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The James Hutton Institute

UNIVERSITY OF LEEDS

Nematodes are important

- Nematodes are everywhere

"Imagine a world where everything except the nematodes had been magically taken away: Our world would still be dimly recognizable…**we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes**"

Nathan Cobb

- Nematodes eat everything

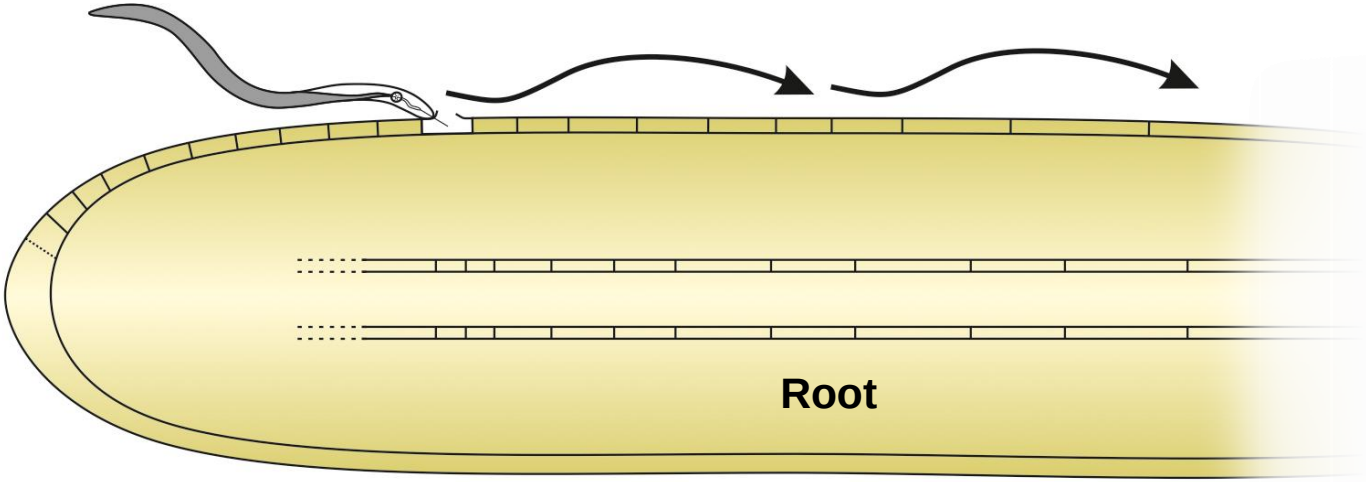
Nematodes can be free-living, plant-, or animal-parasites, predators and necrotrophs.

At least one species of parasitic nematode has been identified for almost every plant and animal species on the planet

