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Genotypic variation in host response to infection affects parasite reproductive rate

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ABSTRACT

Parasite fitness is largely influenced by a variation in host response due to the host's genetic background. Here we investigated the impact of host genotype on pathogen success in the snail vector of its castrating parasite, *Schistosoma mansoni*. We infected five inbred lines of *Biomphalaria glabrata* with two infection doses and followed their growth, reproductive output and parasite production throughout the course of infection. There was no difference in resistance to infection among inbred lines, but lines varied in their responses to infection and the numbers of parasites produced. Snails did not compensate for castration by increasing their fecundity during the early phase of infection (fecundity compensation). However, some lines were able to delay parasite shedding for up to 30 weeks, thus prolonging reproduction before the onset of castration. Here we propose this strategy as a novel defense against castrating pathogens in snails. Gigantism, a predicted outcome of castration due to energy reallocation, occurred early in infection (<15 weeks) and was not universal among the snail lines. Lines that did not show gigantism were also characterised by a high parasite production rate and low survivorship, perhaps indicating energy reallocation into parasite production and costly immune defense. We observed no differences in total parasite production among lines throughout the entire course of infection, although lines differed in their parasite reproductive rate. The average rate of parasite production varied among lines from 1300 to 2450 cercariae within a single 2 h shedding period, resulting in a total production of 6981–29,509 cercariae over the lifetime of a single snail. Regardless of genetic background, snail size was a strong predictor of parasite reproduction: each millimetre increase in snail size at the time of the first shed resulted in up to 3500 more cercariae over the lifetime of the snail. The results of this study provide a detailed picture of variation in hosts' responses to infection and the resulting impacts on parasite fitness, further defining the intricacies of snail-schistosome compatibility.

1. Introduction

When faced with a pathogen, a host can invest in one or a combination of many distinct defense strategies. For example, resistance prevents infection or limits the pathogen burden upon infection, and tolerance limits the damage of the pathogen to the host (Boots and Bowers, 1999). Hosts can also alter their energy allocation and thus life history strategies when challenged by a pathogen, to increase their fitness (Minchella, 1985; Agnew et al., 2000). Host defense strategy has a large impact on pathogen fitness. Often, host and pathogen fitness are negatively related such that increased host defense yields decreased pathogen fitness. However, some defense strategies may have a positive or neutral effect on pathogen fitness, such as tolerance or some life history defense strategies, such as changes in reproductive timing (Roy and Kirchner, 2000; Boots et al., 2009). Thus, in some cases, strategies that are viewed as host defenses may also be viewed from the opposite angle as "parasite offense". In fact, these strategies presumably have been coevolving over time, and in many cases it is difficult to attribute them to either host or parasite. The effect that host defense strategy has on pathogen fitness is of interest because it impacts disease dynamics at multiple scales including an individual's disease risk and disease outcome, the transmission of the parasite throughout populations, and the co-evolutionary dynamics of hosts and pathogens (Boots and Bowers, 1999; Roy and Kirchner, 2000; Restif and Koella, 2004; Miller et al., 2006; Vale et al., 2011; Best et al., 2014). It is therefore of interest to investigate the role of underlying genetic variation on host defense strategy, and how this may create heterogeneity in transmission among

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individuals of a single population. Furthermore, understanding how a host's response to infection can influence pathogen reproduction is paramount to understanding the effects of pathogens on hosts, their co-evolutionary relationship, and the development of targeted and effective control strategies.

To analyse the effects of the host response to infection on parasite fitness, we chose the *Schisosoma mansoni-Biomphalaria glabrata* system. *Schistosoma mansoni* is a bloodfluke that causes a devastating, chronic disease in humans and is transmitted through a snail vector such as *B. glabrata*. A variety of host defense strategies have been reported for the vectors of schistosomiasis, freshwater snails. Genetically based resistance has been well-documented and the underlying genetic mechanisms of resistance are being elucidated (e.g. Knight et al., 1999; Zhang et al., 2004; Goodall et al., 2006; Bender et al., 2007; Hanington et al., 2012; Tennessen et al., 2015). Tolerance mechanisms, on the other hand, have been virtually unexplored. The alteration of life history traits in response to infection has been well documented (e.g. Gerard and Theron, 1997; Sorensen and Minchella, 2001; Blair and Webster, 2007). Schistosomes castrate their snail hosts via biochemical and mechanical means (Pan, 1965; Crews and Yoshino, 1989; Faro et al., 2013) and in some cases infection has been shown to drive enhanced reproduction before castration, termed fecundity compensation (Minchella and Loverde, 1981). Infection can also induce gigantism or accelerated growth (Pan, 1965; Sturrock and Sturrock, 1970; Sorensen and Minchella, 2001). Enhanced growth is considered a byproduct of castration and occurs due to the reallocation of energy away from reproduction and towards growth, but it may also have a selective advantage for the host or parasite (Minchella, 1985; Hall et al., 2007). The advantage to the snail is that increased growth could enhance reproductive effort after clearance of the infection (Minchella, 1985). The advantage to the parasite is an increase in resources to benefit its own reproduction and fitness (Baudoin, 1975).

