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Partitioning average competition and extreme-genotype effects in genetically diverse infections

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Competition between parasite genotypes in genetically diverse infections is widespread. However, experimental evidence on how genetic diversity influences total parasite load is variable. Here we use an additive partition equation to quantify the negative effect of inter-genotypic competition on total parasite load in diverse infections. Our approach controls for extreme-genotype effects, a process that can potentially neutralise, or even reverse, the negative effect of competition on total parasite load. A single extreme-genotype can have a disproportionate effect on total parasite load if it causes the highest parasite load in its single-infection, while increasing its performance in diverse relative to single infections. We show that in theory such disproportionate effects of extreme-genotypes can lead to a higher total parasite load in diverse infections than expected, even if competition reduces individual parasite performance on average. Controlling for the extreme-genotype effect is only possible if the competition effect on total parasite load is measured appropriately as the average difference between the realised number of each parasite genotype in mixed infections and the expected number based on single infection parasite loads. We apply this approach to sticklebacks that were experimentally infected with different trematode genotypes. On average, genetically diverse infections had lower parasite loads than expected from single-infection results. For the first time we demonstrate that competition between co-infecting genotypes per se caused the parasite load reduction, while extreme-genotype effects were not significant. We thus suggest that to correctly quantify the effect of competition alone on total parasite load in genetically diverse infections, the extreme-genotype effect has to be controlled for.

Multi-genotype infections are common in natural host-parasite systems (Woolhouse et al. 2002). In such genetically diverse infections, competition between co-infecting parasite genotypes is widespread (Read and Taylor 2001). Competition, here defined as a reduction in abundance of interacting individuals (Begon et al. 1996), should lead to a reduced total parasite load in diverse infections compared to uniform infections when co-infecting genotypes suffer reciprocally from a fitness reduction. Competitive interactions may also cause a shift in resource allocation from within-host replication to transmission-stage production (Taylor et al. 1998). In contrast, diverse parasite infections may lead to an increased total parasite load (Read and Taylor 2001), for example when the host cannot recognise or respond effectively to several different parasite genotypes, a phenomenon referred here to as facilitation.

Empirical evidence of the influence of infection diversity on total parasite load is variable. Davies et al. (2002) found an increased total parasite load in diverse compared to uniform infections, while other studies were unable to find an effect of infection diversity (Wedekind and Ruetschi 2000, Paterson and Viney 2003, Kurtz and Hammerschmidt 2006). Here we propose a novel approach to quantify negative effects of competition, and conversely, any positive effect of facilitation on total parasite load in genetically diverse infections. The main advantage of this approach is that it allows us to control for extreme-genotype effects, an important but neglected process affecting total parasite load that can neutralise or even reverse the competition or facilitation effect on total parasite load. The extreme-genotype effect is analogous to effects of single extreme plant species in plant biodiversity experiments and in that context dubbed the 'sampling effect' (Tilman 1999). The extreme-genotype effect measures the disproportionate influence on total parasite load of genotypes with a non-average parasite-load in their single infections (Loreau and Hector 2001). Thus, to control for the extreme-genotype effect, the competition or facilitation effect on total parasite load must be measured as the average deviation of each single genotype abundance in the diverse infection from its respective single-infection abundance.

For simplicity, we first discuss the case where an extreme genotype has the highest parasite load in single infections. Here, a 25% increase or decrease in abundance in diverse infections relative to its single-infection leads to a stronger change in total parasite load then a 25% change of a

genotype causing an average load in its single-infection. We dub this disproportionate effect that is not covered by the average competition or facilitation effect as 'extreme-genotype effect' (see Fig. A1a and A1b in Appendix 1 for a graphical illustration of the difference between the average competition and the extreme-genotype effect). Note that a performance increase of a single extreme-genotype can have such disproportionate effects that total parasite load in diverse infections is increased, although on average, competition among genotypes reduces relative genotype performance (Fig. A2e in Appendix 1). Conversely, a frequency decrease of an extreme-genotype can reduce total parasite load, although the relative genotype performance of most parasite genotypes is not reduced (Fig. A2d). Likewise, facilitation effects can be overridden by the extreme-genotype effect (not demonstrated here in further detail). Thus, in order to quantify the average effect of competition or facilitation alone on total parasite load in genetically diverse infections, the extreme-genotype effect must be quantified and controlled for.

