly, the degree to which that diversity was partitioned among human definitive hosts was quite distinct. Regardless of the hierarchical level at which we tested subdivision, measures of F_{IS} and F_{ST} were consistent with higher levels of gene flow in VdG than in Melquíades. While it can be argued that values of F_{ST} are not directly comparable between studies, by standardizing those we corrected for differences in markers and heterozygosities (Hedrick, 2005) and found that Melquíades patient infrapopulations and hamlets are, in fact, more genetically differentiated. The amount of subdivision observed among infrapopulations and hamlets in Melquíades accounted for nearly two and four times the maximum possible subdivision, respectively, relative to that observed in VdG.

Bayesian clustering analysis further supports the conclusion of greater genetic differentiation in Melquíades than in VdG. Where parasites from VdG were assigned to clusters indiscriminately and without association to patient or locale, parasites from Melquíades were more likely to be assigned to a cluster that, while not always unique, was at least associated with the patient. Furthermore, trends of isolation-by-distance were stronger in Melquíades and significantly so among male patients, while no such patterns were observed in VdG. These results are surprising given that overall study area and distances among households were similar between VdG and Melquíades. However, Melquíades appeared to be more geographically variable, with a more complex system of ridges and valleys within the study area, all of which could serve as potential deterrents to regular far-ranging and/or overlapping host movement for water usage. Also, Melquíades has a more complex system of waterways, with at least two rivers running independently through the 40 km² area studied. All of the houses in VdG are situated along one central stream and its few tributaries. These are the only permanent sources of flowing water in the 60 km² area and the primary water contact point for households (Kloos et al., 2004; Gazzinelli et al., 2006a).

In Melquíades, parasites originating from hamlets located on separate rivers were more genetically differentiated than those from the same river, as evidenced by Bayesian clustering analysis. For example, parasites from the second, southeastern stream (represented by Hamlet V) were the most genetically distinct, clustering into their own unique group. Parasites from households along the other stream and its tributaries, though, tended to overlap in their assignment to genetic clusters, indicating some degree of allele sharing among infrapopulations regardless of geographical distance among households. However, such trends were not exhibited among VdG hamlets, despite hamlets being separated by comparable distances. Therefore, it would seem that while distance among households can contribute to genetic differentiation of parasite infrapopulations in some cases (as indicated by isolation-by-distance in males patients from Melquíades), distance alone at this scale is insufficient to explain the entire pattern. Connectivity of water sources, though, seems to have a detectable influence on the distribution of parasite genetic diversity.

The degree to which such patterns of parasite gene flow among connected water sources (i.e. along rivers and canals) are influenced by passive movement of larval stages is still questionable. The snail intermediate host, *B. glabrata*, on the other hand, has been shown to have relatively high gene flow within the Cardoso Stream of VdG (Wethington et al., 2007). Therefore, it would not be unreasonable to expect that intermediate host movement could be a contributing factor to parasite gene flow within the VdG area or along rivers, streams or canals in general. While no such genetic assessments are available for the snail hosts in

Melquíades, it would follow that such gene flow also occurs within that population, accounting for the evidence of some parasite gene flow among hosts whose households are located along the same waterway. Furthermore, from the human perspective, although flowing water may generally be a prime water source by virtue of its ability to carry away pollution and remain less eutrophic during the dry season, it is clear that certain areas of a stream or river are preferable to others (i.e. preference for upstream versus downstream sites; Kloos et al., 1998). Therefore, common usage of a few specific sites along one stream could be expected, whether for social or sanitary motivations. In Melquíades these sites are more likely to be distributed over several streams, reducing the amount of overlap among residents. There are documented alternative definitive hosts within Brazil, one of those being the water rat *Nectomys squamipes* (Antunes et al., 1973; Picot, 1992; D'Andrea et al., 2000). Mark-recapture studies of *N. squamipes* indicate seasonally and sexually variable home ranges of 0.5–5km² (Bergallo and Magnusson, 2004), while genetic analysis indicates significant subdivision over a similar area (Maroja et al., 2003). However the presence and subsequent influence of such alternative hosts in either VdG or Melquíades is unknown at this time.