Plant-nematode feeding strategies

Strategy 1

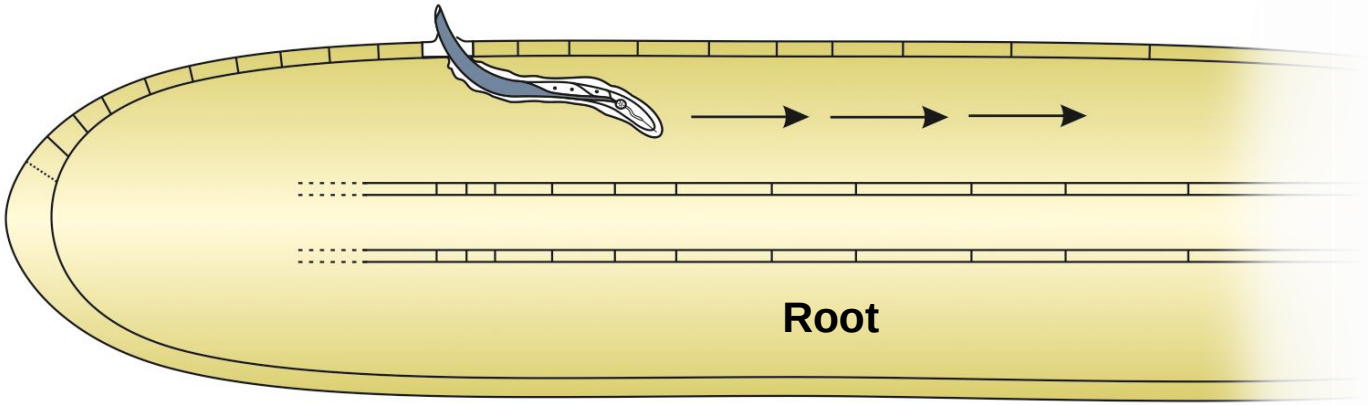
- Simple migratory ecto-parasites



Plant-nematode feeding strategies

Strategy 2

- More specialised migratory endo-parasites

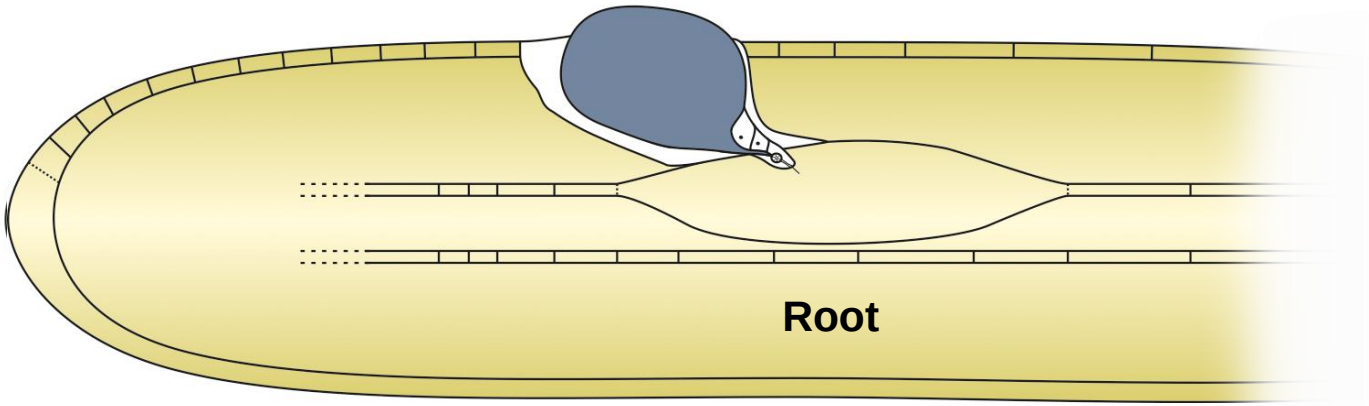


No biotrophic interaction

Plant-nematode feeding strategies

Strategy 3

- Highly specialised sedentary endo-parasites



- Inject a suite of “effector proteins” to modify host root tissue to create a feeding site

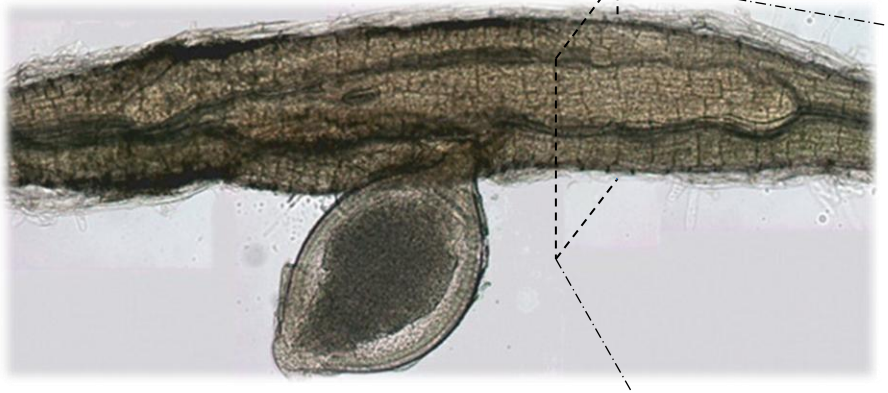
biotrophic interaction

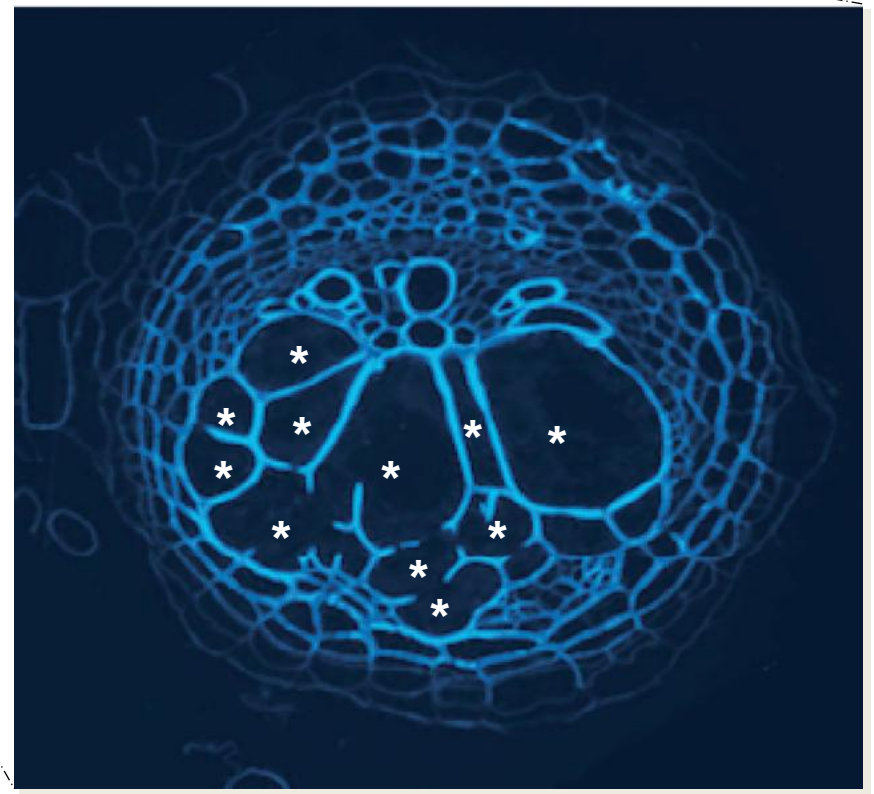
Endoparasitic life cycle

11-13 weeks from egg to egg

http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2012.04238.x/pdf

Feeding site formation





- Cross section through nematode feeding site
- Cell walls are coloured in blue
- Feeding site cells are indicated with *****

http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2012.04238.x/pdf

What does that actually look like? - Potato

What does that actually look like? - Tomato

What does that actually look like? - Carrot

http://photos.eppo.org/albums/pests/Nematodes/Meloidogyne_fallax__MELGFA_/MELGFA_01.jpg

