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Full Length Article

Parasitological and transcriptomic comparison of *Strongyloides ratti* infections in natural and in suboptimal permissive hosts

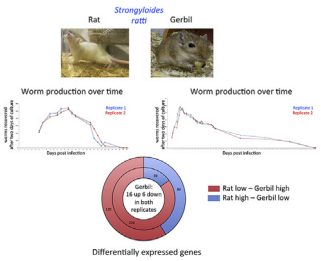
Tegegn G. Jaleta, Christian Rödelsperger, Adrian Streit*

Department for Evolutionary Biology, Max-Planck-Institute for Developmental Biology, Tübingen, Germany

HIGHLIGHTS

- *S. ratti* infect gerbils less efficiently than rats but survive longer in this host.
- *S. ratti* in gerbils reproduce well but produce a high proportion of males.
- Only few genes are differentially expressed between *S. ratti* in rats and in gerbils.
- Several astacins and acetylcholinesterases are differentially expressed.
- Gerbils are good laboratory hosts for the long-term maintenance of *S. ratti*.

GRAPHICAL ABSTRACT



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ABSTRACT

The nematode genus *Strongyloides* consists of fairly species-specific small intestinal parasites of various vertebrates, among them the human pathogen *S. stercoralis*. Between the parthenogenetic parasitic generations these worms can also form single facultative sexual free-living generations. In addition to their primary hosts, several species can also live more or less well in other permissive hosts, which are sometimes not very closely related with the normal host. For example, *S. stercoralis* can also infect dogs and non-human primates.

Here we compare the infection and reproductive success over time and the gene expression profiles as determined by quantitative sequencing of *S. ratti* parasitizing in its natural host rat and in the permissive host gerbil. We show that in gerbils fewer infective larvae successfully establish in the host, but those that do accomplish this survive and reproduce for longer and produced a higher proportion of males during the first two month of infection. Globally, the gene expression profiles in the two hosts are very similar. Among the relatively few differentially expressed genes, astacin-like and acetylcholinesterase genes are prominently represented. In the future it will be interesting to see if these changes in the suboptimal host are indeed ecologically sensible responses to the different host.

1. Introduction

In the genus *Strongyloides,* there are more than 50 species described so far which are all obligate parasites of vertebrate hosts (Speare, 1989; Viney and Lok, 2015). *Strongyloides ratti* is a gastrointestinal parasite of rats (*Rattus norvegicus*) that has been established as a parasite model system for the study of human strongylodiasis and for basic biological research (Viney and Kikuchi, 2017). *S. ratti* has unique and complex life cycles that consist of parasitic and free-living generations (Suppl. Fig. 1; Streit, 2017;

* Corresponding author. Max-Planck-Institut für Entwicklungsbiologie, Department for Evolutionary Biology, Spemannstrasse 35, D-72076, Tübingen, Germany.
E-mail address: adrian.streit@tuebingen.mpg.de (A. Streit).

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Viney and Lok, 2015). In brief: the adult parasites are female only, which live in the mucosa of the small intestine. There has also been a report of isolation from the large intestine of wild rats (Shintoku et al., 2005). The parasitic female produces parthenogenetically female and male offspring that are passed to the environment with the host feces, partially as embryonated eggs ready to hatch and partially as already hatched larvae. The female progenies of parasitic female have two developmental choices: 1) to develop directly to infective third stage (iL3) (direct/homogonic cycle) or 2) to develop to free-living females (indiect/heterogonic cycle). All males develop into free-living adults. The time to reach these stages varies with environmental parameters, in particular temperature but takes under our laboratory conditions about two days. The free-living animals mate and reproduce in the environment and all their progenies develop to iL3 and need to infect a host by skin penetration. This complex life cycle with free-living adults, provides researchers with, for parasites, unique opportunities to apply genetic and molecular experimental tools for the investigation of the basic biology and of the evolution of parasitism in *Strongyloides* spp. (Streit, 2014, 2017).

In several species of *Strongyloides* (Streit, 2017), among them *S. ratti* (Gemmill et al., 1997; Harvey et al., 2000), it was shown that the proportion of males produced is influenced by the immune status of the host such that a stronger immune response is associated with more males produced. Like wise, the switch between the homogonic and the heterogonic development is influenced by the host immune response but also by additional environmental factors acting after the larvae have left the host (Gemmill et al., 1997; Harvey et al., 2000; Streit, 2017). Several authors noticed that raising *Strongyloides* spp. in permissive non-natural hosts also changed the sex ratio and the proportion of homogonic and heterogonic development. Interestingly, the direction of the change differed in different experiments or species. Transferring *S. papillosus* from sheep to rabbits (Brumpt, 1921b; Matoff, 1936; Sandground, 1926; Triantaphyllou and Moncol, 1977) or cattle (Eberhardt et al., 2008) appears to mimic a stronger immune response and led to elevated numbers of males and heterogonic females. However, Crook and Viney (2005) found that rearing *S. ratti* in mice reduced the number of males and heterogonic females.

