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.

Keywords:

Host–parasite interactions; Multiple infections; Mixed infections; Concomitant infections; Microsatellites; Trematoda

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 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 *Lymnaea stagnalis* snails from Lake Huuemonjärvi 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 ($20.0 \pm 0.2^\circ\text{C}$). Containers were perforated with 6 mm holes from all sides to ensure effective water exchange. We maintained the snails under natural light-dark rhythm (16L:8D), 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 μ l 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 the average dissimilarity between the samples that could arise solely from sampling error for each snail. In these calculations, we assumed that the actual genotypic composition of the released cercariae remained unchanged over time, and used the pooled sample from both sampling times as the estimate of the expected genotypic composition. We then resampled each of these distributions with replacement for 500 times (30 observations in each sample). From these samples, we formed 250 pairs of samples per snail and calculated the average expected dissimilarity between them for each snail, which we then subtracted from the observed values of B . Note that these corrections do not include the possible effect of rare genotypes that were missed during sampling. However, since B is more sensitive to changes in the proportions of common genotypes than those of rare genotypes, we considered these estimates of sampling error sufficient for our purpose. We analyzed whether the corrected values of B differed between the feeding treatments (independent samples t -test) and from zero in each treatment (one-sample t -tests). We performed all statistical tests using IBM SPSS 20.0 (SPSS, Chicago) software.

3. Results

The number of parasite multi-locus genotypes infecting individual snails varied between one and nine. At the first sampling time (i.e. before the food manipulation started),

the number of cercaria larvae released from the snails did not depend on the number of parasite genotypes infecting them (linear regression: $R^2 = 0.048$, $F_{1,41} = 2.063$, $P = 0.158$). Five snails were infected with only one parasite genotype. We excluded these individuals from further analyses, which resulted in a total of 21 fed and 17 starved snails. Before the food manipulation started, snails assigned to the different feeding treatments did not differ in the number of released cercariae (*ad libitum* food supply: mean $\pm$ SE = 6068.6 ± 526.4 ; no food: mean $\pm$ SE = 5703.5 ± 539.2 ; independent-samples *t*-test: $t_{36} = 0.480$, $P = 0.634$). The change in cercarial release between the first and the second sampling time was affected by the feeding treatments (Fig. 1; independent-samples *t*-test: $t_{36} = 2.104$, $P = 0.042$). Starvation led to decrease in the number of released cercariae [Fig. 1; one-sample *t*-test (0 as a reference): $t_{16} = -2.443$, $P = 0.027$] whereas no change was observed in the group of fed snails [Fig. 1; one-sample *t*-test (0 as a reference): $t_{20} = -0.090$, $P = 0.929$].

At the first sampling time, snails assigned to the different feeding treatments did not differ in the number of released parasite genotypes (*ad libitum* food supply: mean $\pm$ SE = 5.0 ± 0.5 ; no food: mean $\pm$ SE = 4.7 ± 0.5 ; independent-samples *t*-test: $t_{36} = 0.380$, $P = 0.706$) or the genotypic diversity of cercariae (Stoddart's index, G_0 ; *ad libitum* food supply: mean $\pm$ SE = 3.5 ± 0.3 ; no food: mean $\pm$ SE = 3.3 ± 0.3 ; independent-samples *t*-test: $t_{36} = 0.550$, $P = 0.586$). Furthermore, the changes in the number of released parasite genotypes and in the genotypic diversity of the cercariae between the sampling times were not statistically significant [Fig. 2a-b; one-sample *t*-tests (0 as a reference): number of genotypes: *ad libitum* food supply: $t_{20} = 0.826$, $P = 0.419$; no food: $t_{16} = 0.489$, $P = 0.632$; genotypic diversity: *ad libitum* food supply: $t_{20} = 0.019$, $P = 0.985$; no food: $t_{16} = 1.294$, $P = 0.214$], and did not differ between the feeding treatments (Fig. 2a-b; independent-samples *t*-tests: number of genotypes: $t_{36} = 0.087$, $P = 0.931$; genotypic diversity: $t_{36} = -0.949$, $P = 0.349$). Power analyses indicated that with the observed standard deviations and current sample sizes the differences between

the feeding treatments that would have been detected as statistically significant at $\alpha = 0.05$ were 0.74 and 0.77, respectively. Such changes in the genotypic composition of co-infecting parasites would still be small indicating that host resource level does not play a major role in determining the outcome of competition.