Also a problem in the field…

http://www.idahoag.us/Categories/NewsEvents/PCN%20photos/PCN%20crop.jpg

Challenges of an endo-parasitic life

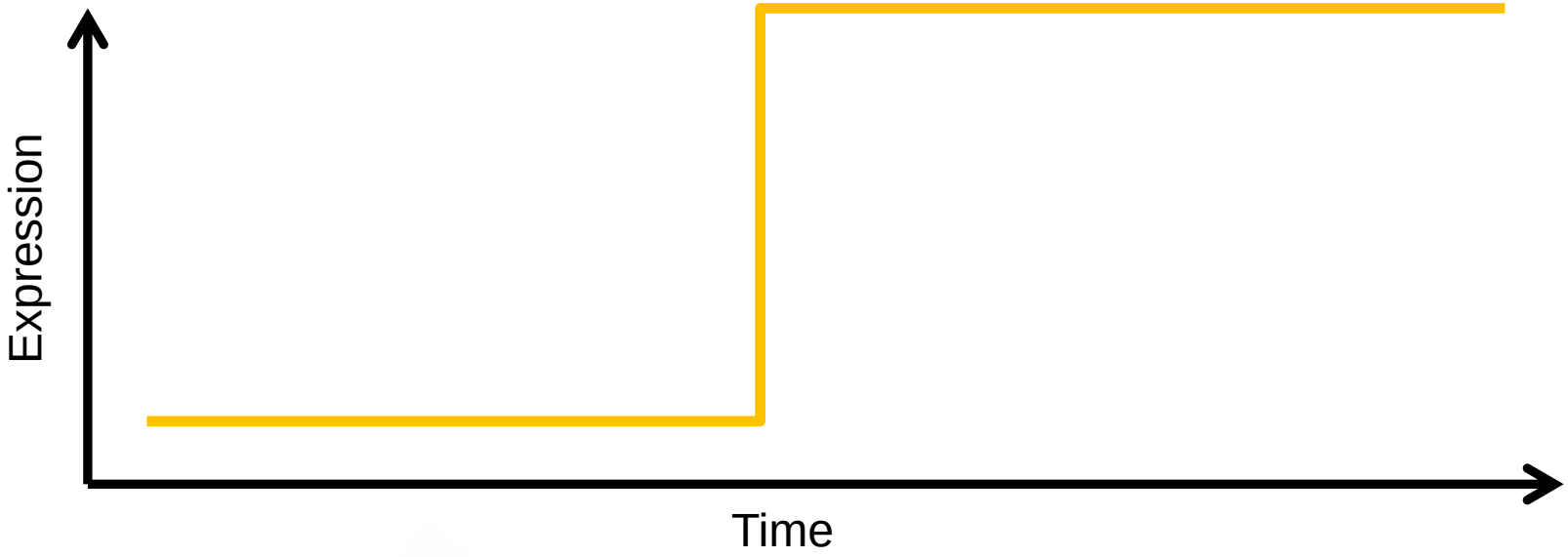
- Upon induction of the feeding site the nematode becomes sedentary.
- Feed from the feeding site every 6 hours without destroying it
- Remain undetected by plant defences for a period of 6 weeks
- **If at any time during these 6 weeks the feeding site dies, the nematode will not survive**

Hypothesis:

There will be a group of “effector genes”, expressed throughout the biotrophic phases, that will be involved in feeding site maintenance and suppression of host defences

