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Effects of intermediate host genetic background on parasite transmission dynamics: A case study using *Schistosoma mansoni*

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Polymerase chain reaction (PCR)

ABSTRACT

For parasites that require multiple hosts to complete their development, genetic interplay with one host may impact parasite transmission and establishment in subsequent hosts. In this study, we used microsatellite loci to address whether the genetic background of snail intermediate hosts influences life-history traits and transmission patterns of dioecious trematode parasites in their definitive hosts. We performed experimental *Schistosoma mansoni* infections utilizing two allopatric populations of *Biomphalaria glabrata* snails and assessed intensities and sex ratios of adult parasites in mouse definitive hosts. Our results suggest that the genetic background of hosts at one point in a parasite's life cycle can influence the intensities and sex ratios of worms in subsequent hosts.

1. Introduction

The dynamics underlying infectious disease occurrence intimately depend on the genetic interaction between parasites and their hosts. For parasites that require a number of hosts to complete their development (complex life cycle), each host species represents a potential filter that can modulate both the infection intensities and the genotypes that ultimately establish in subsequent hosts (Combes, 2001; Shrivastava et al., 2005; Sorensen et al., 2006). Research by both Grosholz (1994) and Wiehn et al. (2002) has demonstrated that parasite establishment in intermediate hosts can vary significantly based on host genetics. More recently, Grech et al. (2006) extended this work to show that intermediate host genetics could potentially impact the transmission of parasite genotypes between hosts in a complex life cycle. More specifically, they found that the transmissible stages of four malaria genotypes were differentially affected by the genetic background of mouse intermediate hosts (four strains). However, very little work has assessed the effects of host genetic background on transmission patterns to subsequent hosts in the life cycle.

Adding to the complexity of transmission in dioecious parasites is the fact that the male and female parasites often express different life-history patterns at a number of points in their life cycle (Boissier et al., 1999). Factors such as parasite longevity, propagule production and infectivity can influence the numbers of females and males that successfully transmit to and establish in different hosts (Boissier and Mone, 2000). This may help explain the distortion in parasite sex ratios reported for a number of parasite groups (Poulin, 1997; Boissier et al., 1999; Stien et al., 2005).

Schistosomes are dioecious trematode parasites that utilize a complex life cycle to complete their development. Adult worms sexually reproduce in the blood streams of mammalian definitive hosts (including humans) and release eggs via host feces or urine into the environment. When eggs enter the water they hatch into a free-living larval stage (miracidium) that infects aquatic snails which serve as their intermediate hosts. Parasites asexually reproduce within these snails eventually releasing thousands of cercariae, the second free-living larval stage. These larvae are then infective to definitive hosts. Previous work using this system has reported that intermediate host genetic background can influence parasite transmission to definitive hosts and that male and female worms have the potential to transmit differentially (Boissier et al., 1999).

In this study, we address the question of whether the genetic background (assessed via microsatellite loci) of the snail intermediate host can alter the transmission and establishment of male

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and female schistosome parasites in subsequent definitive-host mice. We performed this experiment by passaging a field strain of the dioecious parasite, *Schistosoma mansoni*, through two allopatric (field and laboratory) populations of *Biomphalaria glabrata* snails. Parasites that successfully infected snails were used to infect Balb/c mice that were the same sex (male) and age (6–7 weeks), thereby controlling for factors that could otherwise confound our experiment (Caillaud et al., 2006). Although this study is limited to two intermediate host populations and one parasite population, the results presented herein are the first to suggest that intermediate host genetic background can influence general intensities and sex-specific establishment of worms in definitive hosts. These findings may not only have potential implications for parasite population dynamics and transmission, but also practical repercussions for studies assessing epidemiological questions based on field-collected parasites passaged through laboratory hosts.

2. Materials and methods

2.1. Hosts and parasites

Two *B. glabrata* snail populations were used for the experimental *S. mansoni* infections. One group of snails was selected from our laboratory population of *B. glabrata* that has been maintained at Purdue University for over 20 years. The second group consisted of snails collected in a Brazilian field site (Americaninha, 17° 07′ 20″ S, 41° 11′ 21″ W) one year prior to this study.

Eggs of *S. mansoni* parasites were collected from 10 patients at the same time as field snail hosts in the Americaninha site (Brazil, 17° 07′ 20″ S, 41° 11′ 21″ W). During the period prior to experimentation, parasites were maintained in male Balb/c mice as definitive hosts and *B. glabrata* as intermediate hosts. The pool of parasite miracidia that were used for experimental infections (see below) were obtained from Balb/c mice ($n = 3$) that were exposed to *S. mansoni* using standard procedures (Stohler et al., 2004).

