Somaclonal variation in plant-parasitic nematodes as revealed by *Pasteuria* – the potential importance of innate immunity

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Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, UK

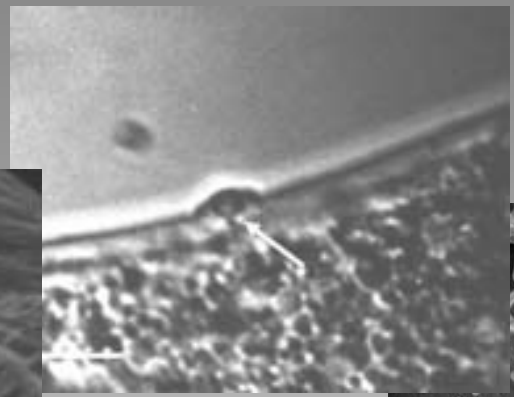
www.rothamsted.bbsrc.ac.uk/ppi/staff/kgd.html

Structure of talk

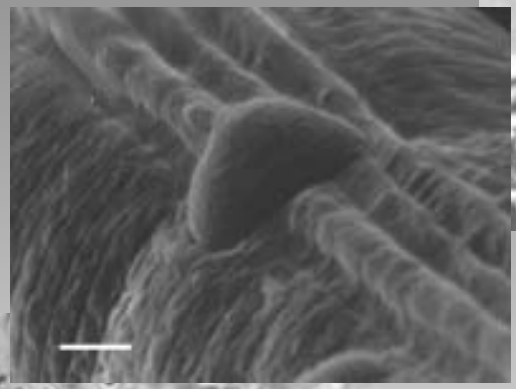
1) Introduction
2) *Pasteuria penetrans* life-cycle and endospore attachment
3) Variation in spore attachment to single juvenile descent lines of nematodes
4) DAF pathway and exogenous peptide manipulation of fecundity
5) Modulation of surface coat using exogenous peptides
6) Summary and conclusions

The life-cycle of *Pasteuria penetrans* on root-knot nematodes (*Advances in Parasitol. In prep.*)