Genotypes with a very low parasite load in their single-infections (yellow genotype in Fig. A1) can also contribute to the extreme-genotype effect, but in the opposite way. If numbers of low-fitness genotypes increase or decrease in diverse infections relative to their single-infection, the competition or facilitation effect would now predict a larger change in total parasite load than what is actually observed. Our approach corrects for this too large competition or facilitation effect.

The illustrative examples used so far assumed for simplicity the parasite load in the single-infection of some genotypes to be equal to the average single-infection parasite load (the grey genotypes in Fig. A1). Sampled genotypes are unlikely to be equal to the average parasite load. Thus, all genotypes contribute to the extreme-genotype effect. Importantly, the greater the deviation of a genotype's single-infection parasite load from the average single-infection parasite load is, the more strongly it influences the extreme-genotype effect.

An analysis of extreme-genotype effects can reveal hierarchy reinforcement, where genotypes with a high parasite load in single-infections have even higher numbers in diverse infections than expected. Such reinforcement results in a positive extreme-genotype effect. If the hierarchy changes, on the other hand, genotypes with a high parasite load in single-infections now perform poorly and genotypes with a low parasite load in single-infections perform best in diverse infections. Such a hierarchy change would produce a negative extreme-genotype effect.

We used the additive partition equation developed by Loreau and Hector (2001) to separate and quantify average competition or facilitation and extreme-genotype effects on total parasite load in diverse infections. The additive partition equation was originally developed for plant biodiversity experiments in order to distinguish 'true' diversity effects from effects of single extreme plant species, also dubbed 'sampling effect' (Tilman 1999). In experimental infections of three-spined sticklebacks *Gasterosteus aculeatus* with either one, three or five genotype(s) of a trematode parasite *Diplostomum pseudospathaceum*, the relative success of each genotype in the diverse infection was assessed at the end of the exposure by identification with microsatellite markers. Genotype-specific abundance measurements are a prerequisite to separate the contribution of pure competition or facilitation and possible additional extreme-genotype effects using the additive partition equation introduced above. In this study, we quantified and controlled for the extreme-genotype effect, in order to correctly quantify the effect of competition or facilitation alone on total parasite load in genetically diverse infections

Methods

Study species

In the complex life cycle of the trematode parasite *Diplostomum pseudospathaceum*, sexual reproduction takes place in the gut of fish-eating birds. Parasite eggs are shed into the water and hatched larvae infect water snails, where asexual reproduction occurs. Snails release free-swimming larvae, which infect fish, such as the three-spined stickleback *Gasterosteus aculeatus*. The parasite penetrates the skin of the fish and migrates within 24 h to the eye lens. No reproduction occurs within the fish and the life cycle is completed when an infected fish is predated by a bird. For fish, infection severely reduces fitness via increased predation risk (Crowden and Broom 1980). Genetically diverse infections are common in natural populations of three-spined sticklebacks (Rauch et al. 2005). In that study, all sampled fish had diverse infections with up to 67 different genotypes found in a single fish and 98% of the genotypes were represented by a single individual, that is, most genotypes have low prevalence.

We established five clonal lines of the parasite *D. pseudospathaceum* by infecting individual water snails *Lymnaea stagnalis* with single parasite larvae (as described in Rauch et al. 2006a). A successfully established parasite reproduces asexually within the snail, so that all parasites released from one snail are of a single genotype. Also, we established five unrelated fish families from crosses between wild caught fish originating from the same lake system in northern Germany as the parasites and snails (for details on crossing procedure and rearing conditions see Rauch et al. 2006b).

Infection design

We applied three diversity treatments with infections comprised of one, three or five genotypes (Fig. A3). All infections were drawn from the same five-genotype pool. Fish were exposed to a constant number of parasite individuals (150) across treatments. In the single- genotype infections, each fish was exposed to 150 parasites of one genotype, in the three-genotype infection to 50 parasites of each included genotype and in the five-genotype infection to 30 parasites of each genotype (Fig. A3). All five genotypes were tested separately in single-genotype infections. For the three-genotype diversity treatment, we tested five different three-genotype combinations. We randomly chose three genotypes from the five-genotype pool. As soon as one genotype was already present in three combinations, it could not be chosen anymore, ensuring equal representation of genotypes. In the five-genotype treatment, we mixed

all five genotypes together. We exposed five fish from each of five families to each of these 11 infection applications (except for one fish family, where only four fish were used in the diverse infections and one family, where only four fish were used in two of the three-genotype infections due to small offspring number in that family) resulting in a total of 265 infected fish. For a discussion of specific fish family by parasite genotype interactions in the single-genotype infections see Rauch et al. (2006a).