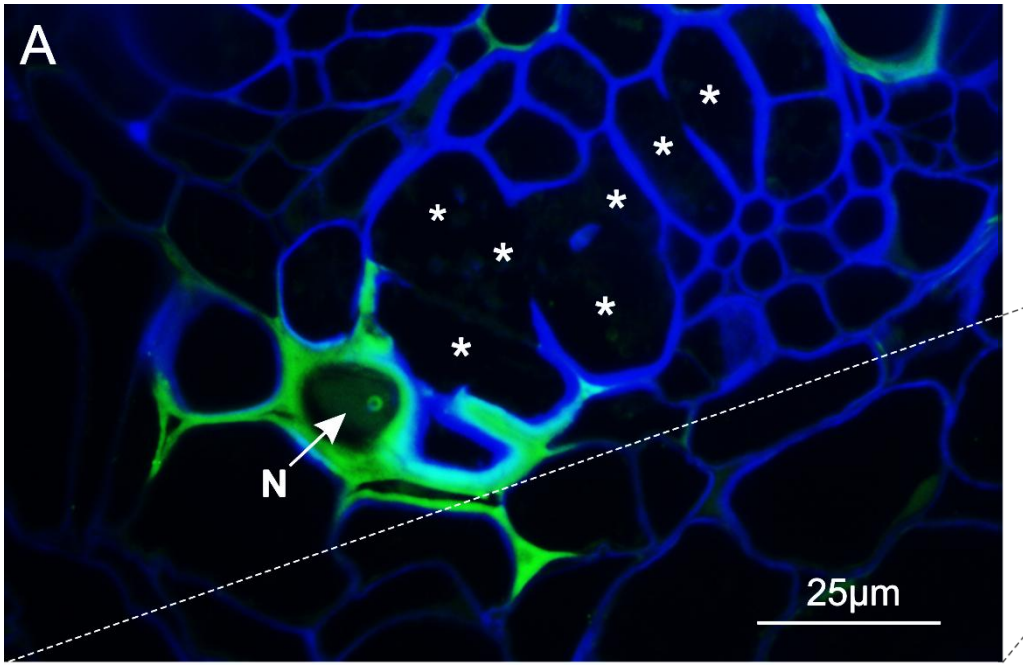
Effector gene identification

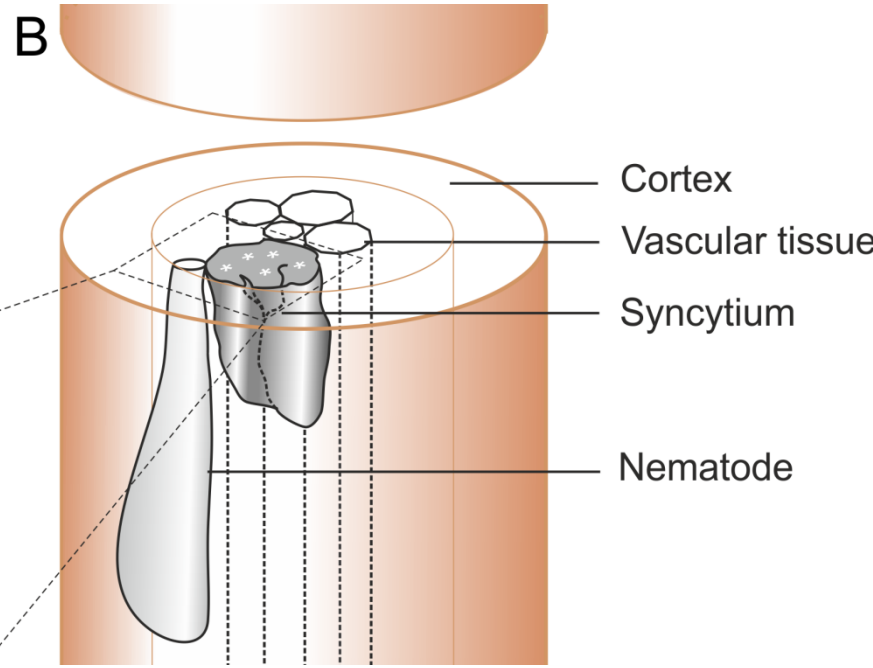
- **Make use of recently assembled genome sequence of the potato cyst nematode *G. pallida***



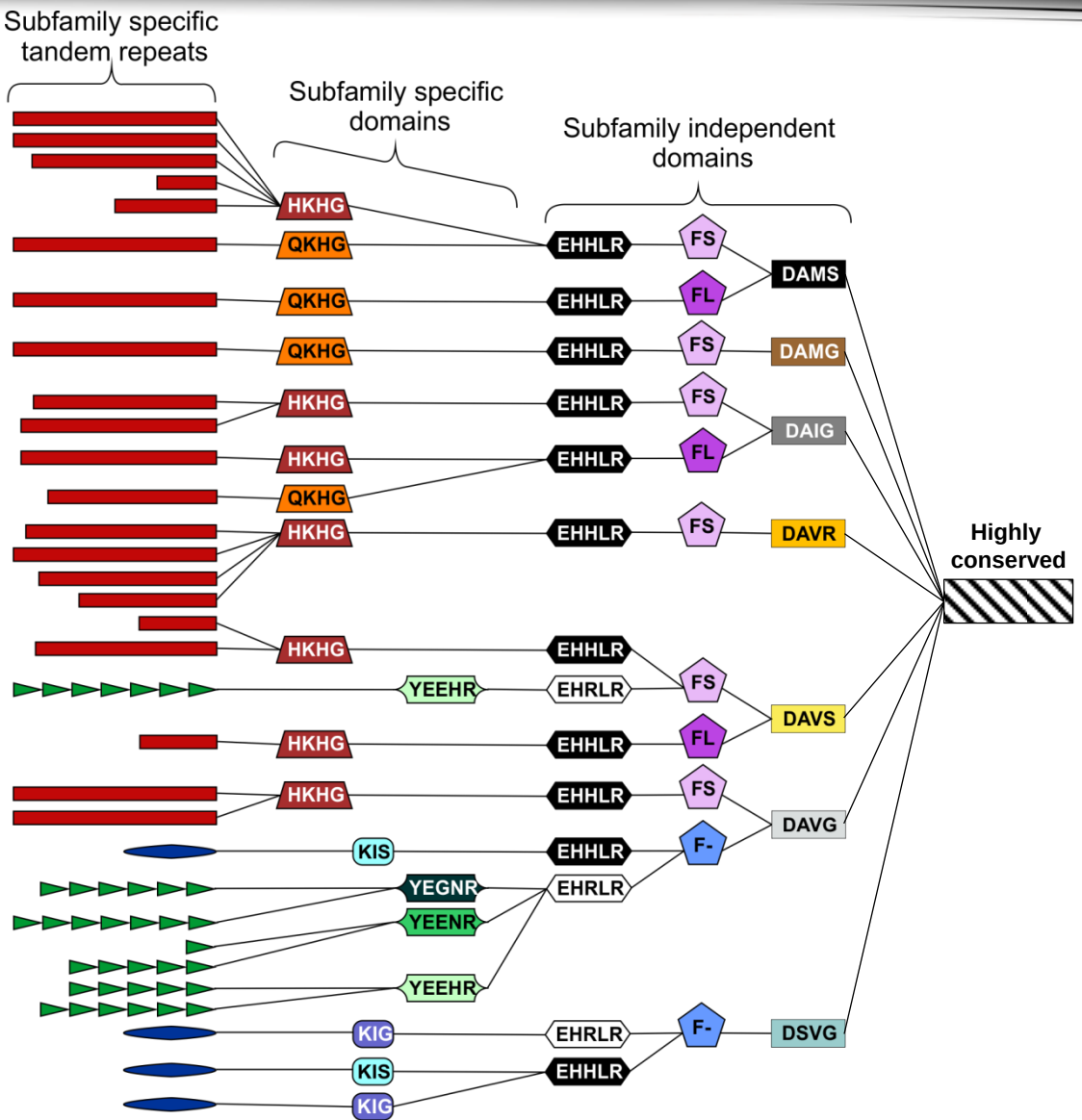
Secreted into the plant?

