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Relative reproductive success of co-infecting parasite genotypes under intensified within-host competition

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ABSTRACT

In nature, host individuals are commonly simultaneously infected with more than one genotype of the same parasite species. These co-infecting parasites often interact, which can affect their fitness and shape host–parasite ecology and evolution. Many of such interactions take place through competition for limited host resources. Therefore, variation in ecological factors modifying the host resource level could be important in determining the intensity of competition and the outcome of co-infections. We tested this hypothesis by measuring the relative reproductive success of co-infecting genotypes of the trematode parasite *Diplostomum pseudospathaceum* in its snail host *Lymnaea stagnalis* while experimentally manipulating snail resource level using contrasting feeding treatments (*ad libitum* food supply, no food). We found that food deprivation constrained the overall parasite within-host reproduction as the release of parasite transmission stages (cercariae) was reduced. This indicates intensified competition among the parasite genotypes. The genotypic composition of the released cercariae, however, was not affected by the feeding treatments. This suggests that in this system, the relative reproductive success of co-infecting parasite genotypes, which is an important component determining their fitness, is robust to variation in ecological factors modifying the strength of resource competition.

1. Introduction

Individuals of free-living organisms are typically simultaneously infected with several parasite species and genotypes that can interact (reviewed by Holmes and Price, 1986; Read and Taylor, 2001). These interactions often modify parasite fitness and thus contribute, for example, to parasite community ecology (e.g. Lello et al., 2004), evolution of parasite virulence (e.g. de Roode et al., 2005; Staves and Knell, 2010), plasticity in parasite life-histories (Koella and Antia, 1995; Wargo et al., 2007), and maintenance of genetic variation in parasite traits (Andersson et al., 2013; Seppälä et al., 2009, 2012). Co-infecting parasites can interact both through direct mechanisms such as competition and predation, and indirectly through host immune function (Graham, 2008; Johnson and Buller, 2011; Mideo, 2009; Pedersen and Fenton, 2007). Many of the above effects of co-infections are, however, assumed to be caused by variation in parasite competitive ability for limited host resources, which also underlies most of the theory developed on the ecological and evolutionary consequences of co-infections (e.g. Bremermann and Pickering, 1983; Chao et al., 2000; Frank, 1996).

The outcome of interactions among co-infecting parasites may depend on ecological factors that modify the intensity of within-host competition between them. One potentially important factor is variation in host resource level (e.g. food availability). This is because hosts in poor condition often provide fewer resources for parasites, thus impairing their growth and reproduction (Bedhomme et al., 2004; Ebert et al., 2000, 2004; Takken et al., 2013; Vale et al., 2013) and possibly intensifying competition. However, experimental tests on the effect of host resource level on the outcome of co-infections are rare and typically focused on co-infections of different parasite species (Duncan et al., 2015; Fellous and Koella, 2009, 2010). In the mosquito *Aedes aegypti*, co-infection of the microsporidium *Vavraia culicis* and the protozoan *Ascogregarina culicis* prolongs larval developmental time (age at pupation), but only under food shortage (Fellous and Koella, 2010). Other effects of co-infections on host and parasite performance such as host survival and parasite reproduction have, however, not been found to depend on host resource level (Duncan et al., 2015; Fellous and Koella, 2009, 2010).

In this study, we focused on a novel aspect of consequences of host resource level for co-infections by examining the competition among genotypes of a single parasite species. To our knowledge, the role of host resource level in determining intraspecific competition among parasites has not been investigated earlier. The outcome of intraspecific

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competition could, however, strongly depend on host resource level since parasites often share the same resource niche in the host. We measured the relative reproductive success of genotypes of the trematode parasite *Diplostomum pseudospathaceum* in their snail host *Lymnaea stagnalis* while experimentally manipulating snail food availability and hence the strength of resource competition among parasite genotypes. In nature, snails are commonly infected with multiple *D. pseudospathaceum* genotypes (up to 90% of infected individuals per population; Louhi et al., 2013a; Rauch et al., 2005), and host exploitation by the parasite is extensive. The parasite reproduces asexually (i.e. clonally) within snails producing parthenitae called sporocysts. Sporocysts eventually fill snail gonads and produce thousands of free-swimming transmission stages (cercaria larvae) per day for several weeks (Karvonen et al., 2004; Seppälä et al., 2008). This leads to castration and increased mortality of the snails (Karvonen et al., 2004; Seppälä et al., 2013). Since food shortage constrains the reproduction of *D. pseudospathaceum* by reducing the number and quality of the produced cercariae (Seppälä et al., 2008; but see Louhi et al., 2013b) we expected that reducing food availability intensifies the resource competition among parasite genotypes. Therefore, we hypothesized that the genotypic diversity of released cercariae decreases in food deprived snails as the genotypes that are the strongest competitors outcompete the weaker ones.

2. Material and methods

2.1. Experimental design

In mid-July 2008, we collected adult *L. stagnalis* snails from Lake Huumonjärvi in Finland (65°06′N, 26°08′E), a lake in which snails are commonly infected with *D. pseudospathaceum* (Louhi et al., 2010). We brought the snails into the laboratory and separated individuals infected with *D. pseudospathaceum* from snails infected with other species by observing the morphology and behavior of the released cercariae (Niewiadomska, 1986; Niewiadomska et al., 1997).

