Title: Experimental evolution of parasitic host manipulation

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**Abstract**

Host manipulation is a parasite-induced alteration of a host’s phenotype that increases parasite fitness. However, if genetically encoded in the parasite, it should be under selection in the parasite. Such host manipulation has often been assumed to be energetically costly, which should restrict its evolution. Evidence of such costs, however, remains elusive. The trophically-transmitted cestode *Schistocephalus solidus* manipulates the activity of its first intermediate copepod host to reduce its predation susceptibility before the parasite is ready for transmission. Thereafter, *S. solidus* increases host activity to facilitate transmission to its subsequent fish host. I selected *S. solidus* for or against host manipulation over three generations to investigate the evolvability of manipulation and identify potential trade-offs. Host manipulation responded to selection confirming that this trait is heritable in the parasite and hence can present an extended phenotype. Changes in host manipulation were not restrained by any obvious costs.

**Key words:** Host manipulation, *Schistocephalus solidus*, energetic costs, extended phenotype, response to selection, experimental selection
Background

The phenotype, including the behaviour, of an infected and an uninfected individual often differs. Such alterations that are caused by an infection can benefit the host, the parasite, both or neither. Trophically transmitted parasites for example can reduce or even reverse their intermediate host’s innate fear of (certain) predators to facilitate transmission [1–4]. Ensuing benefits to the parasite can be accidental due to host responses or side-effects, but the parasite might engage in true host manipulation, that is alter its host phenotype, such as its behaviour, to increase its own fitness. Central to this definition is that the trait in question is controlled by the parasite to some degree; it is an extended phenotype, i.e. a trait that is genetically encoded in one organism, the parasite, but whose phenotype is expressed elsewhere, the host [5,6]. In practise, determining whether any trait expressed by an infected host which seems to benefit the parasite, is indeed controlled by the parasite or a side-effect with accidental benefits to the parasite, can be challenging [3,7–12]. Host manipulation that is encoded by the parasite’s genes should be under selection acting on the parasite rather than the host. This is usually assumed to bear a cost for the parasite, which could either be physiological, i.e. a cost due to the very process of manipulation which might be energetically costly for the parasite [3,10,13–15] or ecological e.g. through increasing mortality through dead-end predation [16–19]. Clear evidence for energetic costs has been elusive and is restricted to correlational evidence of potential trade-offs with other traits [20–23], which might not always be related to costs [21,24].

To better understand how readily host manipulation might evolve, I conducted an experimental selection experiment using the cestode Schistocephalus solidus and its copepod host Macrocyclops albidus. Schistocephalus solidus is known to manipulate its first intermediate copepod host to modify its predation susceptibility according to its need. Before the parasite becomes infective to its subsequent fish host, it reduces host activity and predation susceptibility [21,25–29]. Once the parasite is infective, host manipulation switches to increase host activity and hence enhance predation to facilitate transmission to the next host [25,30,31], albeit this seems to differ between different parasite populations [27,29]. I selected the parasite either for high or low levels of host manipulation and measured various other fitness-related parasite traits to assess potential trade-offs. Selection indeed resulted in altered host manipulation throughout the parasite’s development.

Methods

Hosts

Copepods (Macrocyclops albidus) stemmed from a lab culture that originated from the Neustädter Binnen See, northern Germany (54°06'49.6"N 10°48'28.0"E). On the day prior to infection, copepods were distributed to individual wells of 24-well cell culture plates with about 1 mL of water each. I
used new copepods from the same lab culture for each generation. Only adult male copepods were used to reduce variability with regards to the host. I checked for dead copepods, cleaned wells if necessary and fed copepods every second to third day with 5 Artemia sp. naupili each.

Parasites and infection

Schistocephalus solidus originated from the same population as the copepods (Neustädter Binnen See, northern Germany). Parasites for the initial generation (F0) came from 9 different parasite families, parts of them half sibs that had been bred in an in vitro system in the lab [32]. Their parents originated from a total of 7 different parasite families bred from parasites dissected from fish caught in 2013 and 2014 (see Fig. S1 for a pedigree of parasite families). In subsequent generations, I used eggs from selected parasites to infect the next generation (see below). After breeding, parasite eggs were stored in the fridge (4°C) [33]. Prior to use, they were incubated for 3 weeks and exposed to light overnight to induce hatching. One hatched coracidium was given to each copepod for infection.