The Bray-Curtis dissimilarity index (B) suggested changes in the genotypic composition of the released cercaria larvae in both feeding treatments [one-sample t -tests (0 as a reference): *ad libitum* food supply: $t_{20} = 9.205$, $P < 0.001$; no food: $t_{16} = 8.670$, $P < 0.001$]. However, at least some of these differences could arise from stochastic effects in sampling (see Materials and methods). After controlling for such effects, changes in the genotypic composition of the released cercariae remained statistically significant in the snails fed *ad libitum* [Fig. 2c; one-sample t -test (0 as a reference): $t_{20} = 2.692$, $P = 0.014$] and were close to statistical significance in the starved snails [Fig. 2c; one-sample t -test (0 as a reference): $t_{16} = 2.072$, $P = 0.055$]. These changes, however, did not differ between the feeding treatments (Fig. 2c; independent-samples t -test: $t_{36} = 0.462$, $P = 0.647$). With the observed standard deviation and current sample sizes the difference between the feeding treatments that would have been detected as statistically significant at $\alpha = 0.05$ was 8.8%. Together with the above results, these changes indicate that some parasite genotypes increased and some decreased their cercarial release, but this did not alter the genotypic diversity of cercariae. Thus, no genotypes were superior competitors and able to outcompete the others.

4. Discussion

Consequences of multiple-genotype and multiple-species infections have received wide interest in recent research on evolutionary ecology of host–parasite interactions. This is not only because co-infections are common in nature (reviewed by Holmes and Price, 1986;

Read and Taylor, 2001), but also because resulting interactions among parasites affect numerous ecological and evolutionary processes between hosts and parasites (e.g. Andersson et al., 2013; de Roode et al., 2005; Koella and Antia, 1995; Lello et al., 2004; Seppälä et al., 2012). Many of the interactions among co-infecting parasites take place through competition for host resources (reviewed by Mideo, 2009). Competition among parasite genotypes is probably the main form of interaction also in our study system. This is because no difference in mean cercarial output from snails infected with one or two parasite genotypes was observed in a previous study (Karvonen et al., 2012). This indicates that co-infecting genotypes produce fewer cercariae per genotype, which is in accordance with the competition hypothesis. Our data confirm this result as the number of parasite genotypes infecting each snail (range: 1-9) did not affect the number of released cercariae.

When co-infecting parasites interact through resource competition, ecological factors that constrain host resource level could strengthen competition among parasites and modify the outcome of co-infections. By manipulating snail resource level using contrasting feeding treatments we modified the within-host reproduction of the parasite *D. pseudospathaceum*. Reduced release of cercariae from the starved snails suggests intensified resource competition among the co-infecting genotypes. While we also found small changes in the genotypic composition of the cercariae over time, these changes were independent of the host resource level and did not alter their genotypic diversity. This suggests that the relative reproductive success of the parasite genotypes, which is an important component determining their fitness, is not affected by factors modifying the intensity of resource competition in this species. Thus, rather than being due to variation in the competitive ability of parasite genotypes, the observed changes in the genotypic composition may reflect a temporally dynamic reproduction of the parasite. Daily variation in cercarial numbers released from individual snails is typically high and could be driven by the parasite switching between phases of

cercarial production and release (Karvonen et al., 2004). If this happened asynchronously between the co-infecting parasite genotypes, it could lead to the observed changes in the genotypic composition. It is important to note, however, that the observed effect of starvation on cercarial release was not strong (average reduction of 22%) when compared to an earlier study by Seppälä et al. (2008). Thus, also the effect of starvation on the intensity of competition may have been weak. Furthermore, it is possible that the effects of starvation could be seen in other components important for parasite fitness such as cercarial quality (Seppälä et al., 2008; but see Louhi et al., 2013b), which was not investigated in this study.

Evolutionary ecological research on co-infections has largely focused on their role in evolution of parasite virulence. Several theoretical models predict that parasites with the highest host exploitation rate, and thus virulence, are the most successful in resource competition in co-infections, which then leads to evolution towards increased virulence in the parasite population (e.g. Bremermann and Pickering, 1983; Chao et al., 2000; Frank, 1996). Alternatively, co-infections could select for phenotypic plasticity in host exploitation rate (i.e. expressing low exploitation rate in single infections, and increasing host exploitation when the intensity of within-host competition gets higher), which could lead to the highest fitness in host individuals with different numbers of co-infecting parasites (Choisy and de Roode, 2010; Leggett et al., 2013). Our data, however, does not support such plastic responses since cercarial release, which reflects the rate of host exploitation, did not depend on the number of parasite genotypes infecting the snails or did not increase over time.

