Divergence of an introduced population of the Swimbladder-nematode *Anguillicola crassus* - a transcriptomic perspective

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Abstract

The difference of the immune attack on *A. crassus* in the two diffent hosts provides an opportunity to investigate the parasite's response to different "immune environments" on a transcriptomic basis.

To my grandmother Ruth my brother Roman and my wife Silvia

Acknowledgements

I would like to acknowledge the thousands of individuals who have coded for free software and open source projects. It is due to their efforts that code is shared, tested, challenged and improved. Sharing their intellecual property as a general good, they serve progress in science and technology.

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GLOSSARY

Glossary

DNA Desoxy Ribonucleic Acid; a chemical molecule bearing the heritable genetic information in all life on earth

dpi Days post infection; In infection experiments, a point in time given in days after an individual has been infected

ORF Open Reading Frame; a region in a DNA-sequence begining with a start-codon and not containing a stop-condon. For example a region within a processed mRNA transcript being transcribed into a protein

SNP Single Nucleotide Polymorphism; variation occurring in a single nucleotide between two closely related homlogous sequences. Leading to for example to allelic differences within a population or even the homologous chromosomes in an individual

GLOSSARY

1

Introduction

1.1 The study organism: *Anguillicola crassus*

1.1.1 Ecological significance

Anguillicola crassus Kuwahara, Niimi and Ithakagi 1974 (1, 2) is a swimbladder nematode naturally parasitizing the Japanese eel (*Anguilla japonica*) indigenous to East-Asia. After a single introduction (3) to Germany in the early 1980s *A. crassus* has colonized almost all populations of the European eel (*Anguilla anguilla*) (4). Since the 1990s populations of the American eel (*Anguilla rostrata*) have been colonized as novel hosts (5, 6, 7) and finally it has been detected in three indigenous *Anguilla* species on the island of Reunion near Madagascar (8).

In Asia, as well as in the introduced ranges, copepods and ostracods serve as intermediate hosts of *A. crassus* (9), in which L2 larvae develop to L3 larvae, infective to the final host. Once ingested by an eel they migrate through the intestinal wall and via the body cavity into the swimbladder wall (10), i.a. using a trypsin-like proteinase(11). In the swimbladder wall L3 larvae hatch to L4 larvae. After a final moult from L4 to preadult the parasites inhabit the lumen of the swimbladder, where they eventually mate. Eggs containing L2 larvae are released via the ductus pneumaticus into the eels gut and finally into the water(12).

Within the novel range and hosts, conspicuously elevated prevalences and intensities of infection occur (reviewed in (4) and (13)). These differences in abundance of *A. crassus* in East Asia compared to Europe are commonly attributed to the different host-parasite relations in the final eel host permitting a differential survival of the larval and the adult parasites (14). Recently, data from experimental infections of European eels with *A. crassus* have been published (15). They show that the parasite undergoes (under experimental conditions) a density-dependent regulation keeping the number of worms within a certain range.

Anguillicola crassus Kuwahara, Niimi et Itagaki, 1974 (1) is a nematode feeding on blood in the swimmbladder of freshwater eels of the genus *Anguilla*. Originally endemic to East-Asian populations of the Japanese Eel (*Anguilla japonica*), *A. crassus* has attracted interest due to recent anthropogenic expansion of its geographic- and host-range to Europe and the European eel (*Anguilla anguilla*). Soon after it had been recorded for the first time in 1982 in North-West Germany (16), to where it was most likely introduced by live-eel trade (17, 18), *A. crassus* rapidly spread throughout populations of its newly accquired host (for a review see (4)). At the present day it is found in all but the northernmost population of the European eel in Iceland (19).

The impact of *A. crassus* on the European eel has been a major focus of research during the past decades. High prevalences of the parasite of above 70% (e.g. (20)), as well as high intesities of infections were reported, throughout the newly colonized area (21). Based on a broad base of work on its epidemiology *A. crassus* can be regarded as a model for parasite introduction and spread (13).

As in the natural host in Asia prevalences and intesities are lower (22), high epidemiological parameters were attributed to the inadequate immune-response of the European Eel (23). Interestingly the differences in the two host also affect the size and life-history of the worm: In European eels the nematodes are bigger and develop and reproduce faster (14). While the Japanese eel is capable of killing larvae of the parasite after vaccination (24) or under high infection pressure (25), only pathological effects such a thikening of the swimmbladder wall (26) have been found in the European eel.

1.1.2 Evolutionary significance

1.1.2.1 Divergence of *A. crassus* populations

Today, both theoretical arguments as well as field and laboratory data suggest that evolution, including speciation, can occur very rapidly given the right selective pressure. Such situations provide us with the opportunity of examining how evolution and speciation work at the molecular genetic level (Via 2002).