2.2. Infections

The experimental infection protocol is shown in Fig. 1. Briefly, snails from both lab and field populations ($n = 14$ snails/population) were size matched (7–10 mm in diameter) and placed individually into 3 ml wells in exposure trays. Snails were exposed within 3 h of miracidial hatch to ensure high infectivity of the parasite larvae. Hosts of both genetic backgrounds were exposed to larvae (10 miracidia/snail) originating from the same pool of miracidia. Snails were maintained in their wells overnight and placed individually into 150 ml jars the following day. During the remainder of the experiment, snails were maintained on a diet of lettuce fed *ad libitum*, and were kept at room temperature (22 °C) on a 10 h light:14 h dark photoperiod. Host growth was assessed weekly and survival was determined every 2–3 days. Dead snails were crushed to determine infection status.

To assess snail infections, 6 weeks after exposure, snails were individually placed into exposure-tray wells under fluorescent light for 4 h to initiate parasite release. This process was continued at two-day intervals over the next 10 days and at weekly intervals thereafter to determine whether the timing of parasite release (patency) varied based on host background. At weeks 6 and 9, infected snails were removed from their well, wells were filled to the top with water, and a 1 ml sample (containing cercariae) was collected for each host. Each sample was placed in a Petri plate and 70% ethanol was added to fix parasites for subsequent enumeration using a dissecting microscope at 40× magnification. At week 9, within each snail group, 30 cercariae were collected from each infected host ($n = 5$) and combined to infect a Balb/c mouse. This was repeated for five mice for each of the two snail groups. In order to prevent definitive-host attributes from confounding patterns of parasite establishment, all mice were of the same sex (male) and age (6–7 weeks). At seven weeks post-exposure, mice were euthanized using CO_2 and subsequently examined for worms. To acquire worms, the hepatic portal system (including the liver and the entire intestinal system) was first removed from each mouse and then teased apart using fine forceps and a dissecting microscope. Worms were collected and immediately stored in 100% ethanol. Snail tissue from individuals remaining at the end of experiment ($n = 13$ and $n = 12$ for lab and field groups, respectively) was also individually stored in 100% ethanol for subsequent genetic analyses.

2.3. Host genotyping

Total genomic DNA was extracted from snail head–foot tissue using the following protocol: approximately 10 mg of tissue was homogenized in 200 μl of extraction buffer (100 mM Tris–HCl, 10 mM EDTA, 100 mM NaCl, 1% SDS, 0.06 mg Proteinase K, 1.5 mM dithiothreitol) and incubated at 55 °C overnight. To precipitate proteins after incubation, 10 M ammonium acetate (1/3 of the lysate volume) was added to the lysate and vortexed. The lysate was then centrifuged and supernatant containing DNA was precipitated using a double volume of ice-cold 100% ethanol. Following centrifugation, the DNA pellet was washed using 70% ethanol. Finally, the DNA pellet was air-dried and dissolved in sterile Millipore water. Thirteen field snails and 12 lab snails were genotyped using eight microsatellite markers, Genbank Accession No.: AF157700, AF157701, AF157703, AF157704, AF216280, AF216274, AF216272, AF216271 (Jones et al., 1999; Mavárez et al., 2000). The amplification reaction was performed in a 15 μl volume containing 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 0.1% Triton® X-100, 2 mM $MgCl_2$, 167 nM of each primer, 200 μM of each dNTP, 25 ng template DNA and 0.6 U Taq Polymerase in Storage Buffer A (Promega, USA). Polymerase chain reaction (PCR) amplifications were carried out on Mastercycler® gradient (Eppendorf, USA) under the following conditions: 4 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 60 °C for AF216272, AF216271, otherwise at 55 °C, 1 min at 72 °C; followed by extension 20 min at 72 °C. Fluorescently labeled PCR products were analyzed on an ABI 377 sequencer using internal size standard GeneScan™-500 TAMRA™ (Applied Biosystems) and scored using Genotyper® software (Applied Biosystems).

2.4. Genetic and statistical analyses

Prevalence of infection was analyzed via binary logistic regression. General host and parasite life-history traits were assessed via parametric procedures (*t*-tests). In the case of male and female parasite intensities in definitive hosts, we used a paired t-test as previous work has suggested that the infectivity of one parasite sex can impact the establishment of the other (Boissier et al., 1999).