Experimental infection

We placed the fish singly in 1-l aquaria. To each aquarium, we added 150 parasites into the water no later than 12 h after the parasites had emerged from the snails. The 12 h limit was chosen as Karvonen et al. (2003) showed that infectivity of *D. spathaceum* parasites only started to decline when the parasites had emerged from the snail more than 12 h ago. We haphazardly distributed the aquaria on tables in a climatic chamber (18°C, 16 h light). We allowed the parasites to grow for one week. Whyte et al. (1991) showed that there was no increase in number of parasites in the eye-lens from 24 h after infection to 48 h and 72 h. This suggests that no parasites would newly enter the eye-lens one week after the infection. We then killed the fish with methane sulphonate (MS 222), dissected and isolated the eye lens and counted the parasites within the undamaged eye lens. Parasites not found in the eye lens died either before infecting the fish, on the way to the eye lens or once within the eye lens. Competition between parasite individuals of different genotypes can reduce numbers both before the parasites penetrate the fish (either in the open water or on the fish skin) and within the fish. In a previous study (Rauch et al. 2006a), it was not found that numbers of parasites significantly declined from the first to the ninth week after infection. This indicates that once the parasites are established, parasite mortality is not severe anymore.

To count the numbers for each genotype in the diverse infections, we determined the genotype identity of each parasite from all diverse infections. To differentiate between the genotypes, we used a polymorphic microsatellite marker developed for *D. pseudospathaceum* (Diplo09, GenBank accession no. AJ629252, Reusch et al. 2004). DNA extraction and PCR conditions followed methods in Reusch et al. (2004). We genotyped parasites with an ABI 3100 automated sequencer.

For each fish infected with a genotype-mixture, we scored ΔP (deviation from the expected parasite load), the competition or facilitation effect and extreme-genotype effect by comparison with the single-genotype infections from the same family.

The ability to establish within the eye lens that are independent of other genotypes, including survival outside and inside the host and/or genotype-specific penetration capability, differs between genotypes, leading to significant differences between genotypes in their single-infection parasite loads (Rauch et al. 2006a). By using the single-infection parasite-load of each of the included genotypes to calculate the expected parasite load in the diverse infections, we can correct for the genotype-specific infection abilities that are independent of the co-infecting genotypes.

Separating competition from extreme-genotype effects

To separate competition from extreme-genotype effects we used the additive partition equation developed by Loreau and Hector (2001). In a host-parasite system, in each diverse infection the deviation of the observed parasite load in the diverse infections P_O from the expected parasite load P_E is calculated as

$$\Delta P = P_O - P_E \tag{1}$$

P_E is the sum of all single-infection averages divided by the number of genotypes used.
Then

$$\Delta P = \sum_{i=1}^{N} P_{O,i} - P_{E,i} \tag{2}$$

with $P_{O,i}$ as the observed number of individuals of genotype i in the diverse infection and $P_{E,i}$ as the expected number of genotype i. N is the number of genotypes used in the diverse infection. It follows that

$$\Delta P = \sum_{i=1}^{N} RP_{O,i}\ S_i - RP_{E,i}\ S_i \tag{3}$$

$RP_{O,i}$ is the proportion of genotype i actually observed in the diverse infection relative to its single-infection abundance S_i, where $RP_{O,i} = P_{O,i}/S_i$. $RP_{E,i}$ is the expected proportion, or, more simply, the numbers of genotype i used to infect a host in the diverse infection divided by the numbers used in the single-infection. Then

$$\Delta P = \sum_{i=1}^{N} \Delta RP_i\ S_i \tag{4}$$

ΔRP_i is the deviation of the observed proportion from the expected proportion ($\Delta RP_i = RP_{O,i} - RP_{E,i}$). Using standard definitions from statistics for covariance (cov), ΔP is separated into

$$\Delta P = N\overline{\Delta RP}\,\overline{S} + N\ \mathrm{cov}(\Delta RP, S) \tag{5}$$