Behavioural recordings and trait measurements

On day 6, 7, and 8 (when parasites are not yet infective and suppress predation) and on day 13, 14, and 15 (when parasites are infective and enhance predation) post infection, I recorded copepod behaviour by placing a plate with copepods on an apparatus that dropped it by about 3mm to simulate a predator attack [21,25–27,34]. This drop took place once the plate had been on the apparatus for one minute. Video recordings started before the drop, lasted 15 minutes, and were made with a HD-camera (MHD-13MG6SH-D, Mintron, Taiwan). From these recordings, I extracted one image every two second for 90 seconds in ImageJ [35], starting 10 seconds after the simulated predator attack to exclude the initial reaction. I only used this first 90 seconds of the recording after the drop since this should comprise the time when copepods should expect a predator to be present and during which host manipulation would be most crucial. These images were analysed with a custom-made python program (available at: https://github.com/ferhah/copepodtracking) to automatically record copepod position [29]. To exclude random noise, I only considered a copepod moving if it moved by at least 5 pixels (about one copepod length) from one frame to the next. To obtain copepod activity, I calculated the proportion of time each copepod spent moving (i.e. the number of times a copepod moved divided by the number of images) during the 90 seconds following the simulated predator attack for each trial. Then, I computed the average activity of each copepod over the trials before (6-8 days post infection) and after (13-15 days post infection) parasites reached infectivity.

To identify potential trade-offs that could hint at an energetic cost to host manipulation, I recorded a number of fitness-relevant parasite traits. On day 8 post infection, I checked copepods for parasite infection and, if infected, the presence or absence of a cercomer (Fig. S2), which is an indicator of parasite development [27,36,37]. On day 15 post infection, I took photos of copepods under a microscope to measure parasite size (Fig. S2). To do so, I outlined each parasite’s shape without the
cercomer and measured the area within this shape [38] in imageJ [35]. All measurements were conducted blindly with regards to selection line.

**Selection and parasite breeding**

I selected only the parasites for host manipulation without inducing any selection on the copepod host. In the initial generation (F0), I exposed 597 copepods, 148 of which became infected. With these infected copepods, three parasite selection lines were created (Numbers in brackets represent sample sizes of exposed/infected copepods in each generation and treatment): high line (selection for host manipulation, F1: 580/170, F2: 480/153, F3: 260/95), low line (selection against host manipulation, F1: 647/156, F2: 480/145, F3: 260/107) and control (no selection on host manipulation, F1: 213/61, F2: 160/54, F3: 223/70). Additionally, 27 (F0), 48 (F1), 32 (F2), and 49 (F3) copepods were not exposed and randomly distributed over all plates to measure the behaviour of uninfected copepods.

Not-yet-infective *S. solidus* reduce host activity to suppress predation while infective ones increase it to enhance predation. Hence, to obtain a single measure of host manipulation irrespective of parasite development, I calculated the magnitude of the change from predation suppression before the parasite became infective to predation enhancement after the parasite became infective. More precisely, I subtracted the mean host activity before the parasite became infective and suppressed predation (6-8 days post infection) from the mean host activity after the parasite reached infectivity and enhanced predation (13-15 days post infection). This allowed me to select for predation suppression and predation enhancement simultaneously, since ultimately both contribute to host manipulation. Additionally, this ensured that I did not simply select for parasites that increased or decreased host activity irrespective of their life stage.

All parasites whose hosts survived until 15 days post infection were included in the selection pool. To create the F1 generation, initially one quarter of parasites was randomly selected to serve as controls (i.e. no selection on host manipulation). From the remaining parasites the third that exhibited the strongest host manipulation was selected as the high selection line, while the third that showed the weakest host manipulation as the low selection line (Fig. 1). Any remaining parasites (and their copepod hosts) were discarded. Selected parasites from each selection line were randomly assigned to four artificial replicate populations with parasites from each selection line in each replicate. In each subsequent generation, selection and mating took place within these replicates within each line to obtain four replicates for each selection line. While this might have increased inbreeding, it allowed me to control for responses that could have occurred in single lines due to chance which otherwise would have been impossible to distinguish from any actual effects to selection. To set up F2 and F3, I used all parasites that successfully infected copepods in the control lines, the third of parasites who showed the highest host manipulation in the high selection lines, and the third of parasites which exerted the lowest host manipulation from the low selection lines. If too many copepods were
available for any particular selection line and replicate after selection, I randomly selected a subset
(controls) or took the ones with the highest (high) or lowest (low) host manipulation.