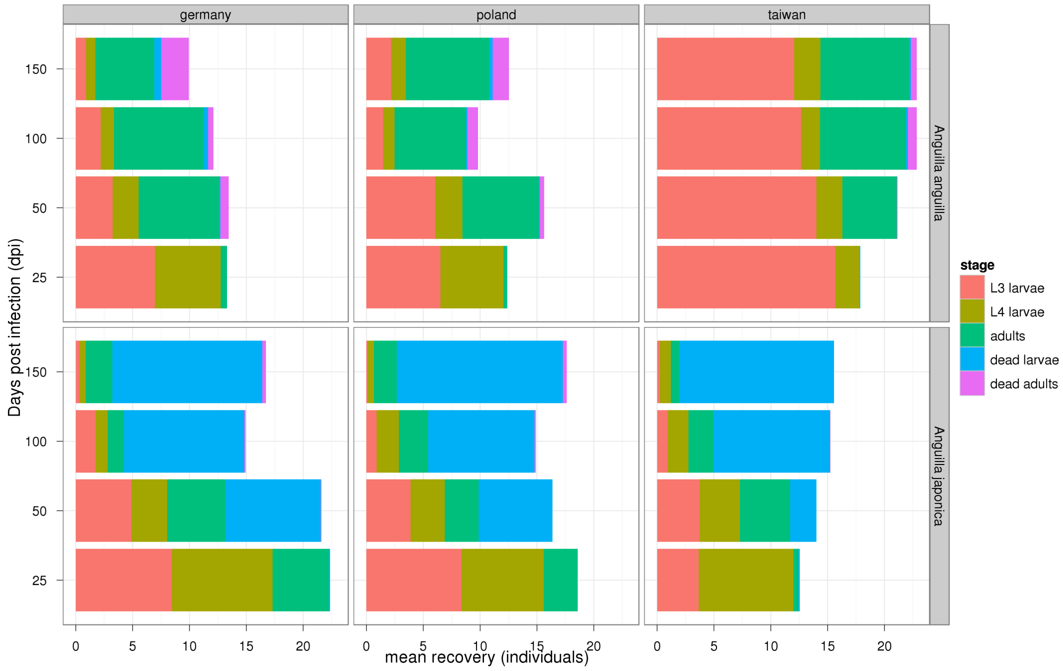

Figure 1.1: Differences in developmental speed - data courtesy of Urszula Weclawski

1.1.2.2 Interest in *A. crassus based on its phylogenetic position in the phylum nematoda*

The genus *Anguillicola* holds a phylogenetic position basal to the Spirurina (clade III *sensu* Blaxter (27)), one of 5 major clades of nematodes (28, 29). The Spirurina exclusively exhibit a parasitic lifestyle and comprise improtant human pathogens as well as prominent parasites of livestock (e.g. the Filaroidea and Ascarididae). This phylogenetic position makes the Anguillicoloidae an interesting system in the endeavour to understand the emergence of parasitism in Spirurina and as an "outgroup" for functional studies of parasitism in this clade. Some functionally interesting genes in this respect are thought to be under diversifying selection in an arms-race between host and parasite(30).

1.1.3 Functional insights from other nematodes used to formulate hypotheses for *A.crassus*

The analysis of ESTs, especially in nematode parasites, has been employed to identify pathogenic factors as potential vaccine candidates in numerous studies. (Blaxter 1995; Blaxter et al. 1996; Daub et al. 2000; Blaxter 2000; Harcus et al. 2004; Mitreva et al. 2004a; Mitreva et al. 2004b; Mitreva et al. 2005).

The complete genome sequence of the nematode Caenorhabditis elegans (The C. elegans sequencing consortium 1998) and Caenorhabditis briggsae (Stein et al. 2003), as well as the draft genomic assembly of Brugia malayi (Ghedin et al. 2007) provide useful sources for mining databases for homologous sequences. Brugia

1.2 Advances in sequencing technology enabeling this study

Recent advances in sequencing technology (often termed Next Generation Sequencing; NGS), provide the opprotunity for rapid and cost-effective generation of genome-scale data.

1.2.1 Pyro-sequencing

The longer read length of 454-sequencing (31) compared to other NGS technologies, allows *de novo* assembly of Expressed Sequence Tags (ESTs) in organisms lacking previouse genomic or transcriptomic data (for a comprehensive list of studies using this approach before Oct 2010 see (32)).

Such transcriptomic datasetes are still less expensive than genomic data-sets in terms sequencing costs and analytical needs.