Variations in life history responses of snails to schistosomes have been well documented in the literature and have been attributed to variances in host developmental states or infection conditions such as host age/size at infection, infection dose and environmental conditions (Pfluger, 1980; Gerard and Theron, 1997; Ibrahim, 2006; Blair and Webster, 2007). Another potential source of variation is the genetic background of the host. Evidence for a genetically based variation in the life history response comes from theoretical works (Gandon et al., 2002) or comparisons among natural populations of snails (Tian-Bi et al., 2013); however, empirical evidence for the effect of host genotype on parasite fitness is lacking in this system (Sandland et al., 2007), although it is well-established as a driving factor in other systems (Salvaudon et al., 2007; Bruns et al., 2012; Gsell et al., 2013). Here we investigate how the variations in defense strategies among distinct genotypes of snails influence pathogen reproduction within the snails.

The life cycle of schistosomes involves a freshwater snail vector and a vertebrate host. Once snails are infected by free-swimming larvae that hatch from eggs deposited into aquatic environments via faeces or urine, the schistosome undergoes a series of asexually reproducing generations within the snail. First, mother sporocysts develop in the headfoot of the snail and these give rise to daughter sporocysts that develop in the digestive gland and ovotestis of the snail. Infective stages, called cercariae, are released through the headfoot of the snail and into the freshwater environment. Cercariae directly penetrate the skin of their vertebrate hosts and establish within the blood vessels lining the intestinal tract or urogenital tract. Because the snail vector is required for parasite development it is an appealing target for control including genetic manipulation of host defense strategies. Reproduction of the parasites within the snail directly determines the number of infective stages that are released into the environment and thus the risk of infection for humans (Carter et al., 1982; Gower and Webster, 2004; Civitello and Rohr, 2014).

We addressed two questions. First, do specific host genotypes invest in different defense strategies when challenged with a pathogen, and second, what are the effects of these strategies on pathogen reproduction? By using inbred lines of snails we were able to characterise variations in host defense traits due to genetic backgrounds. We measured pathogen reproduction as a rate (number of infective stages released per week), and as the total number of pathogens released throughout the lifetime of each snail. We also determined the effect of each host genotype's defense strategy on the fitness of the host; namely reproductive output and fecundity compensation. Together, these analyses allowed us to identify variations in traits upon which natural selection could act, driving the evolution of this host-pathogen system.

2. Materials and methods

2.1. Study organisms

We used five inbred lines of the snail host, *B. glabrata,* which varied in average resistance to the parasite *S. mansoni*. Four of these lines were generated in the laboratory of C. Bayne (Oregon State University, USA) through self-reproduction of individuals from the 13–16-R1 population (Richards and Merritt, 1972) for three generations (Larson et al., 2014) and one line (Newton, 1955; M-line) was obtained from S.M. Zhang, University of New Mexico, USA. The 13–16-R1 population is of hybrid origin from snails collected from Brazil and Puerto Rico and has been maintained at a large population size (hundreds) under laboratory conditions since the 1970s. Our laboratory population of the 13–16-R1 strain is known to be highly heterozygous at all loci examined (Larson et al., 2014). Three generations of selfing results in 87.5% expected homozygosity of alleles due to identity by descent and thus they are highly inbred lines. Susceptibility to infection, as well as the cellular response to infection and transcription of immune relevant genes is known to vary widely among these lines (Larson et al., 2014). The M-line population is a popular laboratory strain that was generated in the 1950s from a breeding scheme to create highly susceptible snails (Mulvey and Bandoni, 1994). The cross occurred between pigmented Puerto Rican and albino Brazilian strains of snails, but is now considered to be highly inbred and highly susceptible to infection with *S. mansoni* (Mulvey and Bandoni, 1994). The inbred M-line was generated from 32 generations of self-reproduction. In our laboratory, both of these strains and all of the inbred lines were maintained without selection pressure from schistosome pathogens.

Resistance was measured previously by exposing two replicate tubs of 24 snails to five to eight schistosome miracidia each using a standard laboratory protocol (as in Bonner et al., 2012) and then measuring infection prevalence. These lines are hereafter referred to as lines 1–5, (1–4:1316R and 5: M-line). Previously established resistance levels varied between 0% and 79%, although we did not see significant differences in resistance in this study. These lines also varied genetically at several loci thought to influence resistance (for example, Superoxide dismutase (Goodall et al., 2006) and catalase (Hahn et al., 2001)).

