

Quantification of the slug parasitic nematode *Phasmarhabditis hermaphrodita* from soil samples using real-time qPCR

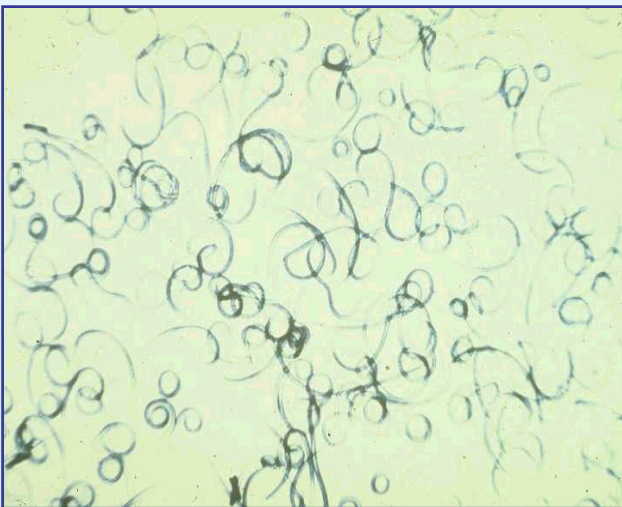
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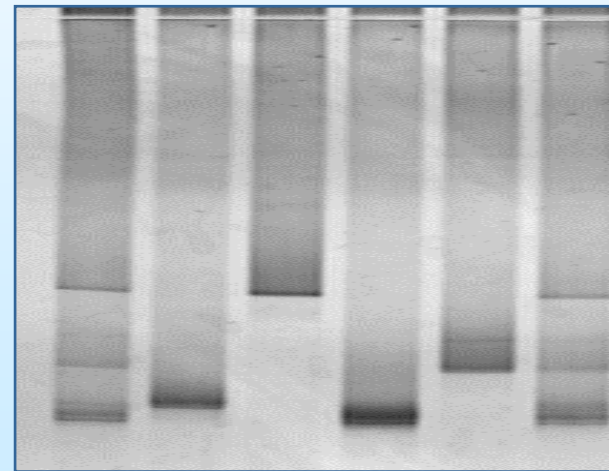
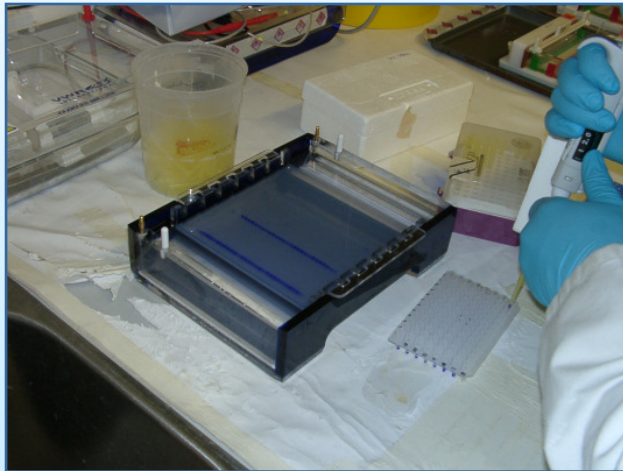
Background

- ***P. hermaphrodita* natural parasite of slugs**
 - Commercially important
 - Scientific study
- **Difficult to study ecology**
 - Identification problem
 - Traditional methods not suitable



