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Following a parasite through its life cycle: how tough is it to cope with different immune systems?

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In host-parasite coevolution, continuing development is needed to maintain fitness. An arms race increasing parasite infectivity and host resistance can lead to various strategies on both sides. For parasites with a complex life cycle, that battle becomes even more complicated since more than one host is necessary to fulfil the life cycle. The tapeworm Schistocephalus solidus is confronted with the immune systems of a copepod, such as Macrocyclops albidus, the stickleback Gasterosteus aculeatus and, as final hosts, any species of fish eating bird. We focused on the two intermediate hosts to analyze the interactions between the parasite and the two different immune systems. Diverse genotypes of the tapeworm were not only expected to be variable in their infectivity and virulence towards both hosts but might also have to trade-off investment to different immune-evasive mechanisms in the two hosts.
Using diverse tapeworm sibships, we found that infection success was highly variable among tapeworm genotypes in the copepod but less so in the stickleback host. Parasite sibships with high infectivity towards the copepod also grew better in this first host, whereas a negative correlation between infectivity and growth in the stickleback host was found. Genotypes, which were highly infective to copepods also elicited a lower innate immune response in the stickleback, and left the fish in better body condition. A possible explanation could be that genotypes are either adapted to cope with innate or adaptive immunity. The first step would be to avoid recognition by any part of the host immune system. Since surface sugars seem to play a major role for immune recognition, we started analyzing variation in such sugar components using fluorescent lectins, proteins that specifically recognize sugar residues.

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Trematodes within host tissues: Diversity of orientation strategies

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Schistosoma mansoni, *Trichobilharzia ocellata* and *Diplostomum spathaceum* invade vertebrate hosts (humans, birds and fish) and reach their final destination via complex pathways. However, there is no information about orientation mechanisms used in this migration. Using a choice-chamber (W-chamber) modelling the early events of infection, we comparatively characterized specific host cues used for localization of the appropriate microhabitats. Chemo-orientation responses of schistosomula were studied by exposing the parasites to chemical gradients of human and animal serum and its components. All species orientated towards low molecular weight fractions of their hosts' serum, and glucose and arginine were identified as the major stimulating cues. The responses of *S. mansoni* and *T. ocellata* were highly specific to the arrangement of hydroxyl groups of glucose. However, *D. spathaceum* responded with lower specificity; mannose and maltotriose were also attractive. Free arginine, in low concentrations as found in the epidermis, only produced orientation responses in the schistosomes, but in different concentration-sensitivities. The effect of bound arginine, present as a main component of proteins in the epidermis, was determined using tetrapeptides containing arginine in terminal or subterminal position. In fact, these also stimulated *D. spathaceum* and, in the schistosomes, even evoked more sensitive responses than free arginine. However, all species responded very differently to its position within the peptides. Our results demonstrate that the species examined respond to specific cues during migration within the host and suggest that the arginine and glucose content of the host's skin tissues are used as landmarks during the parasites' orientation towards deeper skin layers and blood vessels. Apparently, each trematode species has developed its own unique orientation strategy within the host. This might be a mechanism contributing to the host specificity of trematodes.

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Effect of the nematode *Camallanus lacustris* on male traits and female mate choice in three-spined sticklebacks

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The three-spined stickleback (*Gasterosteus aculeatus*) is a fish species with paternal care, in which males exhibit a typical breeding colouration and courtship dance to attract females. Besides this, immunogenetic characteristics (MHC-class-II genes) were shown to be important for females' olfactory recognition of the mates. Theory predicts, that females base mating decision on these traits, which signal reliable information about heritable parasite resistance of the male. Parasites are known to affect a variety of changes in behaviour, colouration or odour of their hosts. Subsequently, females of several animal species avoid such parasitized males.
In this study, we examined the effects of infection with the intestinal nematode *Camallanus lacustris* on visually and olfactory traits of male three-spined sticklebacks, and the resulting impact on female mate choice. *C. lacustris* is supposed to reduce its host's fitness being anquored in the intestine, feeding on blood and gut material. On account of this, infected fish could for example be less effective in brood-care or food uptake and may face high costs by permanent activation of the immune system.
For the experiment, pairs of brothers with identical MHC-class-II genotypes were exposed/sham-exposed to infective *C. lacustris* (L3) in copepods. When both males had built a nest and were ready to mate, we tested

female preference in visual and olfactory set-ups. Finally, the fish were dissected to determine parasite load and compare physiological status by several morphological and immunological measurements. In current analysis we examine the impact of the parasite via male traits on female mate choice and thus expect females to choose the non-parasitized male to gain optimal fitness consequences for their offspring.