For *B. glabrata*, genetic variability was measured by Nei's (1987) unbiased mean heterozygosity (gene diversity). Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested for each of the lab and field groups of hosts. For analyses involving multiple comparisons, the critical probability value for each test was adjusted using the Bonferroni correction (Rice, 1989). All tests were performed using population genetic analysis software FSTAT version 9.3.2 (Goudet, 1995). Software MICRO-CHECKER was used to estimate presence of null alleles (van Oosterhout et al., 2004).

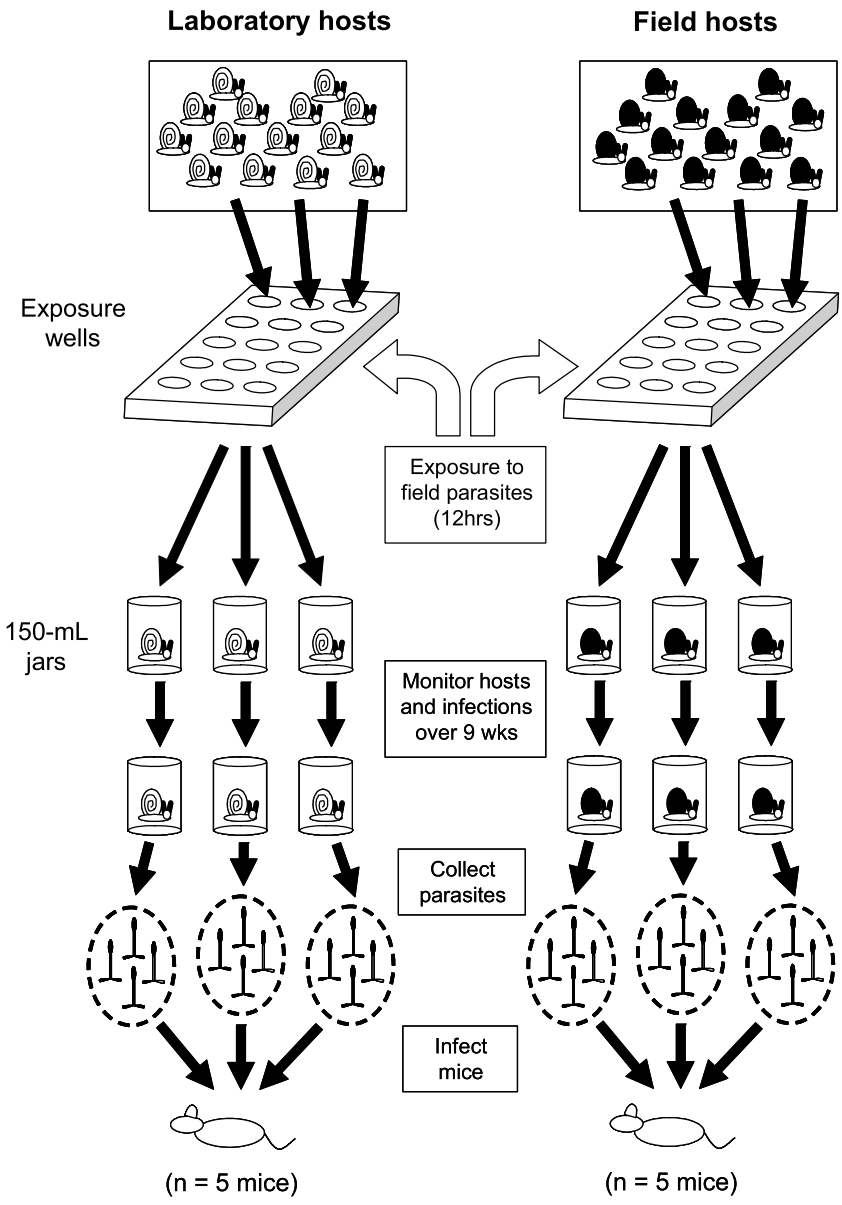

Fig. 1. Outline of the infection design used in the experiment.

Genetic heterogeneity among and within host groups was examined using Wright's F-statistics (Wright, 1965). F_{IS} measures the departure from panmixia within a sample, while F_{ST} measures genetic differentiation between different samples. These parameters were estimated by Weir and Cockerham's (1984) estimators f (for F_{IS}) and θ (for F_{ST}) and computed with FSTAT version 9.3.2. The significance of F_{IS} was tested using a randomization procedure (Goudet, 1995). To test for significance of pair-wise comparisons of genetic differentiation (F_{ST}) between groups, the randomization procedure and the Bonferroni correction were performed using FSTAT version 9.3.2.