- Cell walls are coloured in blue
- Feeding site cells are indicated with * Nematode = N
- **Nematode effector protein in green**





Unique and unusual gene family



Key:
Subfamily - A
Subfamily - B
Subfamily - C

- Over 80 unique genes were identified that all correspond to the same gene family.
- These can be split into 3 subfamilies
- Due to the Hyper variability these genes have been named HYPs
- All HYPs share a common conserved domain.

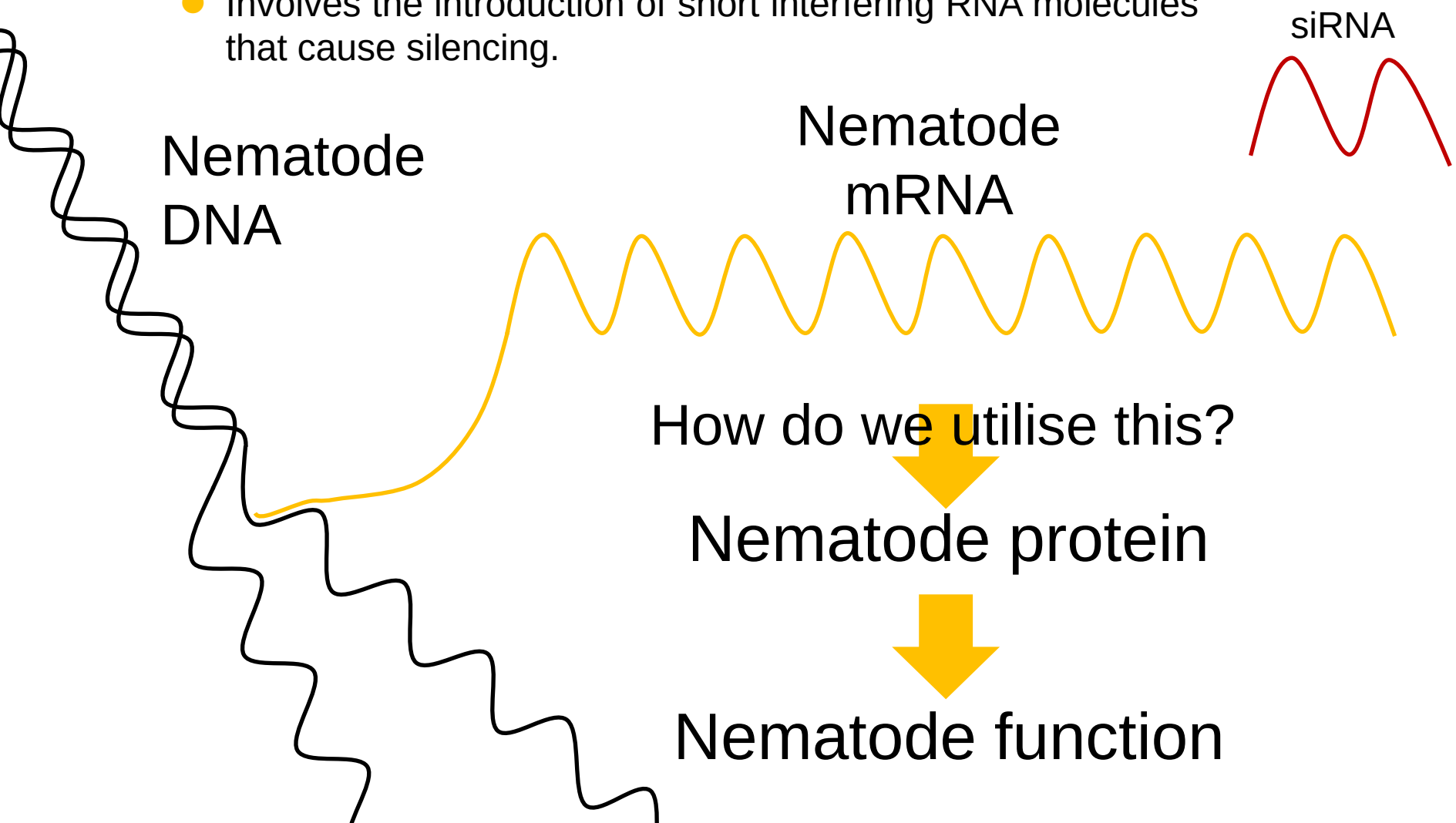
How can this help?

- If we have identified an important nematode gene – we can use targeted silencing to “switch it off”