$N\overline{\Delta RP}\,\overline{S}$ quantifies the effect of competition or facilitation on total parasite load (illustrated graphically in Fig. A2d). A negative value of $N\overline{\Delta RP}\,\overline{S}$ indicates a competition effect, while a positive value indicates a facilitation effect. $N\overline{\Delta RP}$ is the average percent deviation compared to the expected abundance (in Fig. A2d four genotypes show a 25% reduction compared to their expected value, which results in an average reduction over all five genotypes of 20%). The product of $N\overline{\Delta RP}$ and $\bar{S}$ gives the actual deviation, in number of parasite individuals, caused by the competition or facilitation effect. N cov (ΔRP,S) measures the over-proportional effect of extreme-genotypes expressed in numbers of parasite individuals, which is not covered by the average competition effect (Eq. 5, illustrated graphically in Fig. A2d). It measures the covariance between the genotype specific percentage change from the expected abundance and the abundance in single-infections as

$$N\,\mathrm{cov}(\Delta RP, S) = N \sum_{i=1}^{N} \frac{(\Delta RP_i - \overline{\Delta RP})(S_i - \bar{S})}{N - 1} \qquad (6)$$

A change in frequency of a genotype performing average in single-infections does not contribute to the extreme-genotype effect, as in this case, the covariance term $(S_i - \bar{S})$ equals zero. The covariance (and so the extreme-genotype effect) decreases if extreme-genotypes perform worse in the diverse infection than expected (Fig. A2d). The covariance increases if extreme-genotypes perform better than expected in the diverse infection (Fig. A2e).

Statistical analysis

We tested for an influence of the three diversity treatments on total parasite number with a Welch ANOVA. This was necessary because none of the data transformations (log, square root, Box-Cox) achieved variance homogeneity (Levene test). To control for fish family effects, we used the residuals obtained from a Welch ANOVA with the factor 'fish family'.

We tested in three separate analyses whether ΔP, competition or facilitation effect and extreme-genotype effect were significantly different from zero using two-way ANOVA. 'Genotypic diversity' treatment (three- or five-genotype mixtures) and 'fish family' were included as factors. The deviation of the intercept from zero is the overall deviation of the effect tested (averaged over all factor levels). ΔP was log transformed and extreme-genotype effect Box-Cox transformed, to achieve equality of variances and normal distribution.

To test for rank hierarchy changes of genotypes across single and diverse infections, genotypes were ranked according to their numbers within each fish with a diverse infection. Each within fish ranking was compared to the genotype ranking according to the average parasite loads in single-infections at the end of the experiment. We counted the cases where the two rankings were the same, where the two rankings were exactly reversed, where the two rankings changed in two positions (excluding the completely reversed case) and where the two rankings changed in more than two positions. All instances where two or more genotypes had equivalent success were excluded for this analysis. The frequency of these four categories was then compared with the expected frequencies using a chi-square test. With three genotypes, there are six possible rank permutations and the expected frequency ratio of the four categories (no change: reversed ranking: two changes without the reversed ranking: three changes) is 1:1:2:2. In a pair-wise multiple test, the frequencies of the four cases were tested against each other and the p-value was set after Bonferroni correction for multiple testing to 0.0083. With five genotypes, there are 120 possible permutations. As there were only 24 fish infected with 5-genotypes, the high number of permutations leads to extremely low expected frequencies (24/120) for the category no change or reversed ranking. This would lead to expected frequencies far below the commonly applied rule that no expected frequency should be less than 5 and so no test for the five-genotype infections is recommended.

Results

Total parasite number

Genotypic diversity of the infection significantly influenced total parasite number per fish (Welch ANOVA: $F_{2,92} = 5.33$; $p = 0.0065$). Parasite numbers were significantly higher in single-genotype infections compared to five-genotype infections, with the three-genotype infections having an intermediate parasite load (Fig. 1, multiple comparison with pair-wise Welch ANOVA and Bonferroni corrected). The variance was significantly different in the three treatments (Levene test: $F_{2,244} = 12.78$; $p < 0.0001$). The single-genotype treatment had a higher variance than the two infection diversity treatments (multiple comparison with pair-wise Levene tests and Bonferroni correction).