In each generation copepods that had been selected were fed to the subsequent host, three-spined
sticklebacks (*Gasterosteus aculeatus*), to allow them to grow to maturity. This took place 17 days post
infection when all parasites should be able to infect sticklebacks. I used lab-breeding sticklebacks whose
parents had been caught in the Große Plöner See, northern Germany (54°08'09.1"N 10°24'58.9"E). I
randomly used fish from 4 (F0) or 7 (F1 and F2) different fish families. Two days prior to exposure,
fish were spine clipped (first or second spine) for later identification (see below) and distributed to
small individual 1 L tanks. Each fish was then individually exposed to one infected copepod. Two
days later, fish were returned to 16 L tanks with 9-15 fish per tank. Fish that received copepods from
the same selection line and replicate shared tanks. In the F2-generation, I additionally infected fish
from the same families with copepods from the high and low selection lines that had not been
selected. These fish were used only to measure infection rates in fish, but not to obtain the next
generation of parasites. Animal experiments were conducted with permission of the 'Ministry of
Energy, Agriculture, the Environment and Rural Areas' of the state of Schleswig-Holstein, Germany
(reference number: V 313-72241.123-34).

Two to three months after exposure, fish were killed and dissected to obtain mature parasites. I
measured fish weight and length. Unless they were small, I also weighed the gonads to obtain fish
weight without the potentially large weight of the female gonads. Parasites were weighed and bred in
pairs with a size-matched individual from their own selection line and replicate using an in vitro
breeding system [32,39]. Fertility was measured by counting eggs using a Z2 Coulter particle counter
and size analyser (Beckman Coulter Inc., USA). Since parasites were bred in pairs, I was unable to
obtain individual measurements of egg output, but rather obtained one value for each parasite pair.

To identify fish and thereby determine identity of the copepod for each parasite, I took a tail clip
during dissection for subsequent DNA extraction. DNA from tail clips and the spine clips obtained
prior to exposure was extracted with the Qiagen DNeasy 96 Blood and Tissue Extraction Kit,
following the manufacturer’s protocol, and used to type fish for 9 microsatellites to identify fish [40].

**Statistical analysis**

All statistical analyses were conducted in R [41].

I analysed various response variables separately using different subsets of the data. To analyse
copepod behaviour (host manipulation (i.e. the difference in host activity between copepods with not-
yet infective and copepods with infective parasites)), and host activity (i.e. the raw host activity either
in copepods with not yet infective or with infective parasites), I excluded all copepods that had died or
been lost during the experiment or in which exposure had not resulted in an infection. To analyse
infection success in copepods, I used all copepods that had been exposed to a parasite and for which I knew their infection status (i.e. that had survived long enough to be checked for infection). For parasite development and size in copepods, I used all infected copepods for which I was able to obtain the relevant information. To analyse infection success and size in fish, I excluded fish that died within a few weeks after exposure since their parasites might have been too small to determine their infection status or which survived until dissection respectively. To analyse parasite fecundity, I used all parasite families bred during the experiment. Since parasites did not belong to any treatment in the initial generation prior to selection, they were randomly assigned to a selection line which did not necessarily correspond to the selection line for which they were selected in order to facilitate statistical analysis which required that all treatments were present in each generation.

To each parasite trait, I applied general (host manipulation, host activity, parasite size in copepods and fish, and parasite fertility in fish) or generalized linear mixed models with a binomial error family (infection success in copepods and fish and development) using the lme4 package [42]. Mixed models were used in order to account for replicate which was included as a random factor. Generation and selection line and their interaction were included as fixed factors. To analyse host manipulation and host activity, I additionally included infection status of the copepod and all its interactions with fixed factors that did not involve any interaction between infection and selection line for selection on parasites. The model for host activity included two additional factors, a fixed factor parasite stage (i.e. predation suppression by not-yet-infective parasites vs. predation enhancement by infective parasites) and all its interactions with the other fixed factors and a random factor copepod identity to account for the fact that each copepod was measured twice, i.e. before and after reaching infectivity. I choose to analyse host manipulation and activity in two separate models despite substantial overlap since these models serve to answer two different questions. The former served to test whether there were any changes in the actual trait under selection, host manipulation, the latter to test to what extend these changes affected host activity during predation suppression and predation enhancement and whether, as expected, they changed into different directions. To model parasite size in fish and parasite fecundity, I additionally accounted for time spent in fish. If, in the case of parasite fecundity, the two parasites in a pair had spent different amounts of time in their fish host, I used the mean time they had spent in the fish.