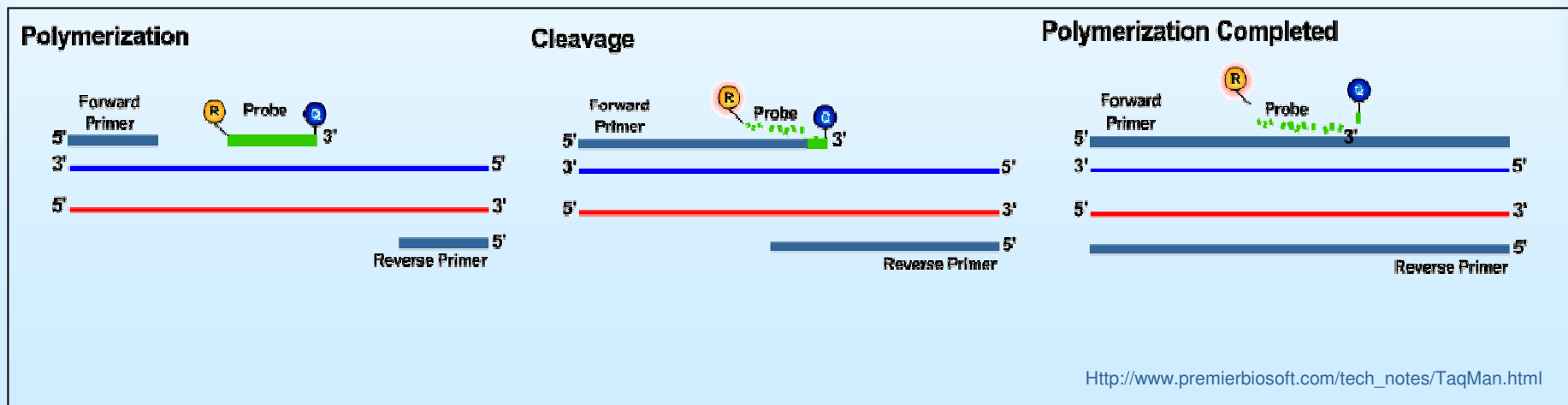
Molecular methods

- **Traditional PCR based**
 - Species specific primers
 - Gel electrophoresis, DGGE
- **Disadvantages**
 - Specificity
 - Non-quantitative



Molecular Methods

- Quantitative real-time PCR
 - Continuous increase in fluorescence
 - Time to exponential phase proportional to the initial quantity of template
 - Quantitation achieved via direct comparison to known standards



Molecular Methods

- **Why use quantitative real-time PCR ?**
 - **Quantitative**
 - **sensitive**
 - **Specific**
 - **Rapid**



Objectives

- **Develop assay for accurate detection & quantification of nematode species in soils**
 - *P. hermaphrodita*
 - *Molecular methods*
 - Sequencing – Primers – qPCR
- **Accurate extraction of your target DNA?**
 - Prior separation of nematodes & soil
 - Direct Soil DNA extraction
 - Nematode DNA persistence in soil

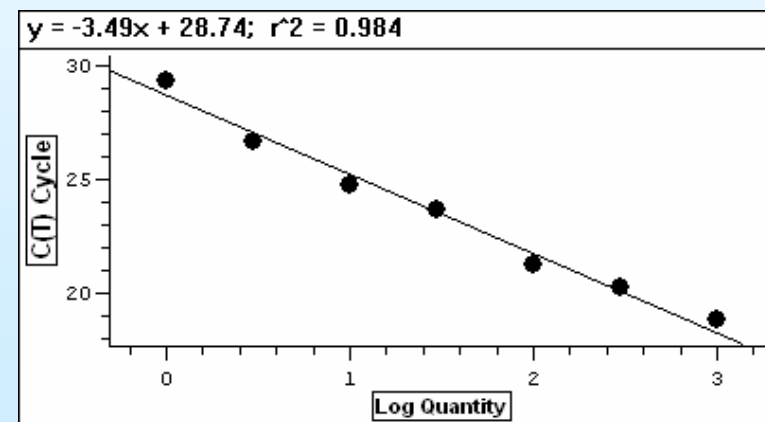
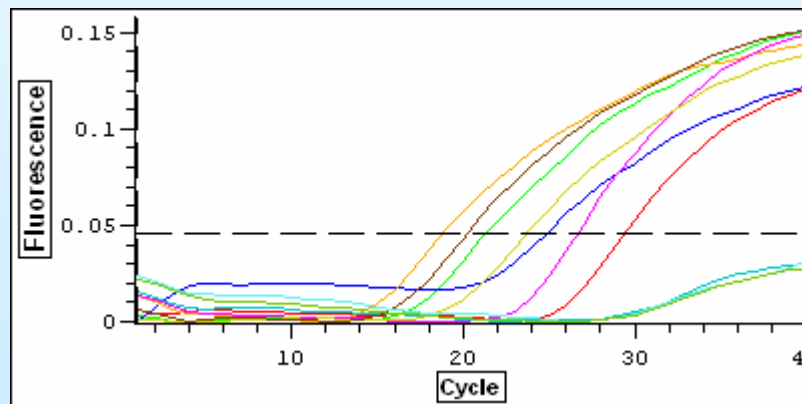
Result

- **DNA sequencing & Alignment**
 - Revealed 97% homology between *P. hermaphrodita* and *P. neopapillosa* 18S gene
- **Primer / probe set design**
 - Primer3 www software
 - NCBI BLAST
 - Probe anneals directly over 2 SNPs

Oligonucleotides	Forward Primer	Dual-labelled probe	Reverse primer
<i>P. hermaphrodita</i> 18S gene	..CGGGCGTAGTTTGTGACT..	..TTCATCCGCTGAAGTCCGGAATTTT..	..TCTATTGGCCTATCATGGTTGT..
<i>P. neopapillosa</i> 18S gene	..CGGGCGTAGTTTGTGAA A T..	..TT T ATCCGCTGAAGT C TGGGAATTTT..	..TCTATTGGCCTATCATGGTTGT..