2.2. Parasite challenges

Snails were challenged with *S. mansoni* strain PR1, which originated from Puerto Rico, and has been maintained at Oregon State University, USA. Juvenile snails ranging in size from 4 to 9 mm were used for all parasite exposures and were randomised among

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treatments such that there were no significant differences in snail size among infection dose treatments (ANOVA, $P = 0.25$; power to detect a difference at the given sample size and variance was 1.00). Although snails ranged significantly in size, only 14 of 440 snails reproduced within the first week of this experiment, suggesting that the majority of snails were still reproductively immature at the time of infection. Size covariates were included in all statistical models to account for differences in initial size. Each snail was individually exposed in artificial spring water to one miracidium ("low dose") or 10 miracidia ("high dose") or sham exposed ("control"). Snails were left overnight in exposure wells. Each infection dose and control group contained approximately 30 snails from each genetic line. Following exposure, snails were housed individually in 500 mL plastic cups containing artificial springwater (Stibbs et al., 1979) and were fed lettuce ad libitum. Water and cups were changed each week. Individuals were randomly allocated to 20-cup trays for storage and kept under a normal light cycle for 5 weeks (12:12 light:dark) at 26 °C. After 5 weeks, snails were maintained under constant dark conditions for the remainder of the experiment to minimize parasite shedding between parasite harvesting days (cercariae are released with the circadian rhythms of their hosts as dictated by the light cycle (Théron, 1984)).

2.3. Measuring parasite production and host fitness

To measure the pathogen burden, once weekly, snails were isolated in individual wells containing 2 mL of artificial springwater and exposed to direct fluorescent light for 2 h to induce parasite shedding. It is our experience that this timeframe is long enough for all developed cercariae to emerge. The infection status was determined by examination of the wells for the presence or absence of cercariae. To enumerate cercariae, and thus the pathogen burden of the snail, the contents of the wells were homogenised using a micropipette and two 100 µL subsamples were collected, stained with iodine solution and photographed through a stereomicroscope for counting. The average of the subsamples was then used to estimate the total number of parasites shed in the 2 mL of water. Weekly assessments of infection status and pathogen burden were continued throughout the life of the snail or up to 55 weeks after infection (after all infected individuals had died). To characterise the infection parameters of the parasite, we measured the prepatent period (time to release of first cercariae), the patent period (duration of cercarial shedding), the rate of cercarial shed (mean number released each week over the course of infection) and total parasite production.

To measure the reproductive effort of the host, egg production was measured for each individual snail each week. Snails laid egg clutches on the hard surfaces of the clear cups and eggs were easily counted by eye. Snail shell height from the base to the top, perpendicular to the aperture, was measured every 2 weeks using digital callipers. Height was used as a measurement because it strongly correlates with width and depth (data not shown) and its measurement caused the least potential damage to the fragile aperture of the shell, particularly in young snails.

2.4. Statistical analyses

The measurements of host fitness and parasite production were used to characterise host investment in several distinct defense strategies among inbred lines. These strategies include: infection resistance, fecundity compensation, sterility tolerance and gigantism. Binomial models were used to compare infection prevalence among lines. Poisson or quasi-Poisson families were assigned in all models involving count data to account for over-dispersion (parasite production and egg counts). The growth rate was log transformed and analysed using a linear mixed effect model. Snail size, either at the time of challenge or during parasite production, was used as a covariate in all models to offset differences in parasite production due to available host resources. Size is known to have an effect on infection susceptibility as well (e.g. (Gerard and Théron, 1995). Differences in survival times among lines and treatments were performed using a Cox proportional hazards model. Normality was assessed with histograms and Schapiro–Wilks tests when tests for normality were considered necessary. Model validation was done using residual plots, Akaike's information criterion (AIC), and drop in deviance tests. All multiple comparisons were made using a Bonferroni corrected alpha level of 0.006. Model estimates are reported here with 95% confidence intervals (CIs) and P values. All statistics were performed in R version 3.0.2 (R Core Team, 2014).

3. Results

3.1. Host response to infection

Overall, we saw differences in responses to infection among genetic lines of snail hosts that greatly impacted parasite fitness measured as cercarial production. Overall, we observed higher infection percentages than expected based on previous infection trials of these genetic lines, which showed differences in infection resistance among the lines. These discrepancies could be due to direct exposure to light during the first 5 weeks after challenge, which can lead to higher rates of infection (Steinauer and Bonner, 2012) or differences in snail size at the time of infection as previous infection trials typically used larger snails which are generally more resistant to schistosomes (Gerard and Theron, 1995).

Within treatments, snail lines showed similar levels of resistance (Table 1). After accounting for size at challenge, dose was the only significant driver of infection status with high dose snails being 5.16 times more likely to become infected than low dose snails (95% CI: 2.80, 10.00; $P < 0.001$).

We found no evidence of sterility tolerance, or the ability to maintain reproduction despite infection. Virtually all snails ceased egg production upon shedding cercariae and never recovered the ability to reproduce. Thus, no lines showed sterility tolerance under these experimental conditions.

We found evidence for gigantism early in the infection and variation for this trait among inbred lines. We compared growth rates within genetic lines among infected and control snails for the first 11 weeks following parasite exposure. This time period corresponded to the time when 95% of infected individuals were still alive and growth was still trending linearly in all treatments. After controlling for baseline differences in growth rates among genetic lines, dose was a significant driver in host growth rate, with low

Table 1
Infection prevalence for each of five genetic lines of *Biomphalaria glabrata* snails exposed to two doses of *Schistosoma mansoni* parasites (low dose = 1, high dose = 10). Only snails that survived until the first assessment of infection at week 5 were included in these results. There were no statistically significant differences in infection prevalence among genetic lines of snail host in either dose treatment ($\alpha = 0.006$, corrected for nine pairwise comparisons).