To obtain p-values for the combined effect of each fixed factor in each analysis, I compared each model to a less complicated model using likelihood ratio tests from the anova function from the R base package [41]. Additionally, I report the estimates for the best model in each case, i.e. the least complicated model that explained the data significantly better than any less complicated model (See Table 2, S2 and S5). To identify between which selection lines significant differences occurred if either selection line and/ or its interaction with any other factor was significant, I fitted separate general or generalized linear mixed models to each response variable within each generation and, for
host activity, parasite stage (not-yet-infective parasites, i.e. predation suppression vs. infective parasites, i.e. predation enhancement), using selection line and infection (host manipulation and host activity only) as fixed factor on which I could subsequently apply post hoc tests using Tukey corrections for multiple testing (glht, multcomp [43]) to compare between individual selection lines. To keep the results easier to read, only the most important details on the statistical outputs of the models are presented in Tables 1-2 and S1-S6.

I Calculated selection differential for each selection line and generation as the difference between the mean host manipulation within each replicate and the mean host manipulation of selected copepods. To estimate heritability of host manipulation by not-yet-infective parasites (suppressing predation), by infective parasites (enhancing predation), and overall, I combined data from all three generations and calculated mid-parent and mean offspring values for each trait. Subsequently, I applied a linear regression in R to obtain heritability.

Results and Discussion

The parasite *Schistocephalus solidus* responded to selection on host manipulation. Selection was based on host manipulation which, in order to obtain a single measurement, is here considered as the change in host manipulation as parasites became infective and switched from predation suppression by not-yet-infective parasites to predation enhancement by infective parasites (see methods). The resulting selection lines had a significant effect on host manipulation (p<0.001, Fig. 2a, Table 1-2); Selection for high host manipulation (high selection lines) increased it by 0.015 ± 0.017 (estimate ± se), while selection against host manipulation (low selection lines), decreased host manipulation by 0.074 ± 0.017 (estimate ± se) compared to unselected controls. This confirms that selection on the parasite only was successful in changing the host’s phenotype. Within infected copepods, differences between those infected by parasites selected for more or less host manipulation appeared within a single generation (t=-3.01, p=0.013, Fig. 2a) and became more pronounced in subsequent generations (p<0.005, Fig. 2a, Table S3). The behaviour of copepods infected with parasites from control lines was intermediate and never differed from high selection lines (p>0.2, Fig. 2a, Table S3) and differed significantly from low selection lines during the second generation only (t=-2.92, p=0.017, Fig. 2a).

This could be some indication that host manipulation more easily decreases than increases. However, given that power to detect differences involving control lines was much lower than power to detect differences between the two selected lines due to a smaller sample size for control lines, variation in the data and the relatively small effect size makes any interpretation of the lack of significant differences between control lines and selected lines difficult to interpret. Somewhat surprisingly host manipulation and the effect of infection on host manipulation decreased between generations (p<0.0001). Some variation in copepod behaviour within and between experiments does not seem uncommon in this system [21,25,26,29,34]. Host manipulation might additionally have been affected
by the breeding regime resulting in some inbreeding. It is unknown whether inbreeding can affect host
manipulation. However, these changes occurred equally in all selection regimes (p=0.1658, Table 1-2), so they are unlikely to change any of the findings with regards to selection on host manipulation.

Differences between selection lines could either be caused by differences in host manipulation before or after the parasite became infective or both. In order to distinguish between these scenarios, I also analysed host activity depending on parasite stage (not-yet-infective vs. infective). There was no main effect of selection line on host activity (p=0.393, Fig. 2 B-C, Table 1-2), but there was a significant interaction between parasite stage (not-yet-infective and suppressing predation vs. infective and enhancing predation) and selection line (p<0.001, Fig. 2 B-C, Table 1-2). Selection line had opposite effects on host activity depending on parasite maturity. More precisely, not-yet-infective parasites from high selection lines induced lower activity (i.e. higher levels of manipulation) than not-yet-infective parasites from low selection lines (Fig. 2b), albeit these differences were only significant during the second generation (t=3.36, p=0.004, Table S3) and seemed to disappear thereafter (t=0.63, p=0.920, Table S3). Once infective, the pattern was reversed in that infective parasites from high selection lines induced higher activity (i.e. higher levels of manipulation) than infective parasites from low selection lines (Fig. 2c). These differences became significant during the second generation (t=-3.03, p=0.013, Table S3) and increased during the third (t=-3.28, p=0.005, Table S3). Hence, both the ability of not-yet-infective parasites to reduce host activity to suppress predation and the ability of infective parasites to increase host activity to enhance predation changed in the expected direction. Controls were mostly intermediate and never significantly differed from either selection line (p>0.1, Table S3).