Methods

- **Quantitative real-time PCR assay**
 - Amplification of DNA from replicate batches of known numbers of *P. hermaphrodita* 1, 3, 10, 30, 100, 300, 1000
 - *Correlation between Ct value and initial nematode number*

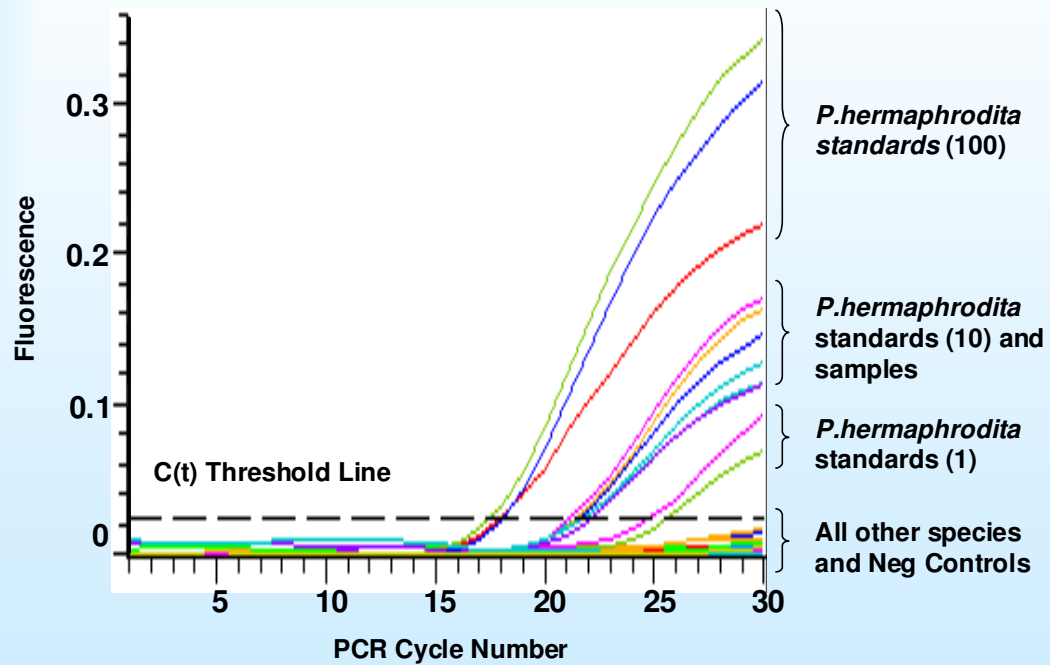


Methods

- **Specificity testing of qPCR assay**
 - **DNA extracted from 10 individuals of a range of nematodes:**
 - *P. hermaphrodita*
 - *P. neopapillosa*
 - *H. megidis*
 - *S. affine*
 - *S. carpocapsae*
 - *S. feltiae*
 - *S. kraussei*

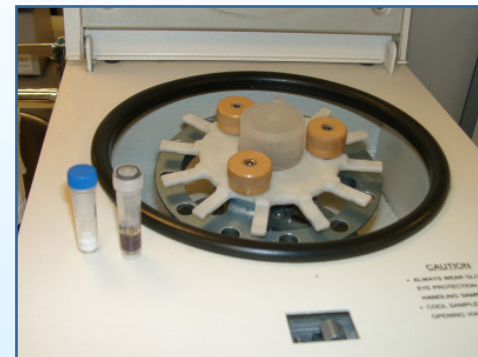
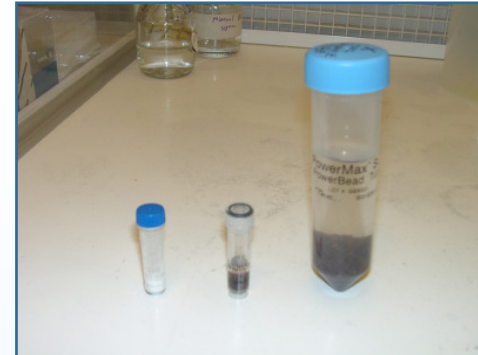
Result

- qPCR assay for specificity



Methods

- **Isolation of genomic DNA directly from soil**
 - 3 Methods
 - **Standard lab method** (Griffiths et al, 2000)
 - 0.5g soil samples
 - **UltraClean Soil TM DNA kit,** (Mobio USA)
 - 1 g soil samples.
 - **PowerMax TM Soil DNA kit,** (Mobio USA)
 - 10 g soil samples.