Our results suggest that co-infecting *D. pseudospathaceum* genotypes have little influence on the division of available host resources. In other words, genotypes do not show superiority in competition or ability to outcompete other genotypes. Thus, instead of being due to variation in their competitive ability, the relative reproductive success of parasite genotypes may depend on the proportion of the host tissues they manage to occupy. Thus,

genotypes with the highest cercarial release may be the ones with the highest initial within-host growth rate, or simply the first ones infecting a snail. In *D. pseudospathaceum*, the development of infection in snails from exposure to cercarial release takes several weeks (Chappell et al., 1994), which may be an adaptation to maximize fitness by first investing in growth rather than reproduction. This is in accordance with the classical age vs. size at maturity trade-off (Stearns, 1989; Stearns and Koella, 1986), which could be most pronounced in systems where investment in growth gives a competitive advantage.

To our knowledge, this is the first study investigating the consequences of host resource level on the interactions among co-infecting genotypes of a single parasite species. The few earlier studies on the topic have investigated ecological factors that modify the strength of competition among co-infecting parasite species. Those studies also suggest that host resource level plays a minor role in determining the outcome of co-infections. In the mosquito *A. aegypti*, co-infection with two microsporidium species *V. culicis* and *Edhazardia aedis* increases parasite virulence by reducing longevity of infected hosts (Duncan et al., 2015). This effect, however, is independent of the host food level (Duncan et al., 2015). In the same host species, the effect of co-infection with *V. culicis* and the protozoan *A. culicis* prolongs larval developmental time under food shortage, but only when the infectious doses of both parasite species are high (Fellous and Koella, 2010). Survival of larvae before adulthood and adult wing size are not affected by such an interaction between infection type and host resource level (Fellous and Koella, 2010). Furthermore, food availability modifies the effect of co-infection on parasite performance so that *A. culicis* is less likely to produce oocysts in co-infections under reduced host feeding, but only if the dose of its competitor is high (Fellous and Koella, 2009). The effect of co-infection on spore production of *V. culicis* is not modified by food availability, but the deleterious effects of co-infection increase with the infectious dose of the competitor (Fellous and Koella, 2009). These findings suggest that

other ecological factors than host resource level that determine the strength of competition among parasites (e.g. infectious dose) may be more important in determining the outcome of co-infection.

Together with the earlier studies mentioned above our results suggest that host resource level does not have a strong influence on the outcome of co-infections. The current knowledge, however, is too limited for broad generalizations on the importance of ecological factors in determining the outcome of co-infections in natural systems. In particular, more studies on parasites with varying characteristics of host use are needed. In trematode–snail interactions, for instance, within-host growth in some species includes also rediae, which are parthenitae with mouthparts (Hechinger et al., 2011; Lim and Heyneman, 1972). Rediae are able to prey upon co-infecting parasites, which could give them a competitive advantage through interference competition. In such systems, the role of host resource level could be more important in determining the outcome of co-infections.

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References

- Andersson, M., Scherman, K., Råberg, L., 2013. Multiple-strain infections of *Borrelia afzelii*: a role for within-host interactions in the maintenance of antigenic diversity? *Am Nat* 181, 545-554.
- Bayne, C.J., Grevelding, C.G., 2003. Cloning of *Schistosoma mansoni* sporocysts in vitro and detection of genetic heterogeneity among individuals within clones. *J Parasitol* 89, 1056-1060.
- Bedhomme, S., Agnew, P., Sidobre, C., Michalakakis, Y., 2004. Virulence reaction norms across a food gradient. *Proc R Soc B* 271, 739-744.
- Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecol Monogr* 27, 326-349.
- Bremermann, H.J., Pickering, J., 1983. A game-theoretical model of parasite virulence. *J Theor Biol* 100, 411-426.
- Buddle, C.M., Beguin, J., Bolduc, E., Mercado, A., Sackett, T.E., Selby, R.D., Varady-Szabo, H., Zeran, R.M., 2005. The importance and use of taxon sampling curves for comparative biodiversity research with forest arthropod assemblages. *Can Entomol* 137, 120-127.
- Chao, L., Hanley, K.A., Burch, C.L., Dahlberg, C., Turner, P.E., 2000. Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. *Q Rev Biol* 75, 261-275.
- Chappell, L.H., Hardie, L.J., Secombes, C.J., 1994. Diplostomiasis: the disease and host-parasite interactions, in: Pike, A.W., Lewis, J.W. (Eds.), *Parasitic diseases of fish*. Samara Publishing, Dyfed, pp. 59-86.