We placed the experimental snails ($N = 49$) individually in 1 l plastic containers and distributed them randomly into six 400 l tanks with continuous flow through of water (20.0 ± 0.2 °C). Containers were perforated with 6 mm holes from all sides to ensure effective water exchange. We maintained the snails under natural light-dark rhythm (16 L:8 D), and fed them *ad libitum* with fresh lettuce for two weeks before the experiment to maximize their body condition. After that, we measured the number of released cercaria larvae from each snail and genotyped the parasites to determine their genotypic composition in each host (see below). We then randomly assigned the snails into two feeding treatments: '*ad libitum* food supply' (24 snails) and 'no food' (25 snails). We maintained the snails in their respective feeding treatments for ten days, which is sufficient time to affect parasite within-host reproduction [Seppälä et al. (2008); the parasite in the previous study was named *Diplostomum spathaceum* but has been later determined as *D. pseudospathaceum* using genetic analysis (Louhi et al., 2010)]. After that, we again sampled the released cercariae to measure parasite reproductive output and determine their genotypic composition.

At both sampling times described above, we placed the snails individually into glass jars containing 0.1 l of water for three hours. We then diluted each suspension to 0.4 l and estimated the number of released cercariae by taking five 1 ml samples. In addition, we haphazardly collected 30 cercariae from each snail, and stored them individually in 1.5 ml Eppendorf tubes in 15 μl of water at −80 °C. Subsequently, we extracted the DNA of each cercaria with Chelex 100 resin according to Criscione and Blouin (2004), and identified multi-locus genotypes of the parasite using three microsatellite markers [Diplo06, Diplo09, Diplo23 (Reusch et al., 2004)] as described by Louhi et al. (2010). These microsatellite markers are highly polymorphic and effective in separating parasite multi-locus genotypes (see Karvonen et al., 2012; Rellstab et al., 2013). The success rate of microsatellite genotyping was 95%. It is important to note that not only exposure to multiple parasite genotypes, but also mutations during their clonal within-host reproduction could increase parasite genotypic variation within individual snails (Bayne and Grevelding, 2003). This, however, was unlikely to affect our results as parasite multi-locus genotypes differing from each other only in one locus, which is the most likely situation in the case of somatic mutations, were not observed in any of the experimental snails. Six snails died during the experiment, and we excluded them from the data.

2.2. Statistical analyses

We first analyzed possible systematic differences in cercarial release among the experimental snails at the first sampling time (i.e. before the food manipulation started). We tested if the number of parasite genotypes infecting the snails affected the number of released cercaria larvae using a linear regression. We then excluded the snails infected with only one parasite genotype from the data, and tested whether the co-infected snails assigned to the different feeding treatments differed in the number of released cercaria larvae using an independent-samples *t*-test. After that, we calculated the change in the number of released cercariae in each snail during the experiment by subtracting their number at the first sampling from the number at the second sampling. We tested if these changes differed between the feeding treatments using an independent-samples *t*-test. We also tested if the changes differed from zero in each feeding treatment using one-sample *t*-tests. We did not use a repeated measures analysis of variance since the treatments did not differ at the first sampling time (see Results) and thus the between factor main effect would become non-informative.

We used three different measures to estimate possible changes in the genotypic composition of the released cercaria larvae followed by food manipulation. First, we counted the number of observed parasite multi-locus genotypes for each snail at each sampling time. We verified that the used sample size of 30 cercariae per snail was sufficient for estimating the number of released parasite genotypes by calculating rarefaction curves for each snail [Supplementary Fig. S1; see Buddle et al. (2005); Gotelli and Colwell (2001)] with the aid of the package vegan 2.3 (Oksanen et al., 2015) in R 3.2.0 (R Core Team, 2015). Second, we calculated the genotypic diversity of the released cercariae for each snail at each sampling time using the Stoddart's index, G_0 (Stoddart, 1983), which varies between one (single genotype) and N (sample size, all different genotypes) and incorporates also the evenness of genotype frequencies within samples. As above, we analyzed whether these variables differed between the snails assigned to the different feeding treatments before the food manipulation started using independent samples *t*-tests. We also calculated the changes between the sampling times for both parameters, and tested if they differed between the feeding treatments (independent samples *t*-tests) and from zero in each treatment (one-sample *t*-tests).

Third, to examine changes in the genotypic composition of the released cercariae that would not be observed as changes in the number of released parasite genotypes or their genotypic diversity, we calculated the Bray–Curtis dissimilarity index, B (Bray and Curtis, 1957), for each snail using the proportions of the different multi-locus genotypes in the samples. B measures the proportional dissimilarity in genotypic composition between two samples, and varies between zero (identical compositions) and one (completely dissimilar compositions). We analyzed whether the values of B differed from zero in each feeding treatment using one-sample *t*-tests. It is important to note, however, that not only the actual changes in the genotypic composition over time, but also those resulting from stochastic variation between analyzed samples due to sampling error lead to values of B that are larger than zero (i.e. two random samples drawn from the same distribution are likely to differ by chance). Therefore, we evaluated the magnitude of such stochastic variation with respect to estimates of B by calculating