Snail line	Low dose		High dose		Control (n)
	Exposed (n)	Proportion infected (n)	Exposed (n)	Proportion infected (n)	
1	21	0.524 (11)	18	0.889 (16)	22
2	20	0.450 (9)	20	0.850 (17)	17
3	29	0.655 (19)	18	0.778 (14)	25
4	23	0.348 (8)	22	0.636 (14)	21
5	20	0.400 (8)	21	0.905 (19)	24

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dose and high dose snails growing 0.07 mm (95% CI: 0.04, 0.09; $P < 0.001$) and 0.05 mm (95% CI: 0.02, 0.07; $P < 0.001$), respectively, faster per week than control snails (Fig. 1, Supplementary Table S1). Thus, gigantism was greatest in the low dose treatment. Not all lines showed a gigantism response. Line 1 control snails had higher mean growth than either low or high dose snails, although the overall line by dose interaction term was not significant (Fig. 1, Supplementary Table S1). The gigantism response manifested early in snail development, but by 15 weeks the control snails appeared to catch up to the infected snails. Thus, infection was not a significant driver of the increase in growth rate when measured across all time points ($P = 0.47$), although it was significant in the early stages of infection. Benefits of gigantism for the snail were not obvious as they did not recover the ability to reproduce. Schistosomes, however, appeared to benefit as snail size had a large effect on parasite production. For each millimetre increase in snail size at first parasite release, the rate of production increased by 11% (95% CI: 4.18; $P < 0.001$). This results in 150–300 additional cercariae per shedding period, depending on the host genetic line.

We found no evidence of fecundity compensation or increased egg production by infected snails relative to controls during any part of the infection (Fig. 1, Supplementary Table S1; $P < 0.001$). Following the onset of shedding, there was a dose-dependent reduction in egg clutch production with low dose and high dose snails laying 4.6 (95% CI: 3.2, 6.8; $P < 0.001$) and 8.9 (95% CI: 6.0, 13.9; $P < 0.001$) times fewer clutches than controls of the same genetic line, respectively. Infected individuals appeared to show a trade-off between growth and reproduction. Snail egg clutch production and growth were significantly negatively correlated in infected individuals but not control individuals during the prepatent period ($r = -0.40$, $P < 0.001$) suggesting energy is more limited for infected individuals due to diversion to parasite growth. Additionally, we found no correlation between snail egg production and cercarial production in either dose group ($P = 0.12$ and $P = 0.23$ for low and high, respectively), suggesting that this energy tradeoff is more complicated than direct resource use by the parasite. A negative correlation might be expected if energy was directly diverted from host egg production to parasite production (Baudoin, 1975; Sousa, 1983; Salvaudon et al., 2007).

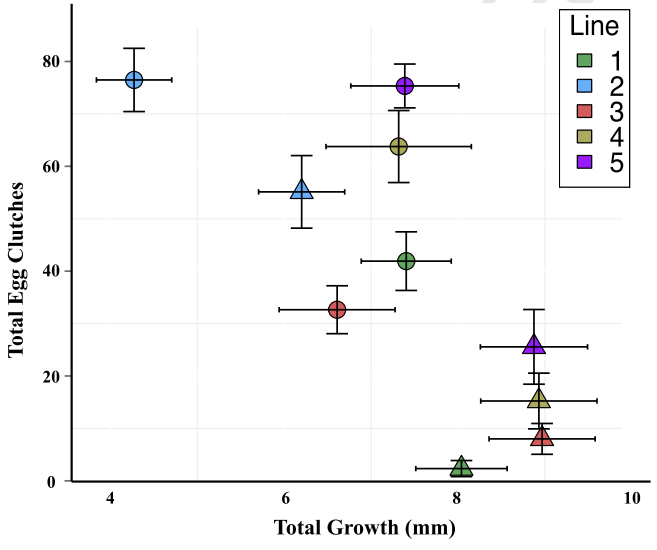

Fig. 1. Total clutch production and growth during the first 11 weeks of the study for *Schistosoma mansoni*-infected (triangles) and control (circles) *Biomphalaria glabrata* snails. A clear energetic shift is visible towards growth once infected. Host genetic lines are coded by colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Although we saw no evidence for fecundity compensation, lines 5 and 2 showed a significantly higher production of eggs during the prepatent period compared with infected snails of other lines (Supplementary Table S1), laying between 4.4 and 43.81 times more eggs before the onset of shedding. These lines also exhibited an increased prepatent period (Fig. 2B, Supplementary Table S2). Thus, the ability to delay parasite development (increase prepatent period), may allow hosts to increase their fitness by extending reproductive time before the parasite fully castrates the snail. This represents a novel host defense strategy that is genetically variable. For line 5, this response was dose-dependent, so the delay was only significant when the host was challenged with a single parasite. Line 2 had a longer prepatent period than most other lines at both doses (Supplementary Table S2, Fig. 2A).

