

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



Zur Erlangung des akademischen Grades eines
DOKTORS DER NATURWISSENSCHAFTEN
(Dr. rer. nat.)

Fakultät für Chemie und Biowissenschaften

Karlsruher Institut für Technologie (KIT) - Universitätsbereich

vorgelegt

Dissertation

von

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geboren in

Schwäbisch Gmünd

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Tag der mündlichen Prüfung:

Abstract

The difference of the immune attack on *A. crassus* in the two different 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 intellectual 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 beginning with a start-codon and not containing a stop-codon. 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 homologous 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. In the last 30 years anthropogenic expansions of its geographic- and host-range to new continents and host-species (all freshwater eels of the genus *Anguilla*) attracted interest of limnologists and ecologists.

First *A. crassus* colonized Europe in the early 1980ies and colonized almost all populations of the European eel (*Anguilla anguilla*) in the following decades (reviewed in (3)):

Wielgoss et al. (4) studied the population structure of *A. crassus* using microsatellite markers and inferred details about the colonization process. From the fact that genetic diversity is highest in northern regions of Germany, and gradually declines to the south they concluded a single introduction event (4) to Germany. This is in agreement with the first record of *A. crassus* in 1982 in North-West Germany (5), and with an import event of Japanese Eels identified as (6, 7).

At the present day *A. crassus* is found in all but the northernmost population of the European eel in Iceland (8)

Since the 1990s populations of the American eel (*Anguilla rostrata*) have been colonized as novel hosts (9, 10, 11) and finally it has been detected in three indigenous *Anguilla* species on the island of Reunion near Madagascar (12).

1. INTRODUCTION

In Asia, as well as in the introduced ranges, copepods and ostracods serve as intermediate hosts of *A. crassus* (13), 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 (14), i.e. using a trypsin-like proteinase(15). 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(16).

Within the novel range and hosts, conspicuously elevated prevalences and intensities of infection occur (reviewed in (3) and (17)). 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 (18). Recently, data from experimental infections of European eels with *A. crassus* have been published (19). They show that the parasite undergoes (under experimental conditions) a density-dependent regulation keeping the number of worms within a certain range.

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 intensities 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 (17).

As in the natural host in Asia prevalences and intensities 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 (18). 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 thickening of the swimbladder wall (26) have been found in the European eel.

1.1 The study organism: *Anguillicola crassus*

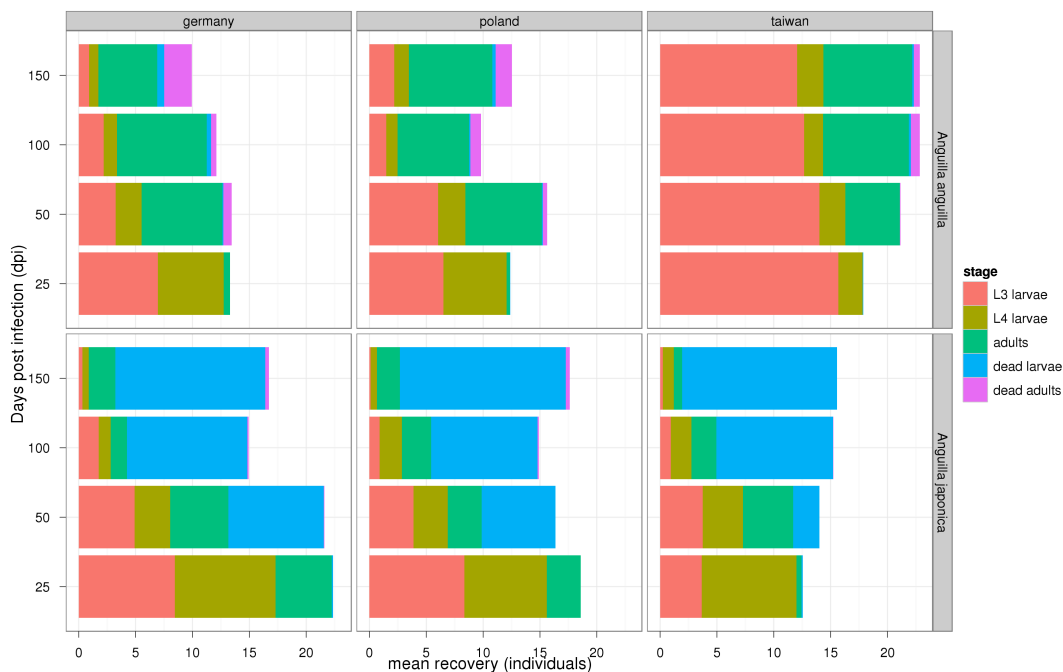


Figure 1.1: Differences in developmental speed - data courtesy of Urszula Weclawski

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).