The selection differential confirmed that selection was similarly strong in either selection line, albeit, in both lines, it decreased over generations (Selection differential for each generation (F0/F1/F2): high: 0.256/ 0.214/ 0.100; low: 0.261/ 0.201/ 0.062) and that there was little selection on control lines (0.016/ 0.004/ 0.006). This decrease could be due to inbreeding and/or because the limit in genetic variation in host manipulation was reached which might well be connected. The fact that *S. solidus* responded to selection indicates that host manipulation must be heritable. Indeed, the heritability estimate (estimate ± se) for overall manipulation was significantly positive ($h^2 = 0.166 \pm 0.042$, t$_{1,78} = 3.91$, p<0.001). Heritability was similar for manipulation by not-yet-infective parasites ($h^2 = 0.147 \pm 0.070$, t$_{1,78} = 2.10$, p=0.039) and by infective parasites ($h^2 = 0.143 \pm 0.056$, t$_{1,78} = 2.56$, p=0.012). Heritability for manipulation by not-yet-infective parasites corresponds well to previous estimates of heritability in this system calculated on comparisons between full sibships [27], indicating that host manipulation is mostly an additive trait. In the same study, however, Benesh [27] found no evidence that host manipulation by infective parasites to enhance predation was heritable. This discrepancy could be due to the fact that Benesh [27] used a different parasite population, whose host
manipulation once parasites are infective differs from the parasite population used in the present study [27,29].

To identify potential costs of host manipulation, I measured potential trade-offs with a number of other traits. Increased ability to manipulate the host did not result in any clear trade-offs between host manipulation and other fitness related traits in parasites (see supplementary information for details). If anything, parasites from the high selection line seemed to perform better during the first and second generation, albeit these differences were not significant and disappeared in the third generation. Selection over further generations would be necessary to judge whether costs would eventually emerge, but they do not seem to be obvious or present a strong hindrance to at least some changes in the level of host manipulation.

The response to selection suggests standing genetic variation for host manipulation in the parasite population. How can such variation be maintained when there seem to be no energetic costs restraining increases in host manipulation? Rather than energetic costs, ecological factors could shape host manipulation. Trade-offs between enhanced transmission to a correct subsequent host and increased risk of dead-end predation (i.e. by predators other than the appropriate subsequent host) [18,44,45] could for example limit the level of host manipulation by infective parasites to enhance predation (and thereby transmission). Host manipulation by not-yet-infective parasites that suppresses predation must have a natural limit as host activity can only be reduced so far, especially since it is also in the parasite’s interest for the host to continue performing some normal functions such as feeding [44]. However, this might not be due to any limit imposed by the host since a not-yet-infective nematode with the same first intermediate copepod host and similar host manipulation reduces host activity much more than S. solidus [34]. The benefits and ecological costs of host manipulation should vary with environmental factors, such as the prevalence of dead-end predators, correct subsequent hosts (and possibly their size [46]), and the availability of alternative food items for these predators. These factors might vary in time and space. The final host of S. Solidus, a bird, provides parasite dispersion resulting in unpredictability with regards to the environment. Additionally, parasites could have to trade-off their ability to manipulate a certain host genotype or species (S. solidus can infect various cyclopoid copepods [33,47]) with their ability to manipulate another host genotype or species. Such genotype-by-genotype interactions are frequent when it comes to host-parasite interactions [48–50].

Conclusions

Host manipulation responded to selection on the parasite and does not seem to be constrained by any obvious physiological costs. This confirms that host manipulation is an extended parasite phenotype that can evolve in response to selection on the parasite rather than on the host. Given ecological
selection pressure, host manipulation can and will respond to selection to better accommodate a parasite’s need for certain host behaviour, even at the expense of the host.