3. Results

3.1. Snail infections

Snail size did not differ among groups at the time of parasite exposure ($t_{26} = 1.3$, $P = 0.21$). Infection prevalence was similar among snail populations (lab: 43% and field: 36%; Wald = 0.15; $P = 0.74$) as was time to patency ($t_9 = 1.0$, $P = 0.34$) and numbers of parasites released at patency ($t_9 = 0.91$, $P = 0.39$). At the time of cercarial collection, (9 weeks post-exposure) there were no

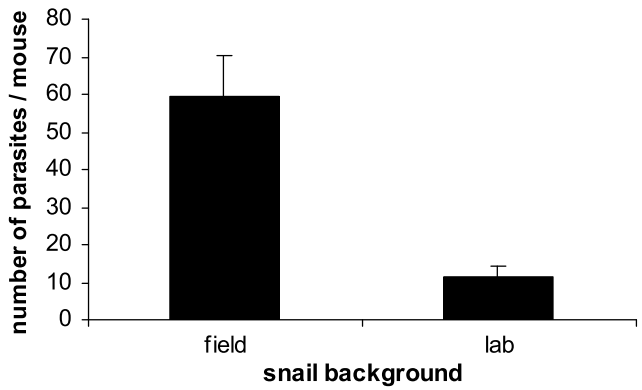

Fig. 2. Mean intensity (± SE) of adult field *Schistosoma mansoni* in definitive hosts after passage through field and lab intermediate host snails.

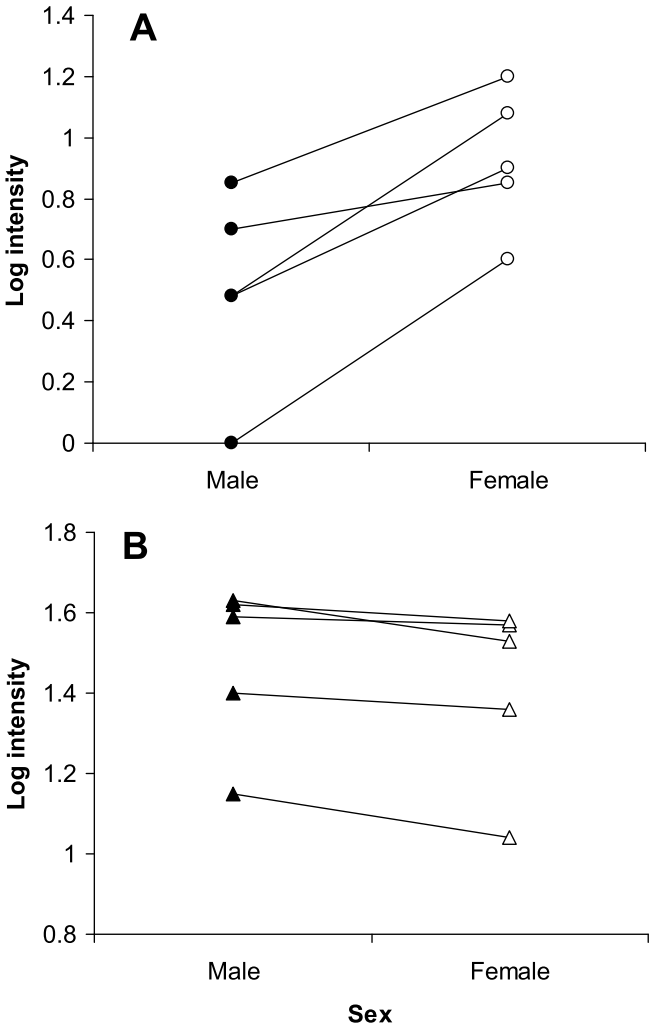

Fig. 3. Mean intensity (log 10-transformed) of male and female *S. mansoni* in definitive hosts after passage through snail intermediate lab hosts (A) or field hosts (B). Lines linking males and females are included to represent individual mouse hosts.

differences in the sizes of infected snails between groups ($t_9 = 0.90$, $P = 0.33$) and the number of cercariae released did not differ between host groups ($t_9 = 0.03$, $P = 0.98$).

3.2. Mouse infections

Significantly greater numbers of adult parasites established in mouse hosts when they were passaged through field snails as opposed to lab snails ($t_8 = 4.40$, $P = 0.002$; Fig. 2), despite the fact that mice were exposed to the same numbers of cercariae. In addition, parasites passaged through lab snails had significantly more females establish than males ($t_4 = 5.06$, $P = 0.007$; Fig. 3A). Conversely, significantly greater numbers of male parasites established relative to females parasites after passage through field hosts; however, the magnitude of this difference was less than that seen between male and female parasites shed from lab snails ($t_4 = 3.52$, $P = 0.024$; Fig. 3B).