1.2.2 Illumina-Solexa sequencing

As shorter read-length but higher throughput of the Illumina-Solexa platform provides superior means for gene expression analyis (**?**):

Expression-tags (SuperSAGE (**?**)) provide the benefit of classical SAGE-analysis .

RNA-seq (**?**)

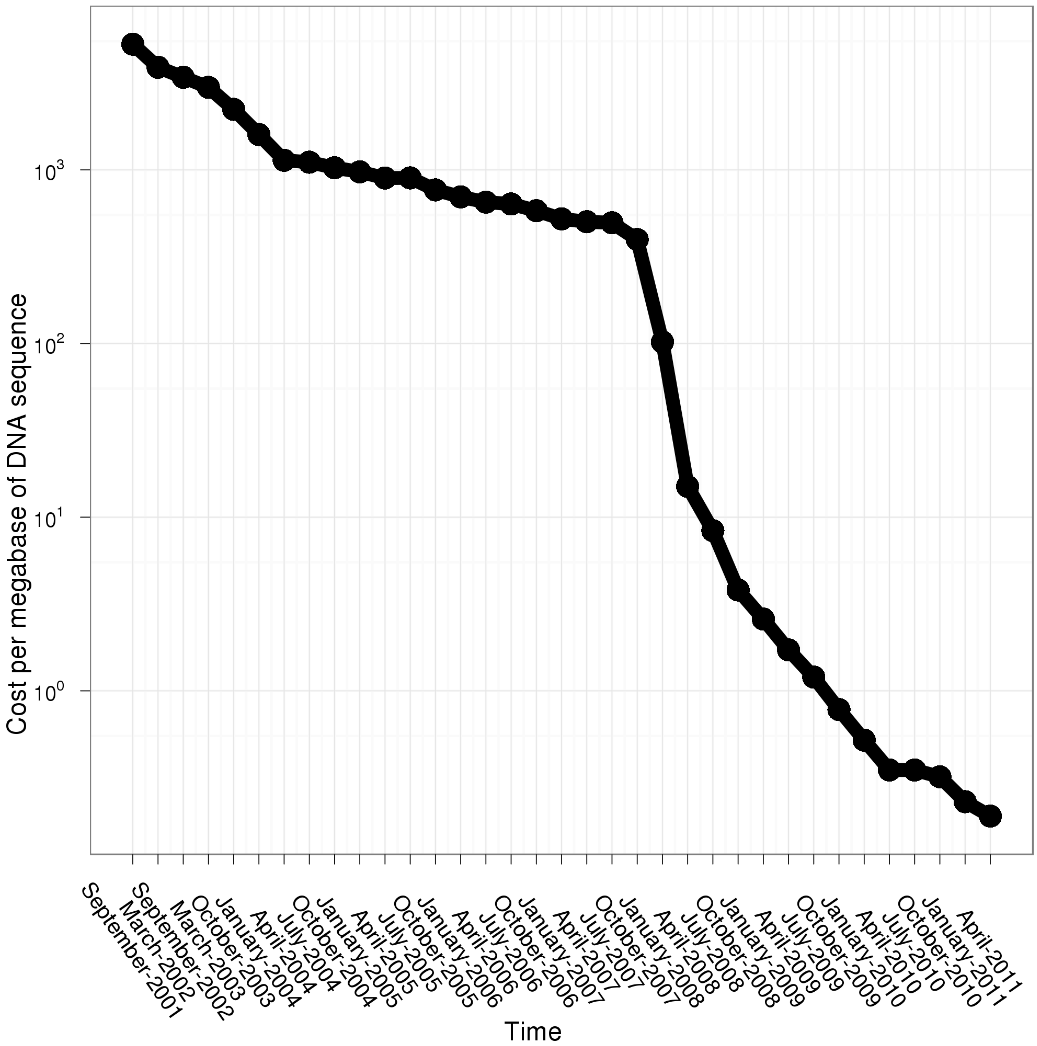

Figure 1.2: Falling seqeuncing costs - Falling into bottomless, Data provided by National Human Genome Research Institute, NHGRI.

Gene	GeneID	Length
human latexin	1234	14.9 kbps
mouse latexin	2345	10.1 kbps
rat latexin	3456	9.6 kbps

Table 1.1: title of table - Overview of latexin genes.

2

Aims of the project

2.1 Final aim

Our ultimate goal is...

2.2 Preliminary aims

There will be several preliminary scientific targets to be accomplished on the way...

3

Discussion

3. DISCUSSION

4

Materials & methods

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The thesis work was conducted from XXX to YYY under the supervision of PI at ZZZ.

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