3.2. Parasite fitness

We found no evidence that delayed development benefitted the parasite. Such a relationship might be expected if increased developmental time led to increased sporocyst size or number that might translate into greater parasite production rate or total reproduction. There was a weak, negative correlation between prepatent length and parasite production rate ($r = -0.26$; $P = 0.05$) and between prepatent length and total parasite production ($r = -0.29$; $P = 0.03$) in the low dose treatment groups, suggesting that parasite fitness was not increased by a longer prepatent period (no significant correlation in the high dose treatment groups). Prepatent period length also correlated negatively with growth rate during that time in both dose groups (low: $r = -0.59$; high: $r = -0.46$; $P < 0.001$). Together, these data show that the length of the prepatent period, or postponing cercarial shedding, does not benefit the parasite through a tradeoff between prepatent development and parasite production.

The total number of cercariae produced by a snail is dependent on the rate of parasite production, length of the prepatent period (incubation time), and length of the patent period. The rate of parasite production differed among lines, with line 2 shedding 2.2 times fewer parasites per week compared with lines 1 and 3 (95% CI: 1.47, 3.44; $P < 0.001$; Fig. 3C, Supplementary Table S2). Dose was not a significant driver in rate of parasite production in four of five lines, as snails in those lines exposed to the low and high doses produced the same number of cercariae per week when size differences were accounted for. However, line 5 produced more cercariae per week in the high dose group than in the low dose group, causing significant statistical interaction between dose and line.

Interestingly, the total number of cercariae produced throughout the lifespan of the snail did not differ among genetic lines in the low dose group (Fig. 3B, Supplementary Table S2), but did differ among certain lines in the high dose group. However, the rate at which those cercariae were produced varied among lines for both doses. There was a significant interaction between dose and snail line such that lines 2 and 5 produced more cercariae in the high dose group than the other lines. Increased production in these lines is due to enhanced survivorship of line 2 and a shorter prepatent period and increased production rate of line 5.

Dose had a significant effect on total parasite production across all lines, with high dose snails producing 2.66 times fewer total parasites over the course of infection. This effect is due to the higher mortality of high dose snails (95% CI: 1.22, 6.17; $P = 0.018$; Fig. 3B, Supplementary Table S2). Indeed, the length of the patent period (weeks of active shedding) correlated positively and strongly with the total parasite production in both low ($r = 0.69$; $P < 0.001$) and high dose ($r = 0.85$; $P < 0.001$) treatments.

Since the majority of hosts continued to shed parasites until death, the length of the patent period strongly correlated with snail

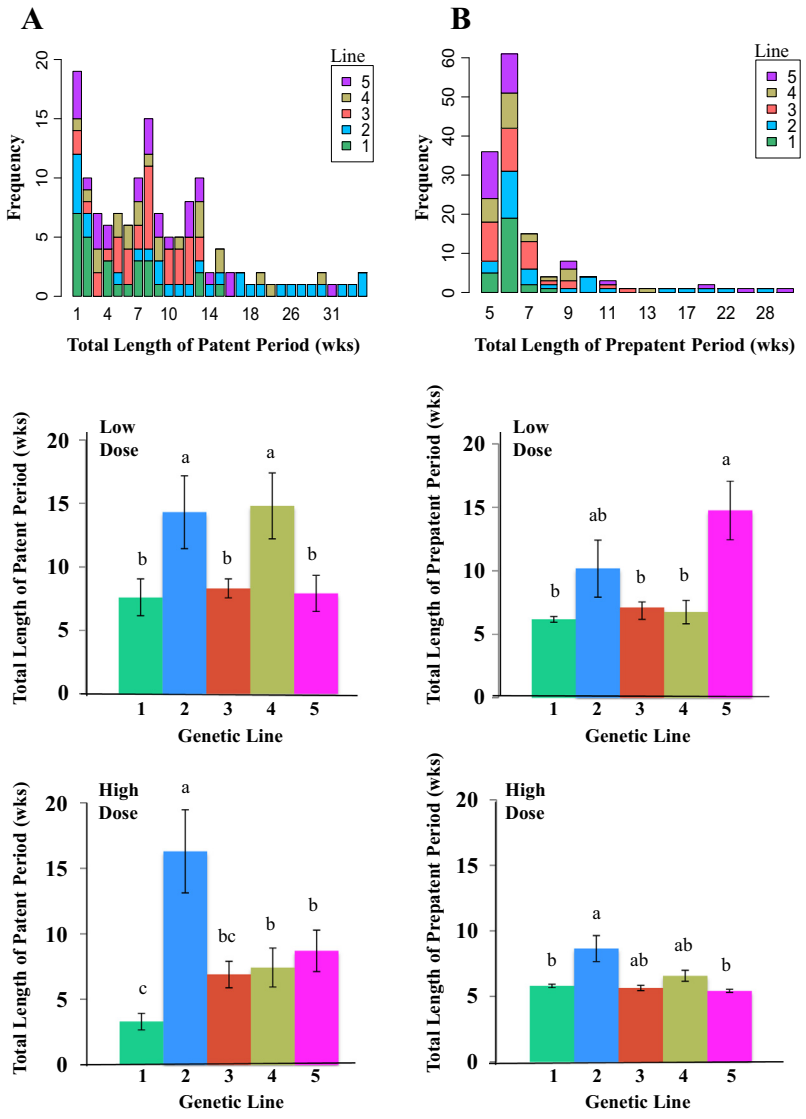

Fig. 2. Total length of the (A) patent period (total time shedding) and (B) prepatent period (total time before the onset of shedding) for *Biomphalaria glabrata* snails exposed to a low (1) or high (10) dose of *Schistosoma mansoni* parasites. Histograms depict broad variations in the total lengths of both patent and prepatent periods in weeks among lines, which contribute to overall parasite production. Bar graphs show significant comparisons among genetic lines in patent and prepatent period length (lines with different letter codes differed significantly at $\alpha = 0.006$). Error bars represent S.E.