Competition and extreme-genotype effects

ΔP was significantly less than zero for the three and five-genotype treatments, demonstrating that the parasite load in the diverse infections was lower than expected from the single-infections (ΔP: t-test that intercept of the two-way ANOVA with factors 'diversity' and 'fish family' is different from zero: Fig. 2, Table 1). The competition effect caused 71% of the total reduction (solid arrow Fig. 2) and significantly reduced the parasite load in the diverse infections (Fig. 2, Table 1). Competition reduced the total parasite load in diverse infections by 14.4% compared to single-infections. In contrast, the extreme-genotype effect explained only 29% of the total reduction (dashed arrow Fig. 2), which did not on its own reduce significantly the parasite load in the diverse infections (Fig. 2, Table 1). There were no significant differences between the three-genotype or five-genotype infections for all three effects (Table 1). The factor 'fish family', was only significant for the extreme-genotype effect (Table 1). None of the 'diversity' by 'fish family' interactions were significant.

The average reduction in diverse infections is illustrated graphically in Fig. 3. For all five genotypes, the relative number (final/initial = numbers of a genotype found at the end of the experiment divided by the numbers used for infection) decreased in the predicted order from the single

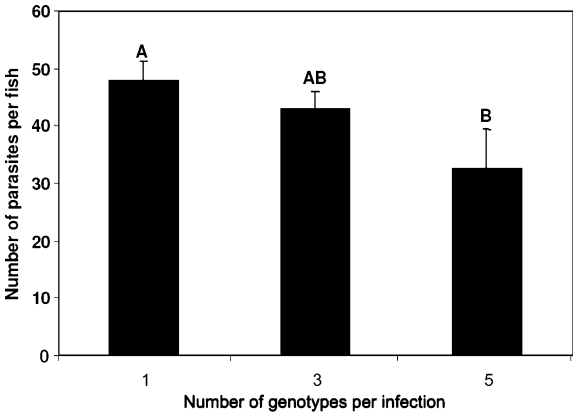

Fig. 1. Effect of genotypic diversity on parasite load. Bars show mean numbers of parasites per fish for each diversity treatment (±SE). Different letters indicate significant differences.

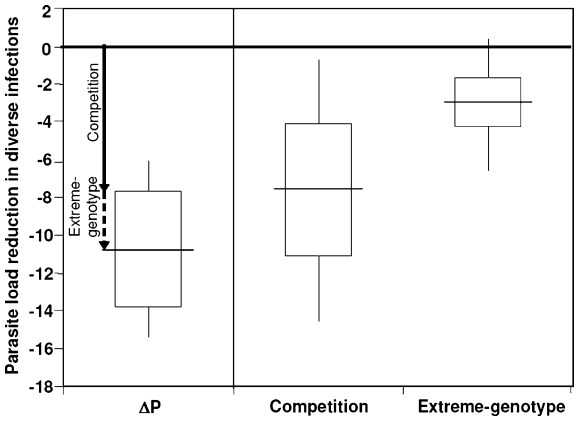

Fig. 2. Reduction of parasite load in genetically diverse infections. The left panel shows total reduction (ΔP) in diverse infections compared to single-infections. Boxes show mean for the pooled three and five-genotype treatments, with upper and lower box boundaries representing the standard error. Vertical lines show the 95% confidence interval. Effects with a vertical line crossing the zero-line are not different from zero. The solid arrow shows the reduction caused by the competition effect, the dashed arrow the reduction by the extreme-genotype effect. The right panel shows that competition significantly reduces total parasite load (95% CI not crossing the zero-line), but not the extreme-genotype effect (95% CI crossing the zero-line).

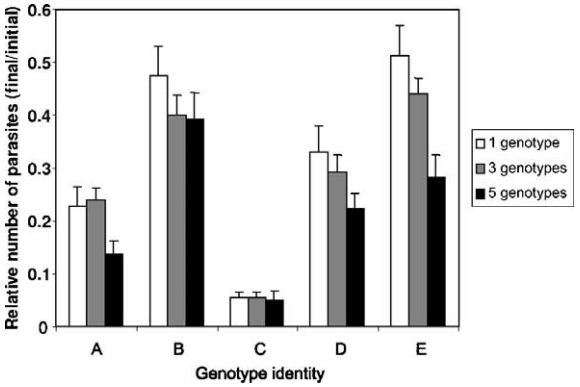

Fig. 3. Relative number of parasites per genotype as a function of the diversity treatment. Bars show mean relative number of parasites (final/initial) for each genotype in the three diversity treatments (±SE).

to the three-genotype to the five-genotype infections except for two cases, where the three-genotype infection is slightly higher than the single-genotype infection.