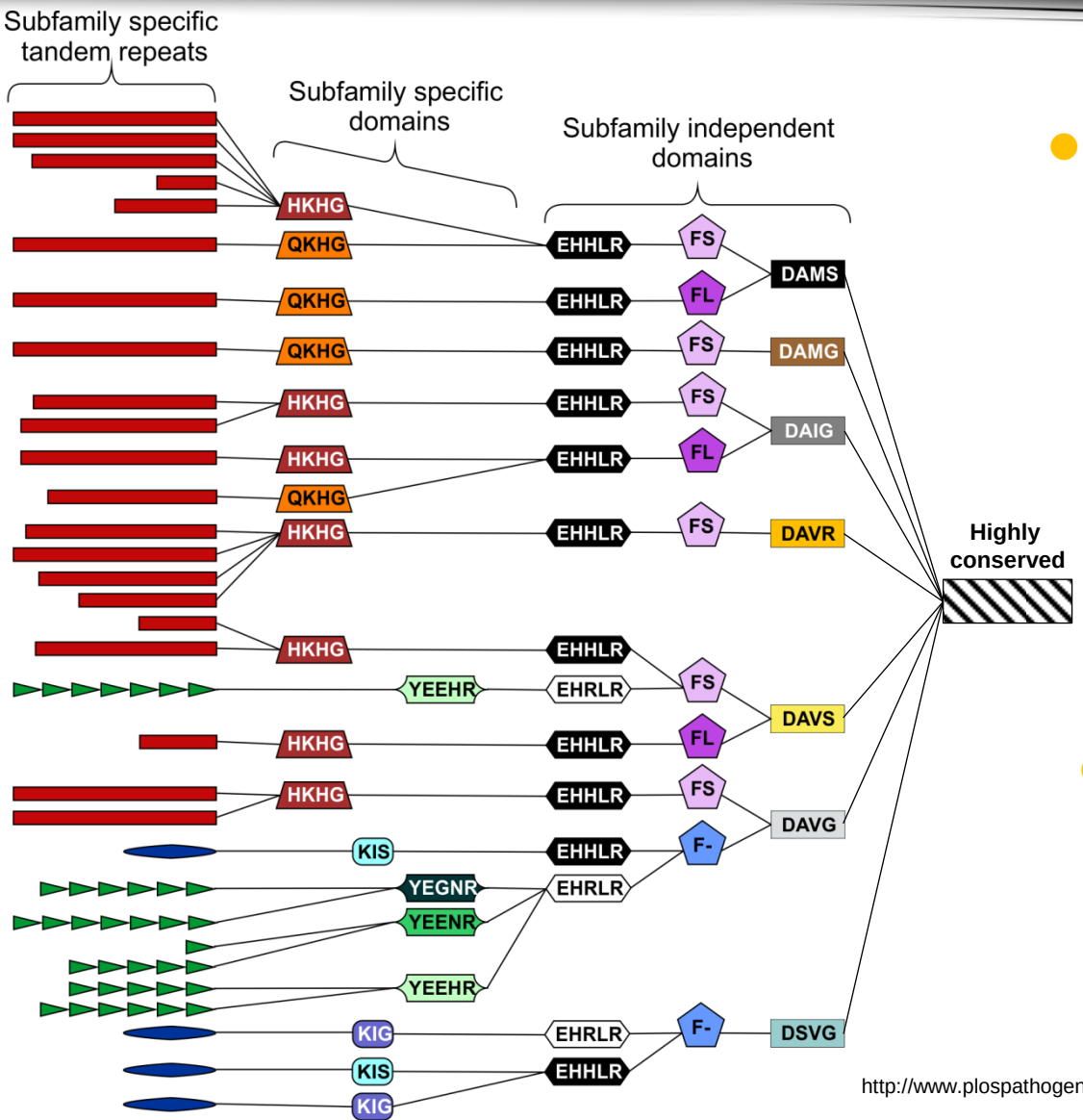
- For this we use a technique called **RNA interference (RNAi)**

RNA interference (RNAi)

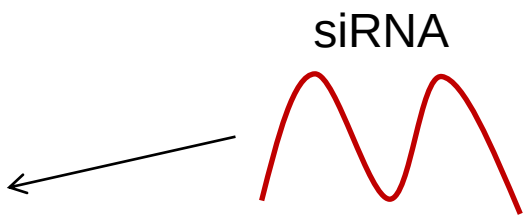
- Involves the introduction of short interfering RNA molecules that cause silencing.



Target the conserved region



- siRNA that causes the degradation can correspond to the highly conserved region

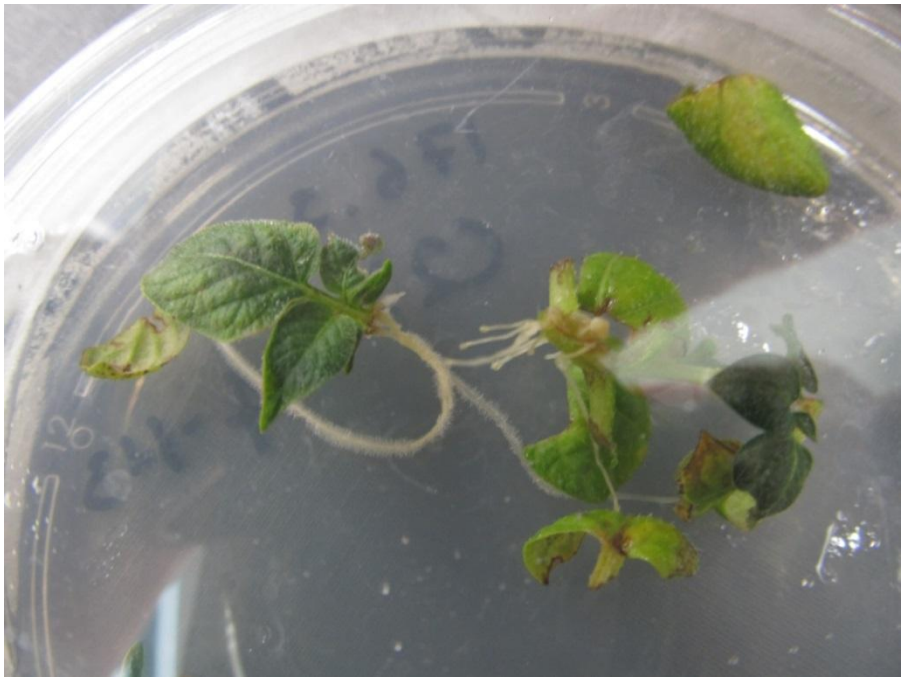


- This way – all members of all subfamilies can be targeted with a single construct.