Given the geographical and hydrological differences between the two villages, the results observed here may not seem surprising. However, the evolutionary and epidemiological implications of such variation should be noted. Firstly, the indications of relatively high gene flow observed in VdG suggest that there is ample opportunity for the spread of advantageous alleles within the parasite population. Thus, if drug resistance or particularly infective strains were to arise within a subset of the population, the potential for it to spread throughout the entire population is high at the scale of this single village. In Melquíades, the spread of alleles throughout the entire village's population would be more limited, particularly between the subpopulations located on separate waterways. However, we would still expect to see eventual exchange of alleles, principally within the portions of the population sharing use of a single waterway. Second, the results presented here indicate that the potential for local adaptation varies between the two areas. The apparently greater level of gene flow among parasites in VdG might be expected to hinder the development of locally adapted strains, while the reduced gene flow within Melquíades could promote local adaptation.

Despite the inherent logistical difficulties of sampling parasites from human populations (Sorensen et al., 2006; Gower et al. 2007), investigations such as the one presented here are crucial to furthering our understanding of the interplay between host behavioral and environmental parameters, and parasite evolution and transmission. Gene flow within and among schistosome populations will vary along a continuum as the array of epidemiological and environmental factors impact upon this complex parasite life cycle. This study's comparability to previous work within a different village setting makes it particularly useful in illustrating how different communities of hosts and parasites may be situated along such a continuum. However, the data herein represent a single snapshot of a dynamic process. To gain a more comprehensive understanding of the magnitude of gene flow within populations, temporal assessments of parasite genetic distribution are still needed.

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Fig. 1. Maps of the two villages sampled in Minas Gerais, Brazil: (a) Virgem das Graças and (b) Melquíades. Topography is detailed by grayscale shading (maps on same elevational scale) and permanent rivers are delineated by solid white lines (fletchings indicate direction of flow). Households are shown as stars and their hamlet groupings are indicated by dashed circles.

Table 1
Summary table of number of genetic diversity for each infrapopulation (patient isolate) of *Schistosoma mansoni* sampled in (a) Virgem das Graças and (b) in Melquiades.

Hamlet	Isolate	Sex	Age	N ^a	A _N ^b	H _S ^c	J ^d
a. VdG							
Taboca	47	M	45	12	5.13	0.66	-0.052
	51	M	9	13	3.75	0.59	-0.313
	144	F	13	26	6.38	0.72	-0.005
	210	M	25	12	4.50	0.62	-0.074
	541	F	43	11	5.63	0.68	-0.002
Suçuarana	261	F	60	10	5.00	0.64	-0.116
	267	M	48	10	4.13	0.57	0.158
	314	M	33	11	5.00	0.61	-0.171
	320	M	27	10	4.63	0.59	0.009
	321	F	23	31	5.50	0.60	-0.041
	330	M	17	30	6.88	0.70	0.003
	331	M	15	23	6.63	0.68	-0.015
Cardoso 1	335	F	63	14	5.38	0.62	-0.077
	338	M	26	21	6.63	0.63	-0.042
	353	M	9	10	3.88	0.60	-0.040
	359	M	13	19	6.25	0.67	0.011
Cardoso 2	386	F	17	23	6.25	0.61	-0.008
	396	M	64	15	6.38	0.67	-0.026
	403	M	36	19	6.38	0.61	-0.020
	404	F	32	17	5.50	0.62	-0.074
	406	M	13	13	5.63	0.65	-0.132
	409	F	8	26	5.75	0.61	-0.004
	422	M	12	17	5.00	0.53	-0.051
	447	F	17	50	8.25	0.64	0.046
	456	F	22	45	6.58	0.64	0.062
	464	M	16	10	4.50	0.65	0.084
	473	M	72	32	6.75	0.66	-0.004
Cardoso 3	523	F	8	11	6.00	0.64	-0.067
	526	F	5	23	7.63	0.61	-0.022
	529	F	19	21	6.63	0.65	-0.051
<i>Total</i>				585	14.13	0.63	-0.019
b. Melquiades							
I	68	M	34	6	4.14	0.71	0.346
	71	M	9	3	2.86	0.76	0.438
	77	M	70	7	2.57	0.54	0.486
	82	M	11	5	3.86	0.72	0.064
II	165	M	58	7	3.43	0.56	0.037
	168	F	11	4	2.71	0.58	-0.093
	180	F	13	7	4.00	0.65	0.307
	181	M	10	12	5.29	0.63	0.202
	182	F	7	20	7.14	0.73	0.116
III	253	M	19	9	5.86	0.74	0.017
	263	M	10	18	7.29	0.70	0.127