**Data availability**

Data is available on dryad (doi:10.5061/dryad.v273bt1).

**Competing interests**

I have no competing interests

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**References**


Tables

Table 1: General linear mixed models to analyse copepod behaviour in response to infection by parasites selected for or against host manipulation. Initial model for host manipulation: response ~ 1 + (1 | population). Initial model for host activity: response ~ 1 + (1 | population) + (1 | copepod identity). Test statistics and p-values were obtained using likelihood ratio tests and are always for the comparison with the preceding (i.e. less complicated) model. N: Host manipulation: 1231 copepods in 4 populations; Host activity: 2462 observations on 1231 copepods in 4 populations. Significant p values are highlighted in bold.

<table>
<thead>
<tr>
<th>Host manipulation (df: 3)</th>
<th>Factor</th>
<th>df</th>
<th>( \chi^2 )</th>
<th>p</th>
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<td>+ Generation</td>
<td>4,1</td>
<td>20.28</td>
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<td></td>
<td>+ Infection</td>
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<td>27.44</td>
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<td>+ Selection line</td>
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<td>+ Infection: Generation</td>
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<td>14.88</td>
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<tr>
<th>Host activity (df: 4)</th>
<th>Factor</th>
<th>df</th>
<th>( \chi^2 )</th>
<th>p</th>
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<td></td>
<td>+ Parasite stage (predation suppression vs. predation enhancement)</td>
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<td>428.66</td>
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<td>1.77</td>
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<td>7,1</td>
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<td>19,2</td>
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Table 2: Summary of the model containing all fixed effects and significant interactions. Infection and its interaction with parasite stage (host activity only) were removed from this model since this information is also contained in selection line. Comparisons are with not yet infective (host activity only) parasites from the control line.

<table>
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<th>Host activity</th>
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<td>0.0060 ± 0.0775</td>
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<tr>
<td>Replicate (intercept)</td>
<td>0.0002 ± 0.0127</td>
<td>0.0011 ± 0.0329</td>
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<td>Residual</td>
<td>0.0431 ± 0.2076</td>
<td>0.0216 ± 0.1470</td>
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<td><strong>Fixed effects</strong></td>
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<tr>
<td>Intercept</td>
<td>0.2354 ± 0.0185</td>
<td>0.2897 ± 0.0216</td>
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<td>Selection line (High)</td>
<td>0.0147 ± 0.0172</td>
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<td>Selection line (Low)</td>
<td>-0.0737 ± 0.0172</td>
<td>0.0271 ± 0.0271</td>
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<td>Selection line (Uninfected)</td>
<td>-0.2261 ± 0.0351</td>
<td>0.2301 ± 0.0366</td>
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<td>Generation</td>
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<td>0.0011 ± 0.0124</td>
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<tr>
<td>Parasite stage</td>
<td>0.2360 ± 0.0174</td>
<td>13.540</td>
</tr>
<tr>
<td>Parasite stage: Generation</td>
<td>0.0286 ± 0.0155</td>
<td>1.849</td>
</tr>
<tr>
<td>Parasite stage: Selection line (High)</td>
<td>0.0138 ± 0.0172</td>
<td>0.806</td>
</tr>
<tr>
<td>Parasite stage: Selection line (Low)</td>
<td>-0.0742 ± 0.0173</td>
<td>-4.298</td>
</tr>
<tr>
<td>Parasite stage: Selection line (Uninfected)</td>
<td>-0.2285 ± 0.0351</td>
<td>-6.516</td>
</tr>
<tr>
<td>Infection:Generation</td>
<td>-0.0646 ± 0.0168</td>
<td>0.0209 ± 0.0135</td>
</tr>
<tr>
<td>Parasite stage: Generation: Infection</td>
<td>-0.0655 ± 0.0168</td>
<td>-3.905</td>
</tr>
</tbody>
</table>
Figure 1: Experimental set up to select parasites for or against host manipulation. Only parasites were selected in different selection lines (presented in different colors) and the copepods harbouring these selected parasites fed to sticklebacks for the parasites to continue their life cycle (bottom, left in the drawing) and breed in pairs in an in vitro system to produce eggs (top, left in the drawing). The offspring that hatched from these eggs of selected parasites was then used to infect naive, unselected copepods from the stock population in every generation (top, middle of the drawing).