Rank hierarchy in single and diverse infections

Comparing the genotype rank hierarchy within each single fish in the diverse infection with the single-infection ranking (the single-infection ranking is always based on average parasite loads), in 56% of the three-genotype infections both hierarchies were identical. In only 2% of the three-genotype infections the ranking was completely reversed. In 37% of the infections two positions were changed (excluding the completely reversed ranking) and in 5% of the infections three positions were changed. The frequency of these four categories of ranking comparisons significantly deviated from the expected frequency ($X^2 = 122.14$; $DF = 3$; $p < 0.0001$). Multiple comparisons showed that the frequency of cases with no changes was significantly higher than expected compared to each of the other three categories of ranking comparisons. Moreover, cases with two changes had higher frequencies than expected compared to cases with three changes and to the reversed cases (statistical details of the multiple comparisons not shown). In the five-genotype infections, in 25% of the infections the ranking was the same as in the single-infections, there were no infections with a reversed ranking, in 50% of the infections two positions were changed and in 25% of the cases more than two positions were changed. No statistical test was performed for the five-genotype infections because expected frequencies were too low.

Discussion

We demonstrate for the first time that competition between co-infecting genotypes alone caused a reduction in parasite load in genetically diverse infections. We could do so because we controlled for any extreme-genotype effects, which we found to be not significant in our trematode-stickleback system. To separate and to quantify competition and extreme-genotype effects, we applied an additive partition equation that was originally developed to partition average from extreme species effects in plant biodiversity experiments (Loreau and Hector 2001).

Three possible mechanisms could account for the identified competition effect. First, direct competition for entry sites into the eye lens can occur with aid of chemical substances that harm only co-infecting genotypes but not the producing genotype itself. Such direct competition

Table 1. Results of two-way ANOVAs (factors 'genotypic diversity' and 'fish family') testing for significant deviation from zero (intercept) of ΔP, and for competition and extreme-genotype effects. The deviation of the intercept from zero (t-test) is the overall deviation of the effect tested (averaged over the factors 'genotypic diversity' (three- or five-genotype mixtures) and 'fish family'.

Effect	Factor	DF	t or F	p
ΔP	intercept	$t_{(130)}$	−4.58	<0.0001
	3- or 5-genotype infection	$F_{(1,130)}$	2.05	0.1549
	fish-family	$F_{(4,130)}$	0.77	0.5490
Competition	intercept	$t_{(130)}$	−2.18	0.0313
	3- or 5-genotype infection	$F_{(1,130)}$	2.91	0.0904
	fish-family	$F_{(4,130)}$	0.38	0.8225
Extreme-genotype	intercept	$t_{(130)}$	−1.78	0.0775
	3- or 5-genotype infection	$F_{(1,130)}$	0.18	0.6712
	fish-family	$F_{(4,130)}$	3.10	0.0179

between parasites of different genotypes can occur both before the fish skin is penetrated as well as within the host. Second, parasites may use the host immune system to harm competitors by releasing antigens similar to those of a co-infecting genotype (Brown and Grenfell 2001). Third, competition can be indirect. Despite of the genotype specific immune defense, some degree of cross-reactivity could be possible. Cross-reactivity is the ability of the same immune defense molecule to react against several genetically different parasites (Kurtz 2005). A specific defense directed against one genotype may also have an effect against a different genotype, albeit much smaller.

Hierarchy reinforcement or reversal between single and diverse infections is revealed by the extreme-genotype effect. In this study, we found no evidence for a genotype's performance to be reinforced. Thus, genotype performance in diverse infections was best predicted by the performance in single-infections. On the other hand, the extreme-genotype effect tended to be negative in our study, which may indicate a moderate level of hierarchy reversal, which was however not substantiated in the rank analysis.