Result

- Isolation of genomic DNA directly from soil

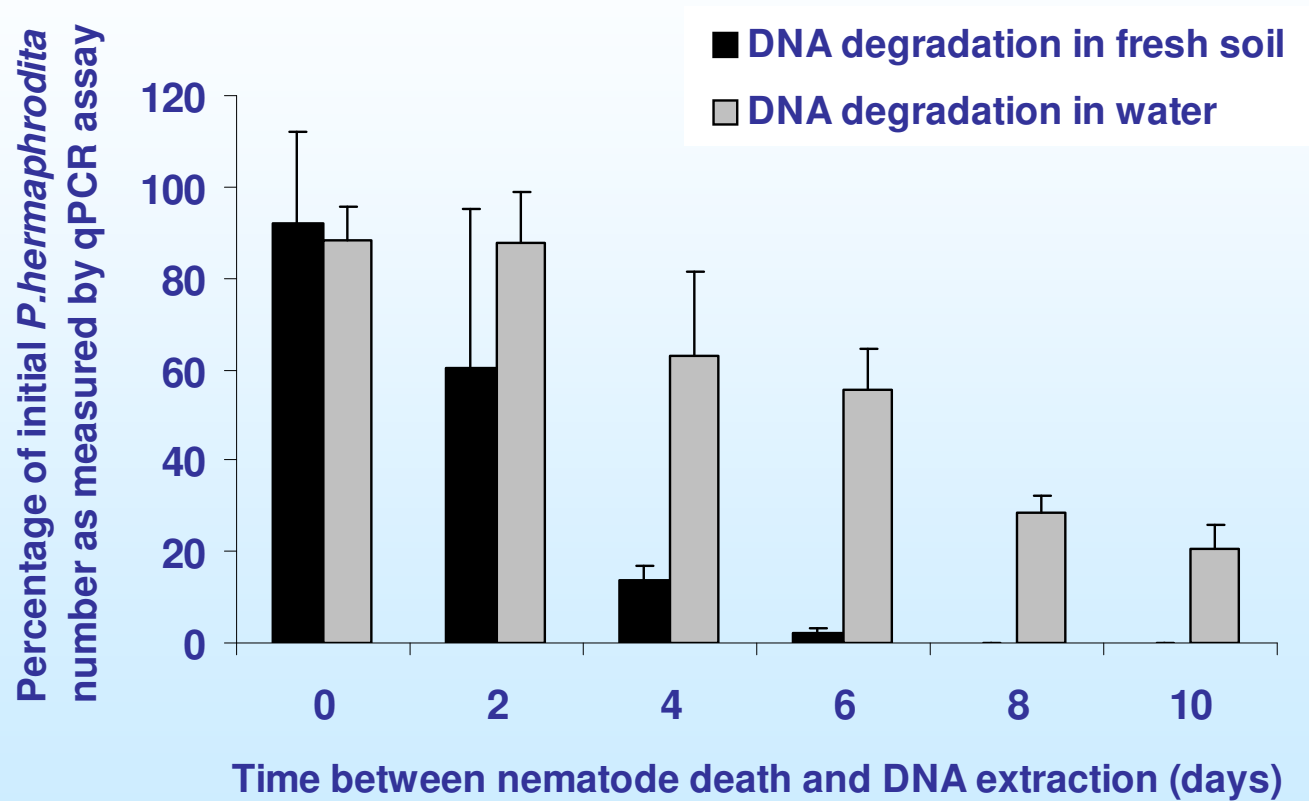
Extraction Method	Soil Sample Size	<i>P.hermaphrodita</i> initial inoculation quantity	Mean quantity measured by qPCR assay. n=10	Standard error	Percentage detection (%)
Standard lab method	0.5g	100	7.6	1.1	7.6
UltraClean Soil™ DNA kit	1g	100	12.2	1.4	12.2
PowerMax™ Soil DNA kit	10g	1000	989.6	89.1	99

Methods

- **Persistence of genomic DNA following nematode death**
 - **Many nematodes die following application to soils**
 - **Does the genomic DNA persist and is it detectable by the qPCR assay?**
 - ***P.hermaphrodita* killed by heating to 40°C for 60 minutes**
 - **Degradation in H₂O Vs. Degradation in soil**
 - **DNA extracted at 0, 2, 4, 6, 8 & 10 days**