Hamlet	Isolate	Sex	Age	N ^a	A _N ^b	H _S ^c	f ^d
IV	326	F	28	13	5.29	0.64	0.044
V	433	F	18	21	7.43	0.77	0.360
	434	M	16	7	4.86	0.78	0.403
VI	531	F	14	8	5.14	0.76	0.396
VII	626	M	23	17	6.57	0.64	0.270
	628	M	13	6	4.29	0.62	0.151
Total				170	15.00	0.67	0.204

^a N, total number of worms genotyped per patient

^b A_N, mean number of alleles observed within the inrapopulation

^c H_S, Nei's unbiased heterozygosity

^d f, Weir and Cockerham (1984) estimate of inbreeding Values in bold text deviate significantly from Hardy-Weinberg expectations of f = 0.

Table 2

Measures of genetic differentiation for various levels of population subdivision of *Schistosoma mansoni* parasites collected from (a) Virgem das Graças and (b) Melquiades (95% confidence intervals in parentheses).

Grouping	F-statistics	AMOVA			P-value
		Number of Groups	Variance components	Percentage of Variation	
a. VdG					
Patient	f^a θ^b F'_{ST}^c				
	-0.019 (-0.004, 0.001) 0.058 (0.047, 0.066) 0.155				
Hamlet	f θ F'_{ST}	5 among infrapopulations within groups within infrapopulations	among groups 5.32 94.15	0.53 <0.001 <0.001	0.024
Patient Sex	f θ F'_{ST}	2 among infrapopulations within groups within infrapopulations	among groups 5.63 94.29	-0.02 <0.001 <0.001	0.418
Patient Age	f θ F'_{ST}	4 among infrapopulations within groups within infrapopulations	among groups 5.83 94.13	-0.15 <0.001 <0.001	0.649
Infection Intensity	f θ F'_{ST}	4 among infrapopulations within groups within infrapopulations	among groups 5.37 94.17	0.46 <0.001 <0.001	0.021
b. Melquiades					
Patient	f θ F'_{ST}				
	0.204 (0.158, 0.286) 0.111 (0.092, 0.127) 0.354				
Hamlet	f θ F'_{ST}	7 among infrapopulations within groups within infrapopulations	among groups 10.21 89.17	0.62 <0.001 <0.001	0.335
Patient Sex	f θ F'_{ST}	2 among infrapopulations within groups within infrapopulations	among groups 10.91 89.33	-0.25 <0.001 <0.001	0.576
Patient Age	f θ F'_{ST}	3 among infrapopulations within groups within infrapopulations	among groups 10.15 89.02	0.83 <0.001 <0.001	0.174
Infection Intensity	f	4 among groups	among groups	-0.28	0.603
	0.268 (0.190, 0.340)				

AMOVA				
Grouping	F-statistics	Number of Groups	Variance components	P-value
θ	0.034 _(0.025, 0.045)	among intrapopulations within groups	11.01	<0.001
F_{ST}	0.134	within intrapopulations	89.28	<0.001

Values in bold text deviate significantly from zero.

- ^a_f, Weir and Cockerham (1984) estimate of the measure of inbreeding F_{IS}
- ^b _{θ} , Weir and Cockerham (1984) estimate of the measure of genetic differentiation F_{ST}
- ^c F'_{ST} , standardized value of F_{ST} (Hedrick, 2005; Meirmans, 2006)