survivorship. In fact, only four snails ceased shedding before death, but those individuals died within 1 week of the end of shedding. Low dose snails were able to survive parasite production for 2.28 times longer than high dose snails (95% CI: 1.59, 3.23; $P < 0.001$). Therefore, dose was the main driver of differences in patent period length due to parasite-induced mortality in the high dose groups. Larger snails were also able to better survive shedding as every millimetre increase in size at the first shed led to an 8% longer patent period (95% CI: 1.03, 1.12; $P < 0.001$).

4. Discussion

In this study we showed that individual host genotype has a significant effect on host defense strategy and the intra-snail reproductive rate of an important parasite of humans, *S. mansoni* (Table 2). Reproduction of the parasite in the snail host is directly related to infection risk for humans because it determines how many infective stages are emitted into the environment. Total cercariae produced, rate of cercarial production, and length of the prepatent and patent periods were all influenced by host genotype and, in some cases, this variation was dose-dependent. To our knowledge, this comparative study is the first to show the importance of host genotype, using inbred lines of snails, on the course of infection of schistosomes in snails, and how these genotype-driven differences affect the fitness of the pathogen.

The differences detected among these snail genotypes presumably are due to differences in their underlying host defense strategies and thus the pathogenesis of the parasites within them. The outcome of infection is the product of the interaction of host and parasite strategies within the environmental context (Dawkins, 1982; Sorensen and Minchella, 2001; Lafferty and Kuris, 2009). In our experiment, we found genetic variation for a potential novel host defense strategy. This strategy was the ability to delay parasite development, or to extend the length of the prepatent period, which was detected in two of the five genetic lines. Although snail reproduction was not high in any infected group, it was the highest in the lines and doses with long prepatent periods (line 2 at both doses and line 5 at a low dose), suggesting that by delaying the development of the parasite, the snails can extend their

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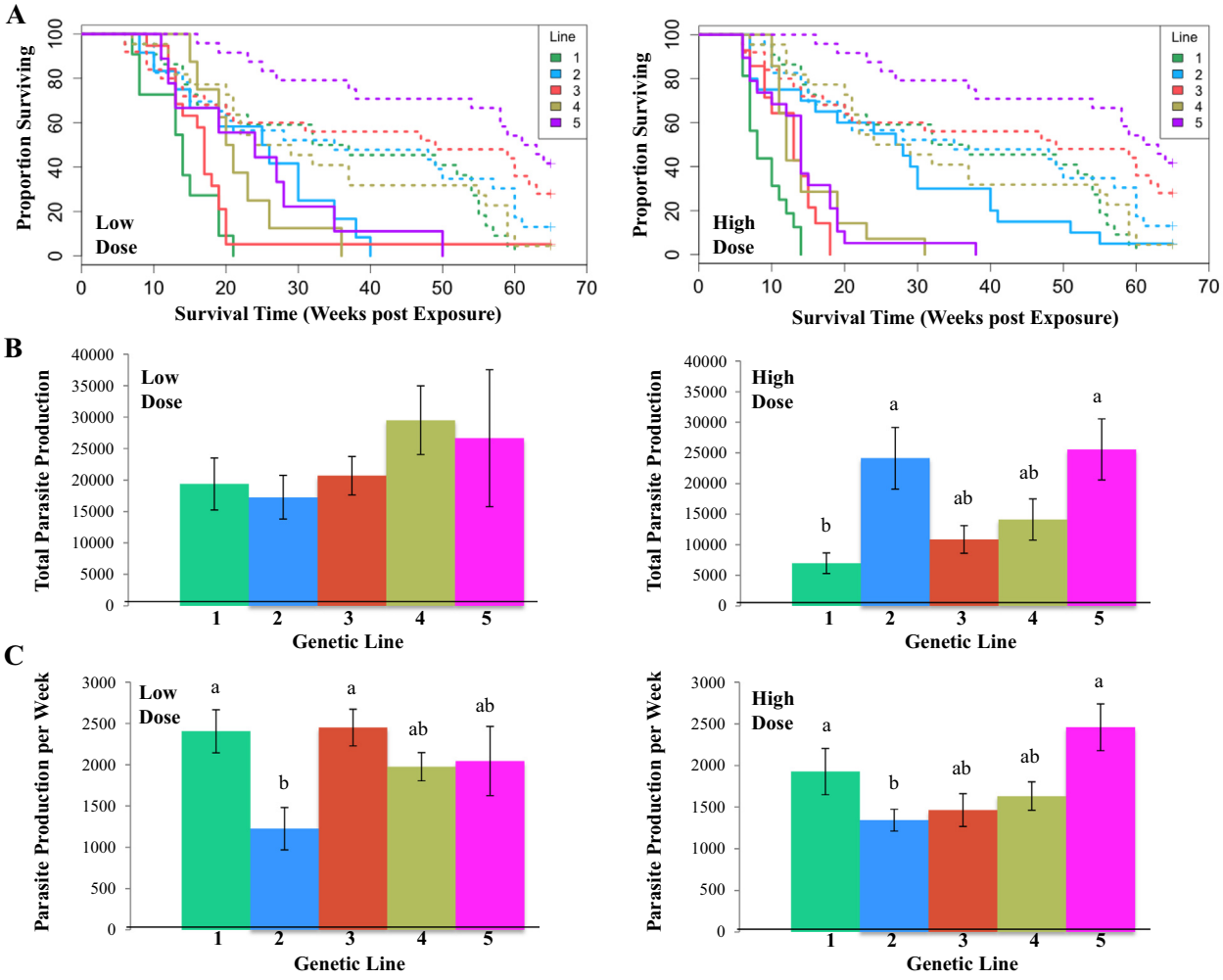