Result

- Persistence of genomic DNA following nematode death



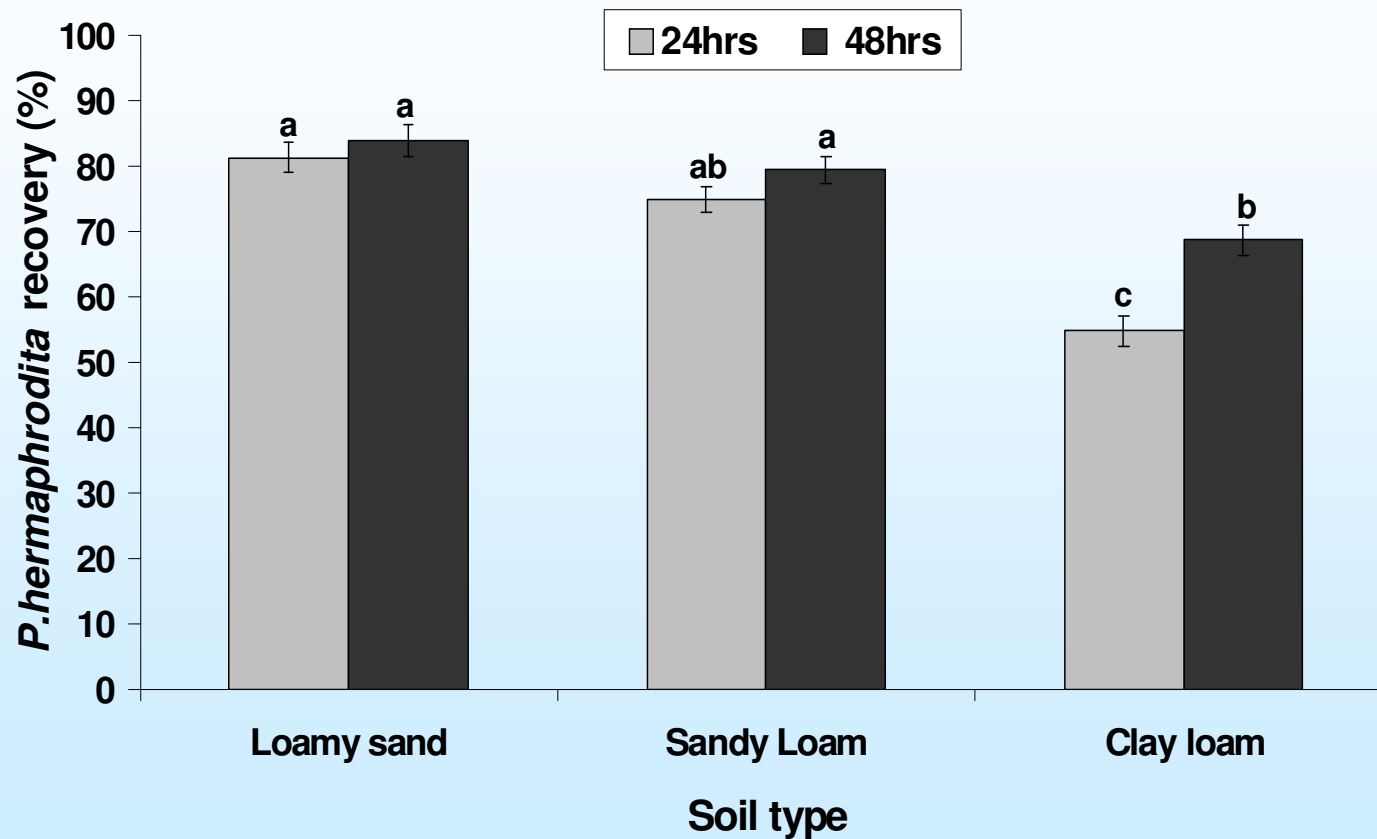
Methods

- **Live nematode extraction from soils**
 - **Nematode/soil separation prior to DNA extraction**
 - **100 *P. hermaphrodita* added to 10 g sterile soil**
 - **3 typical agricultural soils over 24 & 48 hour period**



Result

- **Extraction of live nematodes from soil**



conclusions

- Sequencing revealed high levels of genetic homology between *P.hermaphrodita* and *P. neopapillosa*
- Sequencing allowed development of highly specific qPCR assay which accurately detects & quantifies target
- Method could be simply applied to any nematode species
- **Isolation of genomic DNA directly from soil**
 - Highly variable efficiencies between methods
 - Most efficient method is very expensive
 - Results can be compromised by persistence of DNA within the soil
- **Baermann funnel extraction**
 - an efficient and consistent method of isolating live nematodes to a clean system

Acknowledgements



- **Mark Phillips**
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