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The pathogenic amoeba *Balamuthia mandrillaris* is a host for intracellular multiplication of *Legionella pneumophila* bacteria

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Balamuthia mandrillaris is a free-living amoeba and an opportunistic agent of granulomatous encephalitis in humans and other mammalian species. Other amoebae like *Acanthamoeba*, *Hartmannella* and *Naegleria* can provide a niche for intracellular survival of bacteria, including the bacterial pathogens *Chlamydia*, *Helicobacter* and *Legionella*. So far, the colonization of *B. mandrillaris* by bacteria has not been examined. In this study, we investigated whether the amoeba could host *L. pneumophila* bacteria. *L. pneumophila* is a facultative intracellular parasite of at least 13 different species of amoebae and the infectious agent of Legionnaires disease. Our experiments showed that *L. pneumophila* could initiate uptake by *B. mandrillaris* and, after an initial lag phase, could replicate within the amoeba about 4-5 log cycles from 24 to 72 hours after infection. Approaching completion of the intracellular cycle, *L. pneumophila* was able to destroy its host cell. Observations by light microscopy paralleled our quantitative data and revealed the rounding up, collapse, clumping and the complete destruction of the infected amoebae. Electron microscopic studies unveiled the replication of the bacteria in a compartment surrounded by a structure resembling rough endoplasmic reticulum. The course of intracellular infection, the degree of bacterial multiplication and the ultrastructural features of a *Legionella*-infected amoeba resemble those described for other amoebae hosting *Legionella* bacteria such as *A. castellanii* and *H. vermiformis*. In summary, we have shown that *L. pneumophila* bacteria colonize *B. mandrillaris* amoebae. We hence speculate that the amoebae might serve as a host for bacteria in its natural environment.

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The ameba *Balamuthia mandrillaris* infects immunodeficient mice also by cutaneous and oral routes

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Balamuthia mandrillaris is a free-living ameba and opportunistic agent of granulomatous amebic encephalitis (GAE) in humans and other mammalian species. World-wide, ~110 cases of *Balamuthia* GAE have been reported to date. Both natural source and route of infection remain speculative. In analogy to other free-living, potentially pathogenic ameba, *B.mandrillaris* may reach the brain after nasal/ oral uptake via the olfactory nerve (as *Naegleria fowleri*) or, after lung passage, via hematogenous dissemination (as *Acanthamoeba* spp.). Using highly susceptible immunodeficient mice, we previously provided data for both pathways. Furthermore, we have shown that *B.mandrillaris* also reaches the brain after subcutaneous, intravenous or ocular inoculations.
We now show that *B.mandrillaris* ameba also infected immunodeficient mice through abraded, but not punctured, skin as well as after direct intra-esophageal inoculation. Groups of 12 female 11-weeks-old C57BL/6 *rag*$^{-/-}$ mice received 1x10^4 *B.mandrillaris* (~10% cysts) in PBS either in 20 µl into both nostrils (A) or in 20 µl onto a depilated and abraded ~100 mm^2 area on the back (B), or in two 100 µl portions by gavage into the esophagus (C). Morbidity was monitored in terms of weight, appearance and motility. On days 4, 10, 18, 24, 27, 32, 42 p.i. stool samples were collected and 2 mice group^{-1} sacrificed for macro-pathological, histological and flow-cytometric analysis.
C57BL/6 *rag*$^{-/-}$ mice generally took ~7 d longer to show signs of morbidity resp. to become moribund than the similarly T- and B-cell-deficient BALB/*c*$^{scid/scid}$ mice used previously. All three infected groups showed the typical signs of *Balamuthia* infection. Detailed histological analysis of all major organs will be provided. IF-microscopy revealed *B.mandrillaris* ameba in stool samples for group A and C mice. FACS-analysis showed an increase of inflammatory cells, including NK cells, coinciding with the appearance of *B.mandrillaris* organisms e.g. in the liver.

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Intestinal parasites as biological indicators for environmental pollution

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Intestinal helminths of vertebrates gain on increasing interest as potential bioindicators for heavy metal contamination in aquatic and terrestrial habitats. Among these parasites, particularly acanthocephalans have an enormous heavy metal accumulation capacity exceeding that of established free living sentinels. Metal concentrations several thousand times higher in acanthocephalans than in host tissues were described from field and laboratory studies. Whereas larval stages inside their intermediate hosts are not able to take up high amounts of metals, young worms immediately after infection of the final host begin to take up metals. After