Note that our parasite-host system differs from plant biodiversity experiments in a number of ways. In the latter experiments, plant biomass was often used to measure fitness of the species or genotypes (Reusch et al. 2005, Roscher et al. 2005). The biomass can increase simply by plant growth during the experiment. In our experiment, we replaced biomass with number of surviving parasites per genotype as a measure of fitness. Once in the eye lens, the parasite life stage studied here cannot reproduce. In our study, an extreme-genotype effect arises when extreme-genotypes decrease or increase in their relative proportion of established parasites. In many other host-parasite systems, an increase in numbers is possible, as replication occurs within the host (see for an example the rodent-malaria system in Davies et al. 2002). Although in principle an extreme-genotype effect can be detected in the absence of within-host replication, it is possible that the extreme-genotype effect is more pronounced in parasite species that replicate within their host, simply because the best genotype in single-infections can overgrow other genotypes in the diverse infection. This positive extreme-genotype effect could lead to an increased total parasite load although on average competition reduces relative genotype performance. Further studies that measure the frequency of individual genotypes in diverse infections in parasite species that reproduce within the host are clearly needed to determine the general importance of extreme-genotype effects on parasite load.

In a natural situation, older fish have a higher probability of already being infected when a new parasite attacks (Rauch et al. 2005). This has two important consequences. First, the acquired immune system may already be activated. Kalbe and Kurtz (2006) showed that three-spined sticklebacks already exposed twice to *D. pseudospathaceum* had a lower number of newly established parasites compared to fish without any previous contact to the parasite. The acquired, MHC based immune system played no role in our experiment, as it takes several days to fully activate the adaptive immune system, while the parasites reach the immunologically protected eye lens within 24 h (Rauch et al. 2006a). Second, as most genotypes have a very low prevalence in older fish (Rauch et al. 2005), most parasites will encounter a diverse infection. The situation is different for young, uninfected fish, the situation simulated by our experiment Young, uninfected fish do not possess an adaptive immune system specifically activated for *D. pseudospathaceum*. Also, a single or diverse infection of uninfected fish as tested in our experiment is possible in nature. Most *D. pseudospathaceum* individuals infective to three-spined sticklebacks released from one snail belong to one genotype and different snails release different genotypes (Rauch et al. 2005). Depending on whether a young, uninfected fish encounters a single snail or a group of snails, single or diverse genotype exposure occurs. At our study site, fish only rarely survive a second winter (personal observation). Thus a large part of the population is replaced with young, uninfected fish each year, making our experimental conditions comparable to natural conditions.

An important question in experiments studying the effect of competition or facilitation in diverse and single-infections is the role of infection dose (Gower and Webster 2005). By keeping the total infection dose constant in diverse and single-infections, as in this study, infection numbers of each genotype differ in the diverse and single-infection. Thus, the two treatments differ in two ways, the number of genotypes and the number of individuals used for each genotype. We were fully aware that increasing the number of individuals used for one genotype to infect the host can potentially increase or decrease its relative infection success (established parasites divided with numbers used for infection). However, a study by Whyte et al. (1991) on rainbow trout infected with *D. pseudospathaceum* showed that increasing the number of parasites used for infection from 200 to 500 did not alter the relative infection success. The alternative approach, namely to use equal numbers in the single and diverse infections for each genotype, is problematic because the total infection dose is confounded with diversity (Gower and Webster 2005). For example, exposure to increasing numbers of *D. spathaceum* parasites increased the total parasite load in rainbow trout (Whyte et al. 1991). As we were mainly interested in the consequences of diversity and competition on total parasite load, we decided to keep the total infection dose constant in diverse and single-infections.

The differentiation between average competition or facilitation and extreme-genotype effect is central to understanding and manipulating genetically diverse infections. For example, the effectiveness of drug therapy in combating parasite infection depends critically on the type of mechanism. When competition dominates, the elimination of one genotype reduces total parasite number initially in a multiple infected host. However, as numbers of the remaining genotypes increase due to the reduced competition effect, total parasite load can actually exceed numbers in untreated hosts. In contrast, in the case of a strong extreme-genotype effect, drugs targeting genotypes causing high parasite loads in their single-infections lead to the desired outcome, a drastic reduction of the total parasite load.

In summary, we demonstrated a reduced parasite load in genetically diverse infections. For the first time we show that reduced parasite load was caused only by competition among co-infecting genotypes and not by an extreme-genotype effect. As total parasite load critically depends on

the underlying mechanisms, we recommend to take extreme-genotype effects into account, along with competition and facilitation when studying and predicting the outcome of genetically diverse infections.

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Appendix 1 is available online as Appendix O16301 at www.oikos.ekol.lu.se/Appendix

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