Molecular characterisation of eggshells from the potato cyst nematode *Globodera rostochiensis*

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1. Introduction

- Potato cyst nematodes (PCN) are sedentary endoparasites that hatch in response to root exudates from host plants.
- Hatching stimuli from host plants cause a change in eggshell permeability. Influx of water rehydrates quiescent juveniles prompting metabolic activation.
- Due to their complex structure, not much is known about the compounds initiating hatching in PCN.
- Identifying proteinacious components from the eggshell might give further information on how the eggshell can respond to host root exudates.
- Upon recieving stimulation from host root exudates, there is also a known calcium mediated change in eggshell permeability. However, no calcium binding sites have yet been localised to the PCN eggshell.

1.1 Summary of the PCN hatching cascade

Hatching stimulation
↓
Ca^{2+} mediated change in eggshell lipid layer permeability
↓
Loss of trehalose from perivitelline fluid
↓
Uptake of water by juvenile
↓
Juvenile becomes metabolically active
↓
Enhanced juvenile activity
↓
Exploratory stylet probing
↓
Subpolar slit cut in eggshell by stylet; no enzyme activity
↓
Juvenile hatches from the egg
↓
Further water uptake to full hydration
↓
Emergence of juvenile from the cyst

Modified from Masler and Perry (2018), Hatch survival and sensory perception in Cyst Nematodes, Chapter 3

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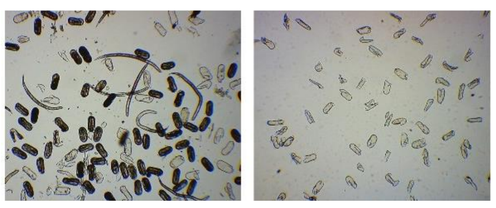
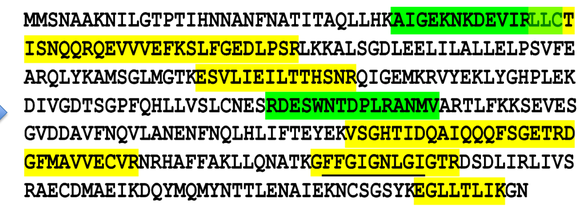
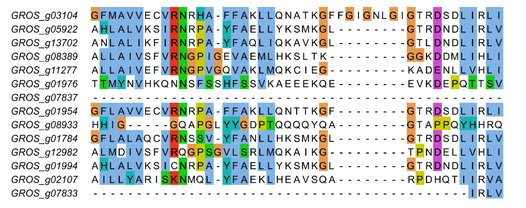
2. Methods – Identifying nematode eggshell proteins



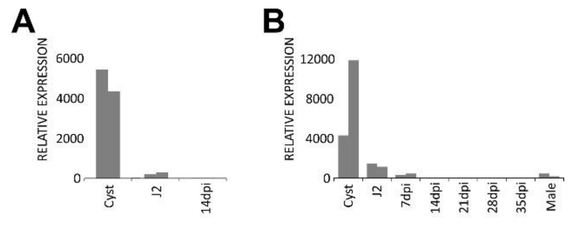
Collection of eggshells following ultrasonication and purification by a sodium-potassium tartrate gradient



Proteins are removed from eggshells using methanol. Protein samples are identified by mass spectrometry. Identified peptides unique to the candidate protein are highlighted



Homologous proteins to the candidate protein are aligned. Motifs unique to the candidate protein are compared to the unique peptides identified by mass spectrometry

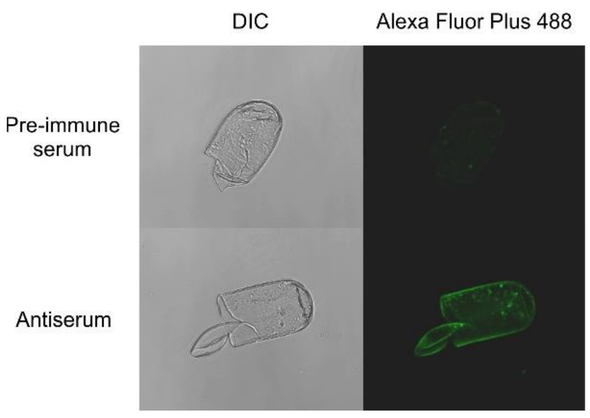


Protein candidates are selected. Eggshell proteins should show very little expression in juvenile stages and increased expression in female/egg producing stages.

The above example is for the annexin GROS_g03104. This protein was of interest due to the calcium dependant lipid binding properties of annexins which could explain the previously identified calcium mediated change in eggshell permeability required for PCN hatching. Other proteins identified included chondroitin proteoglycans which are known *C. elegans* eggshell proteins.

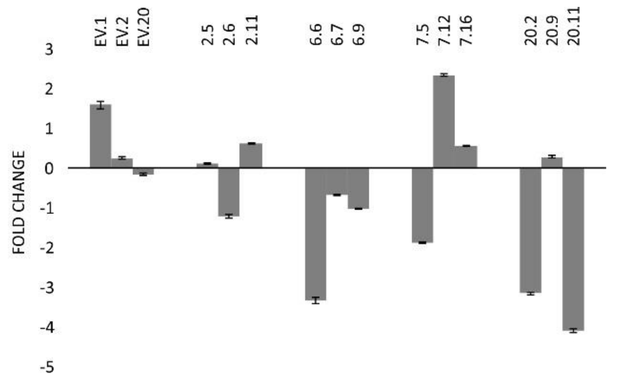
3.1. Localising the nematode annexin GROS_g03104

Antibodies specific to the annexin GROS_g03104 were produced. Peptides used for antibody production are highlighted in green in section 2. Clear localisation to the eggshell was seen.