Fig. 3. *Biomphalaria glabrata* (host) survival and *Schistosoma mansoni* parasite production over the course of infection. (A) Survival curves for infected low dose (1 parasite, solid lines) and high dose (10, solid lines) snails in each line relative to control survival (dashed lines). (B) Total parasite production for each line in low and high dose treatments. (C) Average weekly parasite production for each line in low and high dose treatments. Significant differences among lines in parasite production are denoted with different letter codes ($\alpha = 0.006$, corrected for nine pairwise comparisons). Error bars represent S.E. Note that dose-dependent mortality in A differs among lines, and differences in total parasite production and shedding rate are more obvious in the high dose groups.

Table 2
Summary of the effects of the prepatent period length, patent period length, cercarial shed rate, and total cercarial shed rate on the reproductive output of *Schistosoma mansoni* in five inbred lines of *Biomphalaria glabrata*.

Dose	Snail lines				
	1	2	3	4	5
1 Parasite					
Prepatent	–	↓	–	–	↓↓
Patent	–	↑	–	↑	–
Rate	↑	↓	–	–	–
Total	–	–	–	–	–
10 Parasites					
Prepatent	–	↓	–	–	–
Patent	↓	↑↑	–	–	–
Rate	↑	↓	–	–	↑
Total	–	↑	–	–	↑

Arrows and dashes indicate the hypothesised effects of each characteristic on parasite fitness for each snail line. Upward arrows indicate a relative increase in fitness, downward arrows indicate a decline, and dashes indicate that the value was equivocal with the mean.

reproductive time prior to castration. Also, these snails appeared to allocate less energy to growth, as there was a negative correlation between the prepatent length and growth rate, further evidence that this may be a strategy to increase fitness despite infection, and explains further variation in the gigantism response. Perhaps snails that delay parasite development have also slowed down the castration process, so energy that would normally be diverted to enhanced growth is used instead for reproduction and immune defense. Overall, delayed development did not appear to enhance parasite fitness, thus it appears to be a host defense strategy (e.g. as opposed to gigantism, which one could argue is parasite manipulation of the host). Also, line 5, the dose group that showed delayed development, had a lower cercarial production rate than the dose group that did not delay development (although this was not statistically significant after Bonferroni corrections, $P = 0.02$), adding more evidence that lengthening of the prepatent period enhances snail fitness and not parasite fitness. This effect would be magnified in environments where snail mortality is high due to factors such as predation, habitat instability or infection with other pathogens, resulting in potential host death before the onset of shedding. If delayed development is a host defense

strategy, it appears it can be defeated by the parasites in heavier infections, at least in one genetic line (line 5). If the delay is immune-mediated, then it is possible that in a high infection dose, the parasites are capable of escaping the immune response and developing at a more typical maturation rate (Taylor et al., 1998; Davies et al., 2002). An alternative hypothesis is that when the snail is challenged with high doses, more rapid parasite development is driven by competition between individual parasites (Davies et al., 2002). Consistent with this hypothesis, line 5 produced more cercariae in the high dose group than the low dose group, suggesting that intra-host environment, dictated by genetic background, may play a role in the magnitude of competition among parasites.

Most of the snail lines showed gigantism or accelerated growth after infection. The exception was line 1, in which the mean growth rate of infected snails was lower than that of control snails. Because gigantism due to a castrating parasite is predicted to occur only when the total energy demands of the parasite during castration and the cost of immune defense are much lower than the resources that are freed by castration (Bourns, 1974; Ebert et al., 2004), we hypothesise that in this line, the parasite was able to acquire more resources to enhance its growth or that the immune defense of this line was more energetically costly than those of the other lines. Indeed, in line 1 infections, the parasite had among the highest cercarial production rates and snail survival was the lowest in both dose groups, evidence supporting both of these hypotheses.