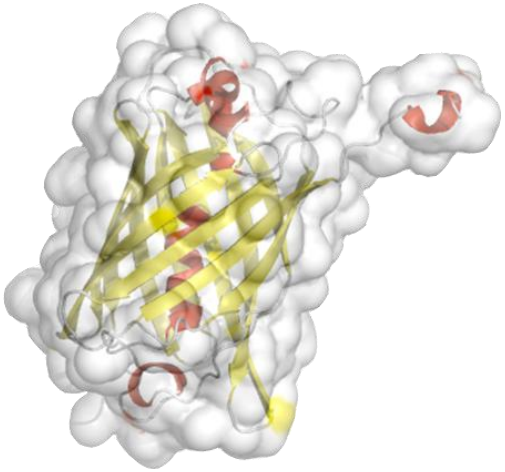
http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1004391

RNA interference (RNAi)

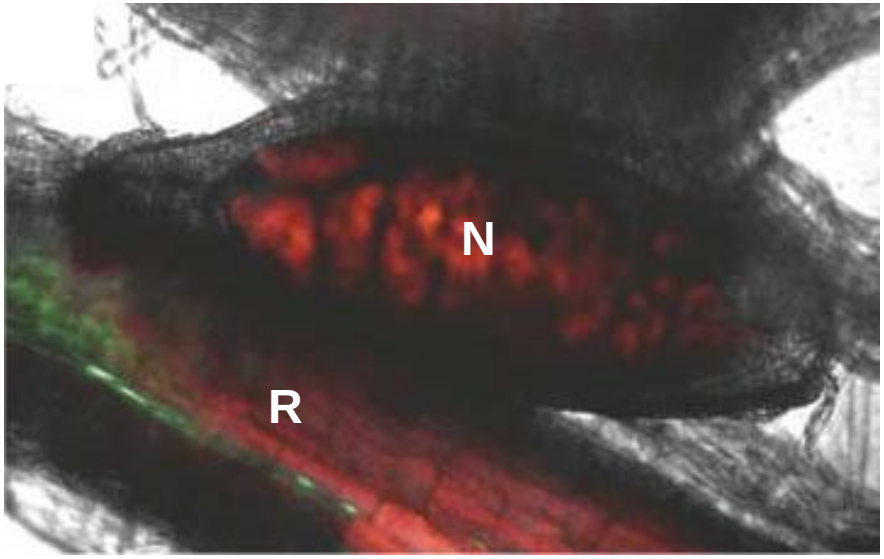
- Transgenic potato roots in sterile tissue culture
- Transfer the siRNA into plants – when the nematode feeds on the plant, it will take up the siRNA, and silence the gene
- How do we know it will work?



Delivery to the nematode - proteins



- Express a red fluorescent protein in the roots of plants (R), can see fluorescence in the digestive system of the nematode (N).

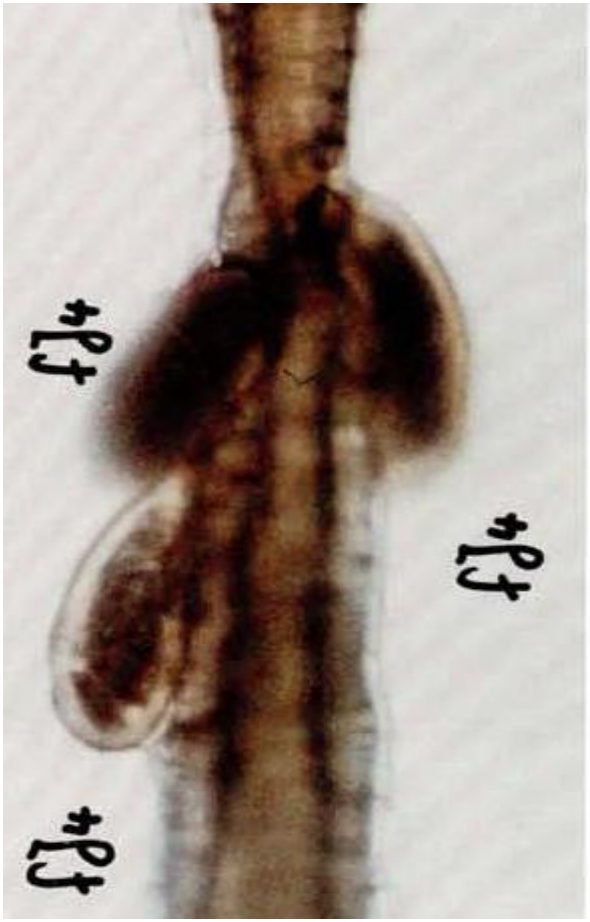


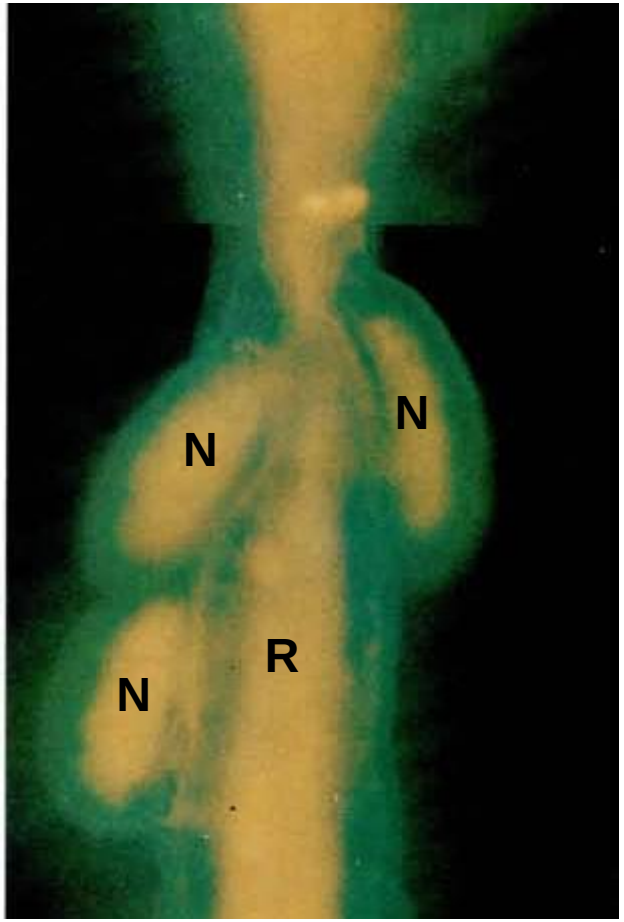
Valentine TA, Randall E, Wypijewski K, Chapman S, Jones J, et al. (2007) Delivery of macromolecules to plant parasitic nematodes using a tobacco rattle virus vector. Plant Biotechnology Journal 5: 827–834.