3.2 Creating annexin knock-down populations

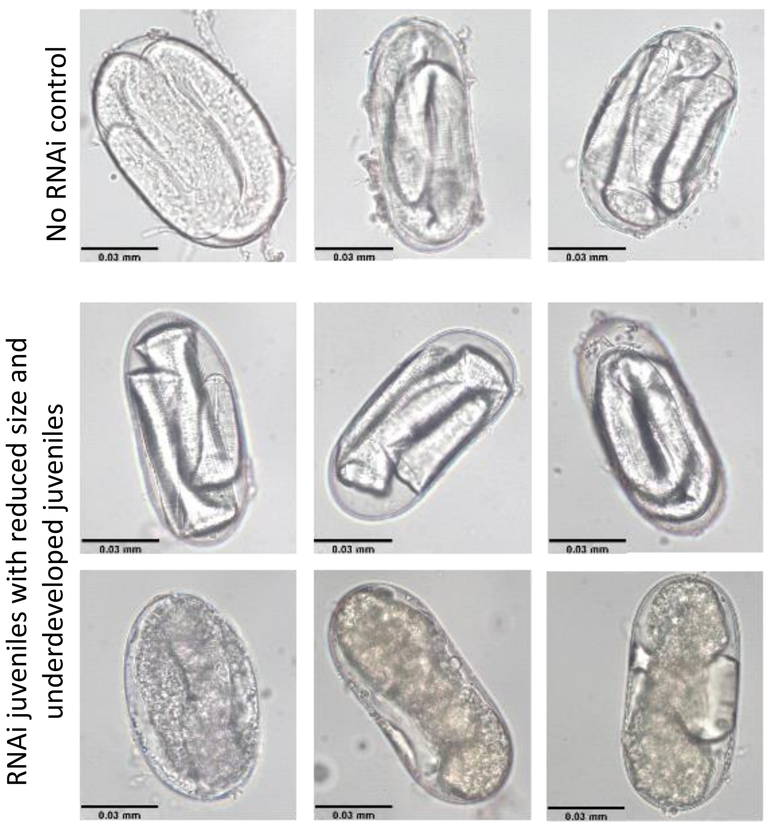
Short-hairpin RNA was expressed in lines of Desiree. 4 plant lines were tested (2, 6, 7 & 20) with one control line that had been through the transformation process using an empty vector (EV). Each line was split into 20 cuttings and each cutting was infected with 20 cysts from a 2012 *G. rostochiensis* population. After 7 weeks, females present on the roots were collected and q-PCR was used to determine the level of annexin knock down.

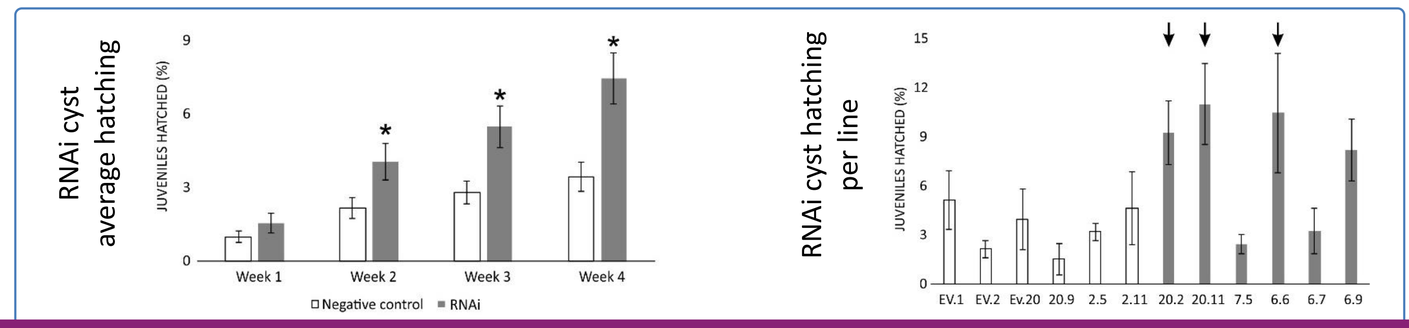


3.3 Testing annexin knock-down populations

Populations collected from modified Desiree lines. Increased hatching was seen in annexin knock down populations (right). Lines showing higher levels of knock down also showed the most hatching (as marked with arrows).

Juveniles from annexin RNAi populations appeared shrunken inside the eggs compared to no RNAi controls (left). Increased numbers of underdeveloped juveniles were also notable.





4. Conclusions

1. The PCN annexin GROS_g03104 is the first protein to be identified and localised in any parasitic nematode eggshell.
2. Identification of chondroitin proteoglycans (CpGs) in PCN eggshell protein extractions suggests that the CpG layer seen in *C. elegans* eggshells is also present in PCN eggshells.
3. Knocking down the eggshell annexin results in delayed juvenile development, decreased juvenile size and increased hatching.
4. These results suggest association of the annexin with the eggshell permeability barrier. Knocking down this protein alters permeability of the eggshell. This allows increased dehydration of juveniles in the eggshells (reducing their size) and increases the ability to rehydrate after dormancy, increasing the number of juveniles hatching.