Overall, the growth rate was accelerated only in the earliest part of infection. After approximately 15 weeks, the control snails began to catch up to the infected snails in terms of size (data not shown). This pattern suggests that early in infection, the parasite requires less energy, and thus resources are available for host growth, but later in infection, as the parasite is undergoing reproduction, its energy demands are higher and more host resources are required (Minchella et al., 1985; Ebert et al., 2004; Lafferty and Kuris, 2009). We also found that growth decreased when hosts were exposed to higher doses of parasites, which is the opposite of the theoretical prediction put forth by Ebert et al. (2004) and the findings of the meta-analysis of Sorensen and Minchella (2001). However, a model based on dynamic energy budgets of hosts infected with castrator parasites predicted that higher doses would lead to less gigantism than low doses, supporting our findings (Hall et al., 2007; Best et al., 2010). Higher doses may yield slower growth due to the energy required to support more parasites initially, immune defenses when challenged with multiple parasites, or competition between individual parasites.

Gigantism had no apparent fitness benefit to the host, especially since infected snails faced a tradeoff between growth and reproduction in the prepatent period before castration (negatively correlated). Gigantism benefited parasite reproduction. Overall, we saw an 11% increase in total parasites shed per millimetre increase in host size, which equated to hundreds of parasites per millimetre of host growth. Parasites benefit from a larger host due to higher resource availability to increase the reproductive output of the parasite (Baudoin, 1975; Dawkins, 1982; Gandon et al., 2002; Bonds, 2006). Interestingly, the total number of cercariae shed over the lifetime of the snail was not significantly influenced by size. This was because accelerated growth slowed down over the course of infection and these snails were perhaps even stunted as the control snails caught up to them in size. Also, other factors such as survival and prepatent period length, which were genotype-specific, had a strong effect on parasite fitness. Even if it is not a defense strategy, gigantism is a response to parasitism that is variable among genotypes of snails, and has a rather large impact on snail fitness.

Genetic background drove differences in parasite production rates, and has been documented in other parasite host systems (e.g. Salvaudon et al., 2007; Vale and Little, 2012). Parasite production was also strongly influenced by infection dose. Over the course of infection, three of the lines produced lower numbers of cercariae in the high dose group than the low dose group, due in large part to differences in survivorship. We observed an almost threefold reduction in survival time in the high dose group, which corresponded to a similar reduction in the total number of cercariae produced. Thus, higher doses at challenge resulted in reductions in both host and parasite fitness (as seen in Gandon et al., 2002; Blair and Webster, 2007). Interestingly, when size differences were accounted for, the high dose and low dose groups produced cercariae at the same rate (otherwise low dose snails produce more at each shedding period on average), which is contrary to earlier predictions that higher doses should lead to greater control of the host, leading to a higher production of parasites (Ebert et al., 2004). Experimental infections have shown that higher infection doses result in higher numbers of mother sporocysts (Gerard et al., 1993; Théron et al., 1997, 2004); but it appears that these do not necessarily yield more total cercariae. It could be that the parasites are "limiting out" the resource, thus making all the cercariae they can without killing the snail and therefore maximising lifetime transmission success, given the available resources (Jensen et al., 2006). Although low dose snails lived longer and produced more cercariae over the course of infection, there was no general correlation between total cercarial production and snail survivorship in the higher dose group. It is also possible that in a multiple infection, there is more parasite growth early on than in a single infection and the snail expends more energy on immune defense and tissue repair, that it reduces the growth of each individual parasite, resulting in fewer cercariae produced. At high doses, castration was more rapid in the high dose groups and although development times were not statistically shorter, they were numerically shorter.

Because the fitness consequences of castration are so high, hosts are expected to evolve counter adaptations to the effects of parasites (Lafferty and Kuris, 2009; Best et al., 2010). We did not find overwhelming evidence of sterility tolerance to infection. Reproduction in infected snails only occurred during the prepatent period and differed among groups. The two lines producing the most eggs (2 and 5) also had the longest prepatent periods and the highest background fecundity, suggesting that they may not be facultatively altering their energy allocation when infected (Webster and Woolhouse, 1999). It is possible that sterility tolerance is a strategy of older snails which are reproductively mature upon infection, or even if snails were allowed to outcross rather than be restricted to self-fertilisation.

The evolutionary outcomes of host defense investment and energy allocation can have huge implications for disease dynamics. Hosts must find ways to maximise their own fitness in spite of a parasite with the same goal (Anderson and May, 1982). Especially in the case of vector-borne human diseases, studies often focus on identifying resistant genotypes to introduce into natural systems in an effort to reduce transmission (e.g. Riehle et al., 2003). That being said, unrealized heterogeneity in transmission often exists in these natural populations due to tolerance mechanisms (Woolhouse et al., 1997), which can impact the efficacy of control efforts based on a homogeneous host population. Here we have shown that infection dynamics and infected host fitness are highly influenced by the host's genetic background and the parasite dose in the *B. glabrata* – *S. mansoni* system. These differences impacted the number of parasites produced by influencing the prepatent period, patent period and cercarial production rate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2015.10.001.

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