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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