- Choisy, M., de Roode, J.C., 2010. Mixed infections and the evolution of virulence: effects of resource competition, parasite plasticity, and impaired host immunity. *Am Nat* 175, E105-E118.
- Criscione, C.D., Blouin, M.S., 2004. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. *Evolution* 58, 198-202.
- de Roode, J.C., Pansini, R., Cheesman, S.J., Helinski, M.E.H., Huijben, S., Wargo, A.R., Bell, A.S., Chan, B.H.K., Walliker, D., Read, A.F., 2005. Virulence and competitive ability in genetically diverse malaria infections. *P Natl Acad Sci USA* 102, 7624-7628.
- Duncan, A.B., Agnew, P., Noel, V., Michalakakis, Y., 2015. The consequences of co-infections for parasite transmission in the mosquito *Aedes aegypti*. *J Anim Ecol* 84, 498-508.
- Ebert, D., Carius, H.J., Little, T., Decaestecker, E., 2004. The evolution of virulence when parasites cause host castration and gigantism. *Am Nat* 164, S19-S32.
- Ebert, D., Zschokke-Rohringer, C.D., Carius, H.J., 2000. Dose effects and density-dependent regulation of two microparasites of *Daphnia magna*. *Oecologia* 122, 200-209.
- Fellous, S., Koella, J.C., 2009. Infectious dose affects the outcome of the within-host competition between parasites. *Am Nat* 173, E177-E184.
- Fellous, S., Koella, J.C., 2010. Cost of co-infection controlled by infectious dose combinations and food availability. *Oecologia* 162, 935-940.
- Frank, S.A., 1996. Models of parasite virulence. *Q Rev Biol* 71, 37-78.
- Gotelli, N.J., Colwell, R.K., 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* 4, 379-391.
- Graham, A.L., 2008. Ecological rules governing helminth-microparasite coinfection. *P Natl Acad Sci USA* 105, 566-570.
- Hechinger, R.F., Wood, A.C., Kuris, A.M., 2011. Social organization in a flatworm: trematode parasites form soldier and reproductive castes. *Proc R Soc B* 278, 656-665.

- Holmes, J.C., Price, P.W., 1986. Communities of parasites, in: Kikkawa, J., Anderson, D.J. (Eds.), Community ecology: pattern and process. Blackwell, Melbourne, pp. 187–213.
- Johnson, P.T.J., Buller, I.D., 2011. Parasite competition hidden by correlated coinfection: using surveys and experiments to understand parasite interactions. *Ecology* 92, 535-541.
- Karvonen, A., Kirsi, S., Hudson, P.J., Valtonen, E.T., 2004. Patterns of cercarial production from *Diplostomum spathaceum*: terminal investment or bet hedging? *Parasitology* 129, 87-92.
- Karvonen, A., Rellstab, C., Louhi, K.R., Jokela, J., 2012. Synchronous attack is advantageous: mixed genotype infections lead to higher infection success in trematode parasites. *Proc R Soc B* 279, 171-176.
- Koella, J.C., Antia, R., 1995. Optimal pattern of replication and transmission for parasites with two stages in their life-cycle. *Theor Popul Biol* 47, 277-291.
- Leggett, H.C., Benmayor, R., Hodgson, D.J., Buckling, A., 2013. Experimental evolution of adaptive phenotypic plasticity in a parasite. *Curr Biol* 23, 139-142.
- Lello, J., Boag, B., Fenton, A., Stevenson, I.R., Hudson, P.J., 2004. Competition and mutualism among the gut helminths of a mammalian host. *Nature* 428, 840-844.
- Lim, H.K., Heyneman, D., 1972. Intramolluscan inter-trematode antagonism: a review of factors influencing the host-parasite system and its possible role in biological control. *Adv Parasitol* 10, 192-268.
- Louhi, K.-R., Karvonen, A., Rellstab, C., Jokela, J., 2010. Is the population genetic structure of complex life cycle parasites determined by the geographic range of the most motile host? *Infect Genet Evol* 10, 1271-1277.