Mongolian gerbils (*Meriones unguiculatus*) have been used as a model hosts in a number of parasitological studies due to their high susceptibility to various parasites in the laboratory (see for example Konigova et al., 2008; Tokiwa et al., 2015). Persistent *S. venezuelensis* (Horii et al., 1993; Tsuji et al., 1993) and *S. stercoralis* (Nolan et al., 1993) infections have been reported in gerbils. To our knowledge, there has been no report of natural infection of gerbils with any species of *Strongyloides*. Gerbils are also susceptible to *S. ratti* (Niamatali et al., 1992). However, gerbils appear not to be susceptible to all species of *Strogyloides* since our attempts to infect a total of six gerbils in two independent experiments with *S. papillosus* failed in all cases.

Here we systematically compare the dynamics of *S. ratti* infections in the natural host rat and the permissive non-natural host gerbil. We also compare the transcriptomes of parasitic females residing in rats and gerbils, respectively. The sex ratio differs greatly between the two hosts with many more males produced in gerbils. The comparative transcriptome analyses revealed that only a small proportion of the genes are differentially expressed between the two hosts. Among the differentially expressed genes, two gene families that are highly expanded in the *S. ratti* genome (Hunt et al., 2016) are strongly represented, namely astacin metallopeptidase-like and acetylcholinesterase genes.

2. Materials and methods

2.1. Parasites and hosts

The *S. ratti* strain ED321 (Viney, 1996) was used throughout these experiments. ED321 was received from M. Viney, University of Bristol in 2010 and maintained in Wistar rats as described (Viney et al., 1992). This strain was originally derived from strain ED5 (Viney et al., 1992) through selection for a high percentage of heterogonic development and is the strain, for which the genome sequence has been published recently (Hunt et al., 2016). Host animals (rats and gerbils) were purchased from Charles River (Sulzfeld, Germany). All experiments with animals were in accordance with national and international animal wellfare legislation and guidelines. The permits were granted by the Regierungspräsidium Tübingen (AZ35/9185.82–5). The rats were kept in Techniplast type 4 cages with elevated tops in an in-house animal facility, which is subject to regular inspections by the veterinary authorities of Tübingen (Veterinäramt Tübingen).

2.2. Experiment 1: comparison of the worm production of rats and gerbils infected with S. ratti over time

As a measure for the number and the sex ratios of the worms produced by the infected host animals, we isolated and counted males and females (free-living and infective L3) after two days of culture as described below. At this time point the sexes of the worms can be reliably determined and there are no or only easily recognizable, very small worms of the next generation present. Notice that the numbers of surviving worms present after two days of culture may be different from the numbers of eggs originally produced by the parasitic females. Differences between treatments may be due to different numbers of eggs produced and/or different survival. Procedure: Groups of four weeks old male rats and gerbils were subcutaneously infected with about 500 third stage infective larvae (iL3s) per animal as described (Viney, 1996). The animals of the same species were housed together. Starting from six days of post infection, the animals were placed in cages with metal grid bottoms underneath lined with wet paper towels (over night at 22 °C) for five days per week until we failed to detect worms in two consecutive samples or for two months. If after two months worms were still present (was the case only in gerbils), feces were collected once a month until no worms were detected anymore. The feces were cultured on watch glasses as described (Viney et al., 1992). The feces collected from one group of animals were distributed to six watch glasses. During the first 4 weeks and 8 weeks of infection for rats and gerbils respectively the fecal samples were split in half and the two parts were incubated at 19 °C and 22 °C respectively for 48 h. Later the entire samples were incubated at 19 °C. The worms were recovered from the cultures using Baermann funnels (Lok, 2007). The isolated worms were washed in watch glasses with phosphate buffered saline (PBS) and all worms from one group of hosts and the same temperature were pooled in 1 ml of PBS. The numbers of male and female (includes the very few iL3s from the homogonic cycle produced in this strain) worms were determined for each group of animals as follows. If upon a rough estimate the total number of worms in the sample was estimated to be less than 1000 the males and females were counted directly. If the total number of worms was larger the worms in three separate samples of 5 μl were counted and the total number of worms extrapolated. The experiment as described above was repeated with host animals purchased at a different time and with a different batch of iL3s for infection, resulting in two independent biological replicates.

The nightly worm production determined after two days of culture and the parasitic worm burden in both hosts were