3.3. Host genotypes

Out of the eight microsatellite loci, two loci (AF157704 and AF157701) yielded null alleles and therefore were excluded from further analyses. Four to eight alleles per locus (mean 5.67) were observed among the 25 genotyped snails. A large number of alleles (82% and 85% for field and lab snails, respectively) were not shared between the groups (see private alleles in Table 1), indicating high differentiation between these hosts. Unbiased mean heterozygosity (gene diversity) across six loci ranged from 0.21 to 0.66 (mean 0.53 per locus) in hosts from the field and from 0.39 to 0.78 (mean 0.58 per locus) in laboratory hosts (Table 1). One locus (AF216272) deviated significantly from HWE in the field snail group (adjusted $\alpha = 0.004$), indicating a heterozygote deficiency due to presence of null alleles. After correcting for multiple comparisons, tests for LD did not reveal any linkage between loci.

Overall f (F_{IS}) values (0.041; 95% CI −0.175 to 0.267) indicated no departure from panmixia within each of the host groups. However, genetic differentiation θ (F_{ST}) between the groups was significantly large (0.436; 95% CI 0.348–0.523), indicating substantial genetic differences between these two groups of intermediate hosts.

4. Discussion

To our knowledge, this is the first study to investigate the role of intermediate host genetic background in the transmission of parasite individuals and genotypes to definitive hosts. In general, transmission of field-collected parasites through their native snail hosts was more successful than through lab hosts. This is not overly surprising given that selection is predicted to favor local adaptation in systems where parasites migrate more than their hosts (Gandon et al., 1996; Lively and Dybdahl, 2000) and such a pattern is likely for the host–parasite system in this study. In the field, the introduction of novel host genotypes into populations where parasites are locally adapted to their hosts may reduce disease transmission, especially if migrants either outcross with local host genotypes or self-fertilize.

Passage through different intermediate host genotypes also appeared to modulate parasite sex ratios. For schistosomes, both field and experimental results have reported sex-ratio distortions in definitive hosts that tend to be biased towards males. A number of mechanisms have been presented to help explain this pattern. Through empirical investigations and meta-analyses, Boissier and colleagues (1999, 2001) demonstrated that male and female schistosomes exhibited different life-history patterns (such as infectivity, reproduction and survival) in both snail and mouse hosts

Table 1
Genetic variability among lab and field snail hosts across six microsatellite loci

		AF157703	AF216272	AF216271	AF157700	AF216280	AF216274
Field host $n = 13$	N_A	3	4	3	3	2	2
	N_P	3	3	2	2	2	2
	H_E	0.64	0.66	0.62	0.56	0.51	0.21
Lab host $n = 12$	N_A	3	5	4	3	2	3
	N_P	3	4	3	2	2	3
	H_E	0.64	0.78	0.56	0.47	0.39	0.61

N_A, number of alleles; N_P, number of private (unique) alleles; H_E, gene diversity.

potentially generating altered sex-ratios. Furthermore, Mitchell et al. (1990) suggested that mouse immune responses could differentially target male and female eggs leading to a male-parasite bias in infected snails. In our study, when parasites were passaged through their native intermediate hosts we observed the expected bias towards male worms in definitive hosts (Morand et al., 1993). However, when parasites were passaged through hosts of a different genetic background (lab snails), the sex bias was of greater magnitude and reversed (favoring females). What processes could drive such a pattern? There is evidence that male and female *S. mansoni* exhibit differential gene and protein expression during the life cycle (Fitzpatrick et al., 2004, 2005; Wuhrer et al., 2006). Variation in the immunological responses of each snail strain towards parasite male and female larval forms may have differentially filtered the sexes that successfully transmitted to mouse definitive hosts.

Along with potential evolutionary consequences, there are also practical implications that emerge from this study. Introducing field-collected parasites into laboratory settings often involves cycling the parasite through non-native strains of host and/or different host species. Our results demonstrate that this procedure can substantially reduce general parasite establishment and bias sex ratios in definitive hosts. This not only could make life cycle maintenance infeasible, but it could also lead to erroneous conclusions when extrapolating laboratory findings (after passage) to field-based patterns.

In conclusion, our experimental results suggest that differences in the genetic backgrounds of intermediate hosts can be important for the establishment of parasite individuals within definitive hosts. It is also important to recognize that some aspects of our experimental approach such as the use of mice as definitive hosts (instead of humans) and the use of a single parasite strain constrain the broad applicability of these results to other systems. Nonetheless, these results provide initial insight into an aspect of host–parasite associations and disease epidemiology that is rarely considered.

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