- Louhi, K.R., Karvonen, A., Rellstab, C., Jokela, J., 2013b. Genotypic and phenotypic variation in transmission traits of a complex life cycle parasite. *Ecology and Evolution* 3, 2116-2127.
- Louhi, K.R., Karvonen, A., Rellstab, C., Louhi, R., Jokela, J., 2013a. Prevalence of infection as a predictor of multiple genotype infection frequency in parasites with multiple-host life cycle. *J Anim Ecol* 82, 191-200.
- Mideo, N., 2009. Parasite adaptations to within-host competition. *Trends Parasitol* 25, 261-268.
- Niewiadomska, K., 1986. Verification of the life-cycles of *Diplostomum spathaceum* (Rudolphi, 1819) and *D. pseudospathaceum* Niewiadomska, 1984 (Trematoda, Diplostomidae). *Syst Parasitol* 8, 23-31.
- Niewiadomska, K., Valtonen, E.T., Siddall, R., 1997. Cercariae from *Lymnaea stagnalis* in lake Kuuhankavesi (central Finland). *Acta Parasitol* 42, 132-137.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2015. *Vegan: Community Ecology Package*. R package version 2.3.0., <http://CRAN.R-project.org/package=vegan>.
- Pedersen, A.B., Fenton, A., 2007. Emphasizing the ecology in parasite community ecology. *Trends Ecol Evol* 22, 133-139.
- R Core Team, 2015. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rauch, G., Kalbe, M., Reusch, T.B.H., 2005. How a complex life cycle can improve a parasite's sex life. *J Evol Biol* 18, 1069-1075.
- Read, A.F., Taylor, L.H., 2001. The ecology of genetically diverse infections. *Science* 292, 1099-1102.

- Rellstab, C., Karvonen, A., Louhi, K.R., Jokela, J., 2013. Genotype-specific vs. cross-reactive host immunity against a macroparasite. *Plos One* 8, e78427.
- Reusch, T.B.H., Rauch, G., Kalbe, M., 2004. Polymorphic microsatellite loci for the trematode *Diplostomum pseudospathaceum*. *Mol Ecol Notes* 4, 577-579.
- Seppälä, O., Karvonen, A., Kuosa, M., Haataja, M., Jokela, J., 2013. Are sick individuals weak competitors? Competitive ability of snails parasitized by a gigantism-inducing trematode. *Plos One* 8, e79366.
- Seppälä, O., Karvonen, A., Rellstab, C., Louhi, K.R., Jokela, J., 2012. Reciprocal interaction matrix reveals complex genetic and dose-dependent specificity among coinfecting parasites. *Am Nat* 180, 306-315.
- Seppälä, O., Karvonen, A., Valtonen, E.T., Jokela, J., 2009. Interactions among co-infecting parasite species: a mechanism maintaining genetic variation in parasites? *Proc R Soc B* 276, 691-697.
- Seppälä, O., Liljeroos, K., Karvonen, A., Jokela, J., 2008. Host condition as a constraint for parasite reproduction. *Oikos* 117, 749-753.
- Staves, P.A., Knell, R.J., 2010. Virulence and competitiveness: testing the relationship during inter- and intraspecific mixed infections. *Evolution* 64, 2643-2652.
- Stearns, S.C., 1989. Trade-offs in life-history evolution. *Funct Ecol* 3, 259-268.
- Stearns, S.C., Koella, J.C., 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* 40, 893-913.
- Stoddart, J.A., 1983. A genotypic diversity measure. *J Hered* 74, 489-490.
- Takken, W., Smallegange, R.C., Vigneau, A.J., Johnston, V., Brown, M., Mordue-Luntz, A.J., Billingsley, P.F., 2013. Larval nutrition differentially affects adult fitness and *Plasmodium* development in the malaria vectors *Anopheles gambiae* and *Anopheles stephensi*. *Parasite Vector* 6.

- Vale, P.F., Choisy, M., Little, T.J., 2013. Host nutrition alters the variance in parasite transmission potential. *Biol Lett* 9, 20121145.
- Wargo, A.R., de Roode, J.C., Huijben, S., Drew, D.R., Read, A.F., 2007. Transmission stage investment of malaria parasites in response to in-host competition. *Proc R Soc B* 274, 2629-2638.

Figure legends

Fig. 1. Change in the number of released *Diplostomum pseudospathaceum* cercariae between the two sampling times (the number of released cercariae after food manipulation minus the number of released cercariae before food manipulation; mean $\pm$ SE) for *Lymnaea stagnalis* snails maintained in 'ad libitum food supply' ($N = 21$) and 'no food' ($N = 17$) treatments. Food manipulation started after the first sampling and lasted for ten days. At each sampling time, snails were allowed to shed cercariae for three hours.

Fig. 2. Change in (a) the number of multi-locus genotypes (mean $\pm$ SE), (b) genotypic diversity (Stoddart's index, G_0 ; mean $\pm$ SE) and (c) genotypic composition [Bray-Curtis dissimilarity index, B ; mean $\pm$ SE] in *Diplostomum pseudospathaceum* cercariae released from *Lymnaea stagnalis* snails maintained in 'ad libitum food supply' ($N = 21$) and 'no food' ($N = 17$) treatments between the sampling times. Food manipulation started after the first sampling and lasted for ten days. At each sampling time, snails were allowed to shed cercariae for three hours.

Fig. 1

Figure(s)
Fig. 2