Delivery to the nematode - sugars

- Fluorescently labelled dextrans produces similar results.

- Root (R)
- Nematode (N)

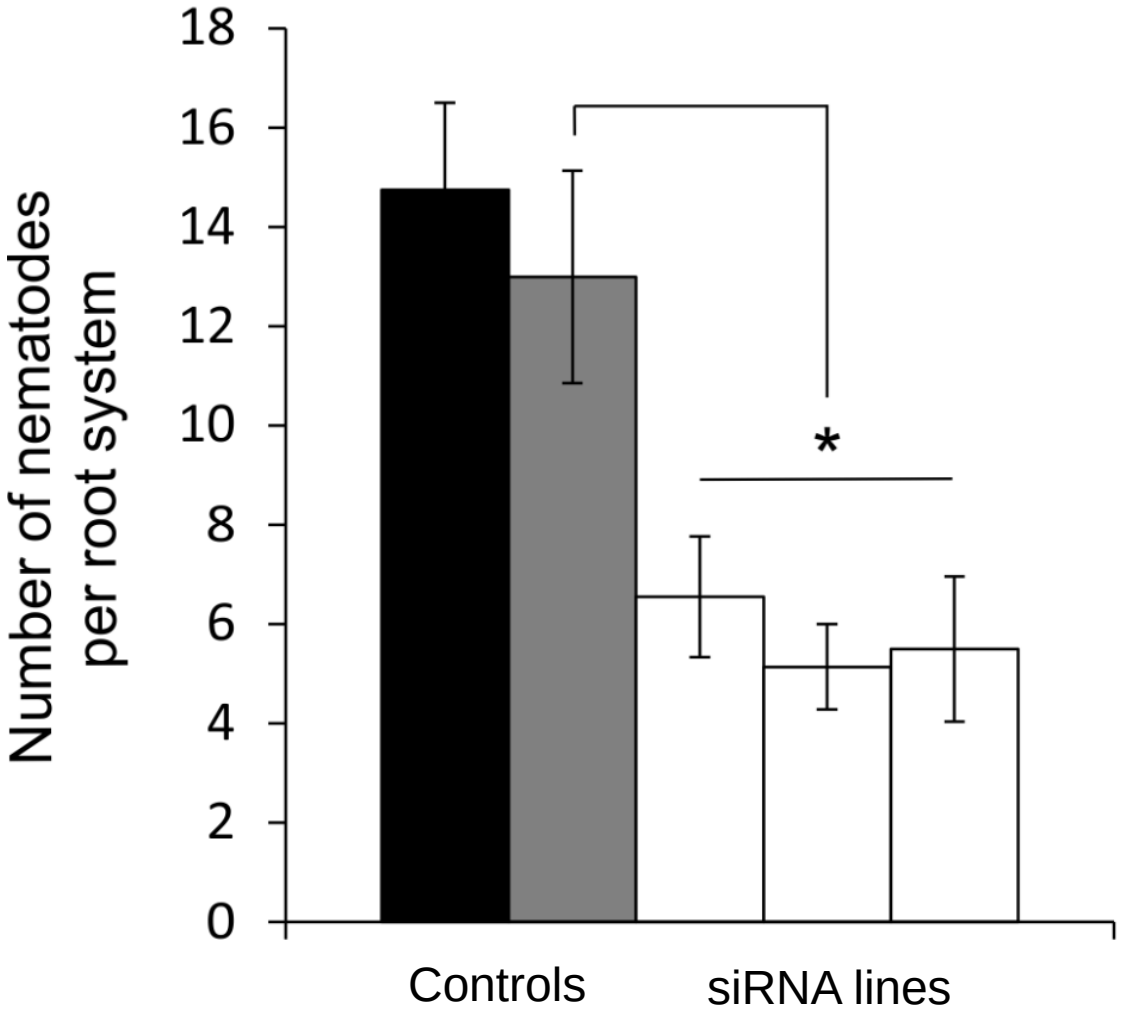




Bockenhoff A, Grundler FMW (1994) Studies on the nutrient-uptake by the beet cyst-nematode Heterodera-schachtii by in-situ microinjection of fluorescentprobes into the feeding structures in Arabidopsis-thaliana. Parasitology 109: 249

RNA interference (RNAi)

- Significant reduction in total nematode infection by approximately 55 - 65% ($p<0.05$)
- This can also inform function



David Miller

- With the aid of the David Miller Travel Award I was able to attend and present my PhD findings at the International Symposium on Molecular Plant-Microbe Interactions (IS-MPMI) in Rhodes, Greece.

- The ideas and knowledge acquired, made possible by the David Miller Travel Award, have been directly translated into current grant applications.

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