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 important human pathogens as well as prominent parasites of livestock (e.g. the Filarioidea and Ascarididae). This phylogenetic position makes the Anguillicolidae an interesting system in the endeavour to understand the emergence of parasitism in Spirurina and as an “outgroup” for func-

1. INTRODUCTION

tional 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* (Ghedini 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 previous 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 analysis (?):

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

1.2 Advances in sequencing technology enabeling this study

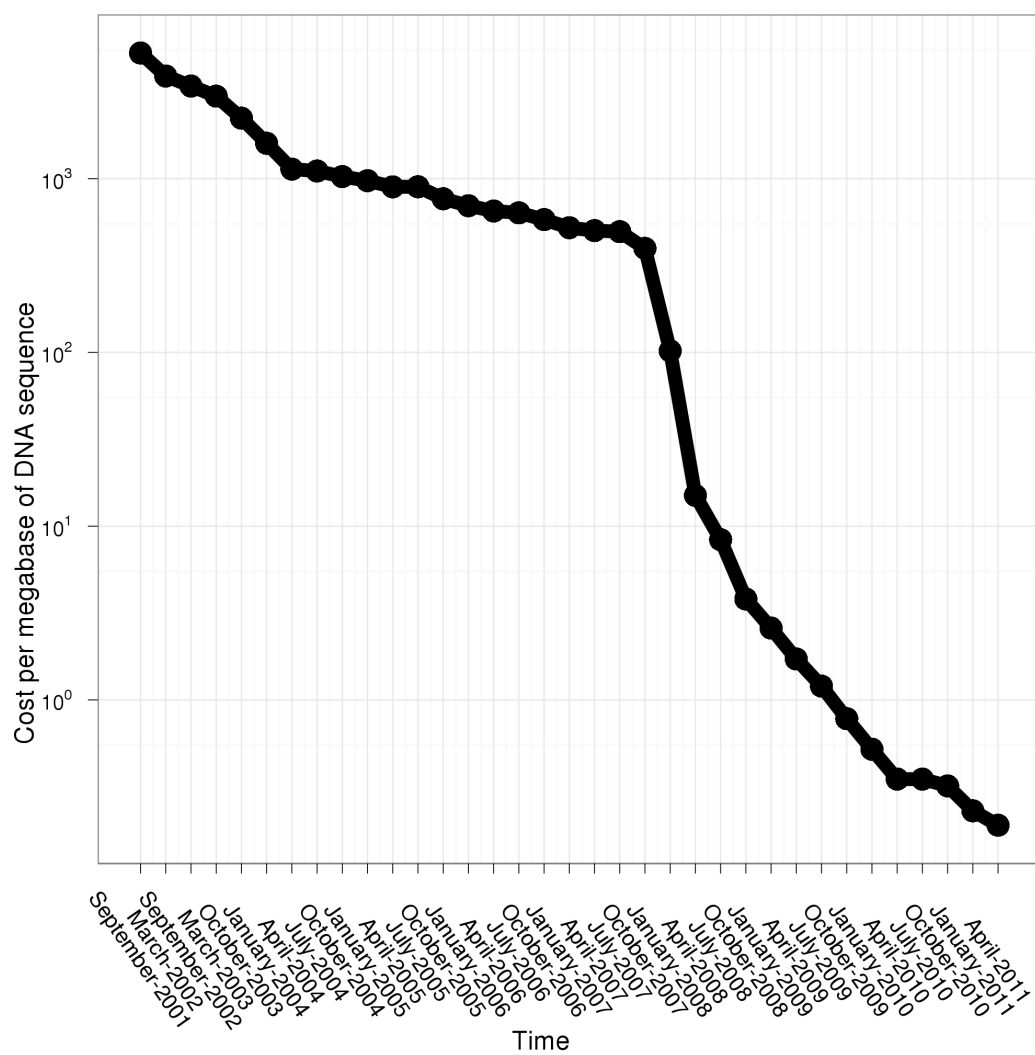


Figure 1.2: Falling sequencing costs - Falling into bottomless, Data provided by National Human Genome Research Institute, NHGRI.

1. INTRODUCTION

RNA-seq (?)

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

The source of my interest in *A. crassus* and the ultimate goal of the study presented here is the identification of .

And followed my interest in

2.2 Preliminary aims

Establishment of transcriptomic and genomic resources,

2. AIMS OF THE PROJECT

3

Pilot sequencing (Sanger method)

3.1 Parasite sequences

rRNA

3.2 Host sequences

Screening

3. PILOT SEQUENCING (SANGER METHOD)

4

Pyrosequencing of the *A. crassus* transcriptome

454

4. PYROSEQUENCING OF THE *A. CRASSUS* TRANSCRIPTOME

5

NlaIII-tag sequencing (Super-SAGE)

5.1 Comparison with pyrosequencing-data

5. NLAIII-TAG SEQUENCING (SUPER-SAGE)

6

Transcriptomic divergence inferred from expression differences in common garden experiments

6.1 Infection experiments

6.2 Examination of data-quality

6.3 Expression differences between male and female

6.4 Expression differences between worms in European
and Japanese Eels

6.5 Expression differences between worms in from the Eu-
ropean and Taiwanese worm-population

6. TRANSCRIPTOMIC DIVERGENCE INFERRED FROM EXPRESSION DIFFERENCES IN COMMON GARDEN EXPERIMENTS

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Discussion

7. DISCUSSION

8

Materials & methods

8. MATERIALS & METHODS

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Declaration

I herewith declare that I have produced this paper without the prohibited assistance of third parties and without making use of aids other than those specified; notions taken over directly or indirectly from other sources have been identified as such. This paper has not previously been presented in identical or similar form to any other German or foreign examination board.

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

CITY,