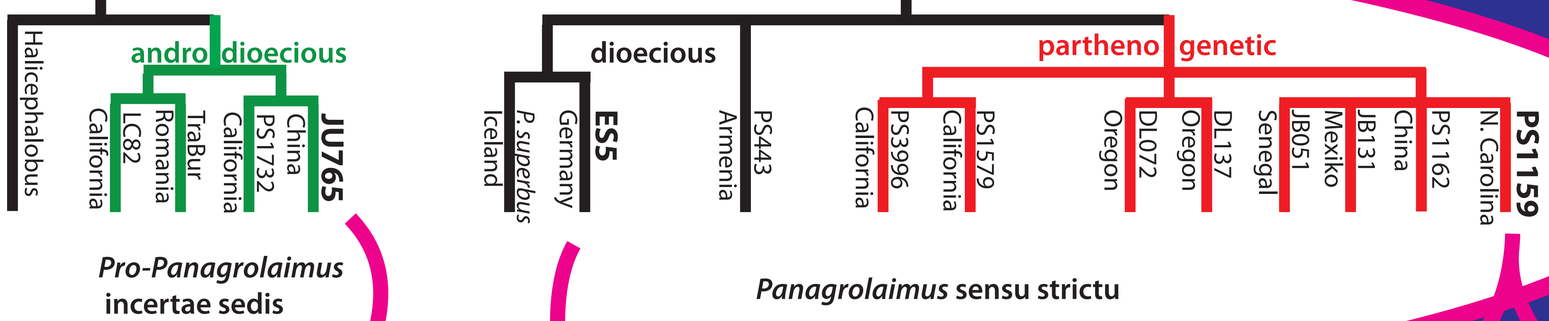
On the fast track to extinction?
Origin, divergence and mutation rates of asexual organisms

Philipp H. Schiffer, Gary Stier, Michael Kroiher and Einhard Schierenberg
Universität zu Köln, Zoologisches Institut, Köln, Germany
Corresponding author: PHS - ORCiD:0000-0001-6776-0934 - philipp.schiffer@googlemail.com

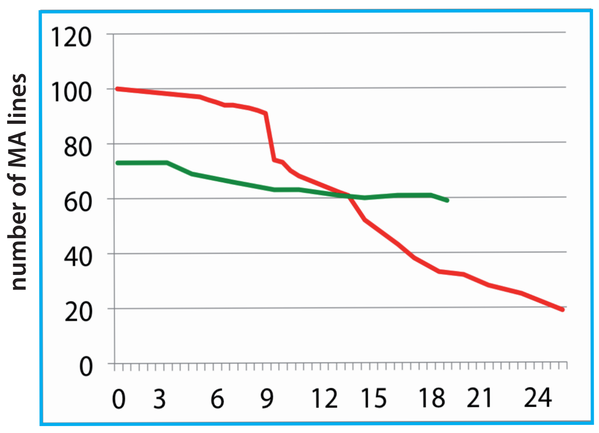
Recent research explored the parameters under which sexual reproduction is favourable. However, it is not clear whish genomic background allows for asexual reproduction to be established and which mechanisms confine parthenogenetic species to terminal phylogenetic branches with evolutionary short lifespans. One genetic condition observed in asexual species is polyploidy arising through hybridisation. The possession of multiple copies per gene could temporarily aid in buffering against detrimental mutations that cannot be eliminated by outcrossing in asexuals and thus accrue in genomes. Still, losing the 'best' genotype forever once it has mutated, asexual organisms might be trapped in a feedback loop culminating in extinction - especially as low quality genotypes might evolve elevated mutation rates. Conversely, elevated mutation rates could help in adaptation to novel and extreme environments where asexuals are often found.

Employing a mutation accumulation (MA) experiment I have explored the mutation rate in a parthenogenetic species of the cosmopolitan nematode genus *Panagrolaimus*, analysing a closely related hermaphroditic species in comparison. I sequenced and assembled the genomes of both species in order to construct references against which genomic sequences of MA lines were mapped. I then differentially screened for mutations and directly calculated rates. To analyse the origin and genetic background of parthenogenesis in *Panagrolaimus* I also generated genomic and transcriptomic data of two dioecious and several parthenogenetic species.
Here I am presenting results from these analyses, giving a first estimate of the mutation rates and divergence. My genomic and cellular data indicate that asexual *Panagrolaimus* species are tetraploid hybrids. This study will aid in a better understanding of evolutionary forces acting on asexual organisms and is a basis for research on molecular and cellular mechanisms leading to the establishment of parthenogenesis.

Depicted in this phylogeny are only those species/strains within the genus ***Panagrolaimus* s.l.** that have been or will be sequenced. The dendrogram adapted from Lewis et al. (2009) is supplemented with own data.



MA line experiments with PS1159 and JU765

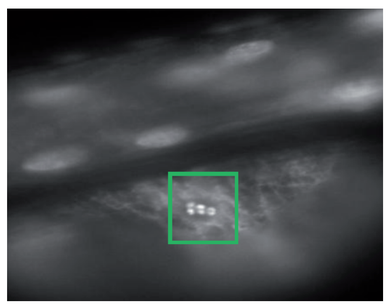


After two years (31 to 40 generations) 80% of PS1159 MA lines had been lost. The lines of the androdioecious JU765 appear to decline much slower.

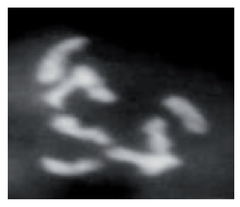
A fitness assay comparing lifetime fecundity between the the MA line founder populations and remaining bottlenecked MA lines is presently conducted. First observations indicate a dramatic fitness loss in parthenogenetic MA lines.

Evolution of parthenogenesis after Hybridisation?

Panagrolaimus spec. Bornheim



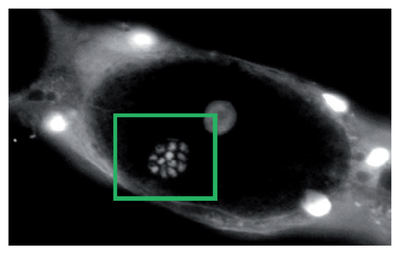
Fertilised egg outside of mother
2n = 6



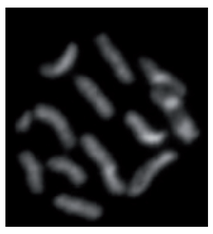
Pre-meyotic zone
2n = 6

C-Value ≈ 0.09 => Genome size ≈ 8,225*10^7 (n=3, ± 5%)

Panagrolaimus spec. PS1159



Post-meyotic activated egg
2n=12



Mitotic phase oogonium
2n=12

C-Value ≈ 0.159 => Genome size ≈ 1,455*10^8 (n=4, ± 5%)

Chromosomes were stained with DAPI and visualised with a Zeiss Apotome Microscope. C-values were measured with RTDetection-PCR as describend in Wilhelm et al. (2003).

Genomic data from the parthenogenetic species *P. spec* PS1159 yields divergend copies for many genes. The species has twice as many Chromosomes as the dioecious species *P. spec* Bornheim. This corresponds to a doubling in genome size. This indicates a hybrid origin of parthenogenesis in these nematodes.

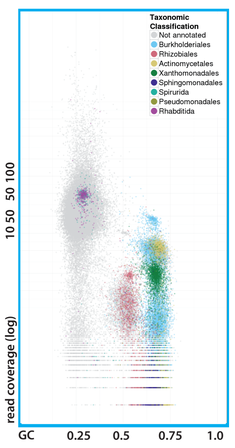
Workflow differential mutation calling

Illumina sequencing of G_0 at ~ 50X Coverage

Illumina sequencing of derived MA line genomes at ~ 50X Coverage

clc assembler

clc assembler



Removing contamination employing GC + coverage Kumar (2011) and BLAST+ filters

Pooled assembly of reference genome

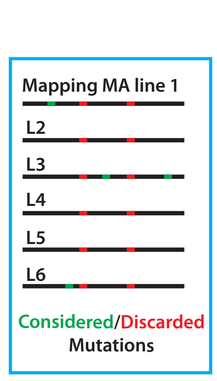
clc mapper

Select contigs with coverage 100 - 500

Mapping MA line reads to reference genome assembly

VarScan

Custom Scripts



Differentially call mutations

Mutation rate in parthenogentic MA lines!?

1.08e-7

Based on READs mapping with ≥ 15X coverage

Rate seems high? Check here for updates:

To reliably calculate a mutation rate an error free reference genome is needed. However, this is practically impossible to construct from 2nd Generation Sequencing data. Thus an approach using all obtained READS (maximising coverage) is choosen and mutations are then called differentially: only snps/indels unique to a single MA line are considered for calculation.

Available data

Species	PS1159	PS1806	PS1162	JB051	DL137	PS1579	JU765	ES5	ES1
Origin	California	California	China	Senegal	Oregon	California	China	Germany	Germany
Reproduction	partheno.	partheno.	partheno.	partheno.	partheno.	partheno.	androdio.	gonochor.	gonochor.
Genome	yes	yes	yes	yes	yes	yes	yes	yes	yes
Transcriptome	yes				yes	yes	yes	yes	

Genome and transcriptome data from wild type cultures will allow to analyse rates of evolution in parthenogenetic species.

Next steps?

1. Increase MA line coverage
2. Robustly infer mutation rate
3. Compare to wild type data
4. How do they evolve in the first place?
5 . What is the molecular background?

References

• CLC bio, White paper on de novo assembly in CLC Assembly Cell 3.0, CLC bio, Aarhus, Denmark, (2010)

• Kumar, S., & Blaxter, M. L. (2011). Simultaneous genome sequencing of symbionts and their hosts. Symbiosis, 55(3), 119–126.

• Lewis S., et al. (2009) Molecular evolution in *Panagrolaimus* nematodes: origins of parthenogenesis, hermaphroditism and the Antarctic species P. davidi BMC Evol. Biol. 9, 15.

• Wilhelm, J., Pingoud, A., & Hahn, M. (2003). Real-time PCR-based method for the estimation of genome sizes. NAR, 31(10), e56.