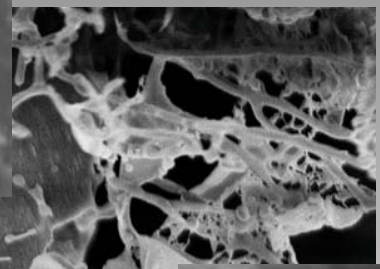
Penetration peg



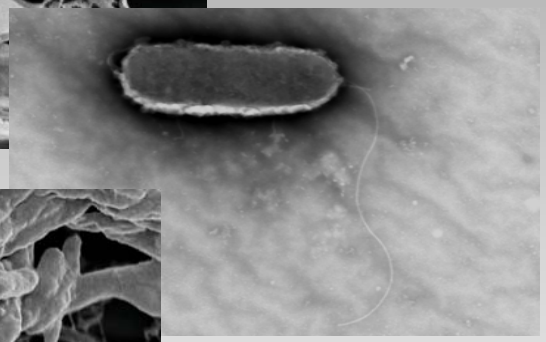
Adhesion to cuticle

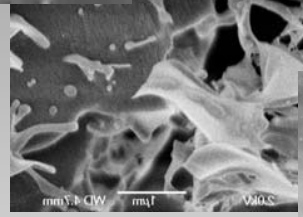


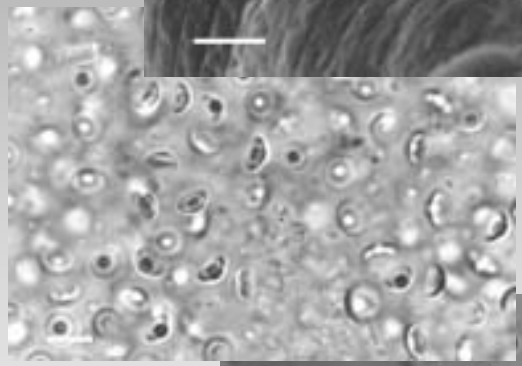
Rhizoids



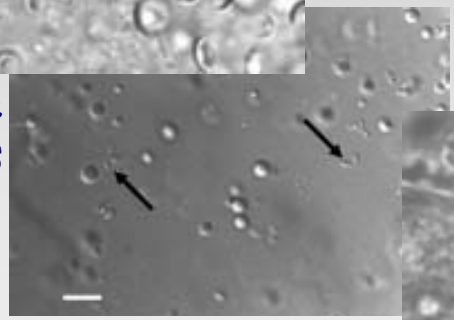
Rod



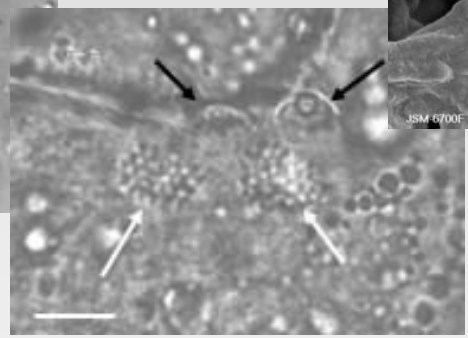




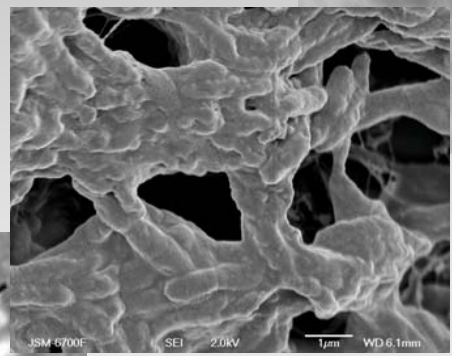
Mature endospores



Maturation

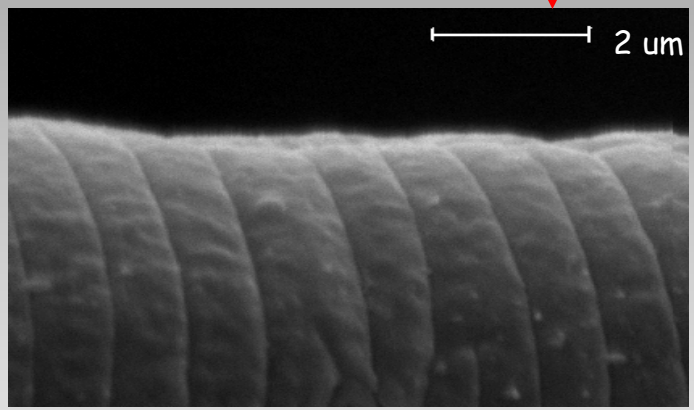


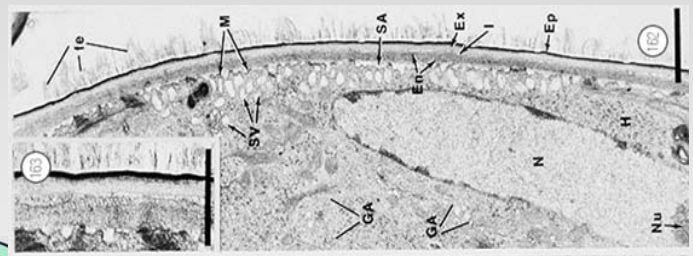
Sporulation

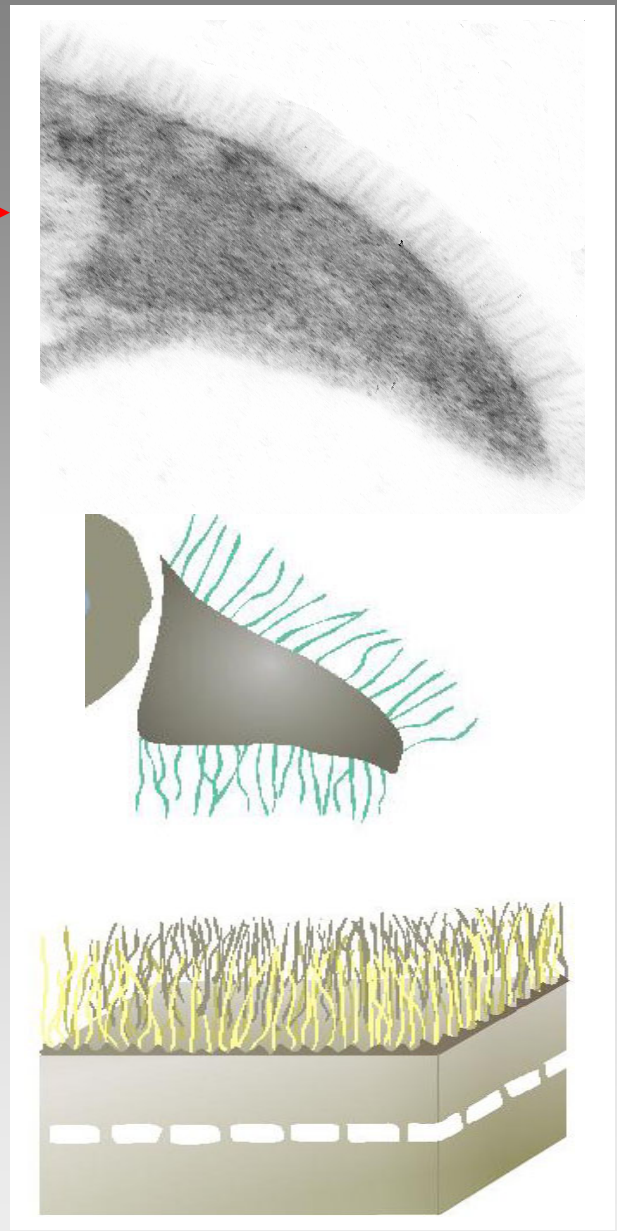


Granular masses

Spore attachment: *Velcro*-like mechanism

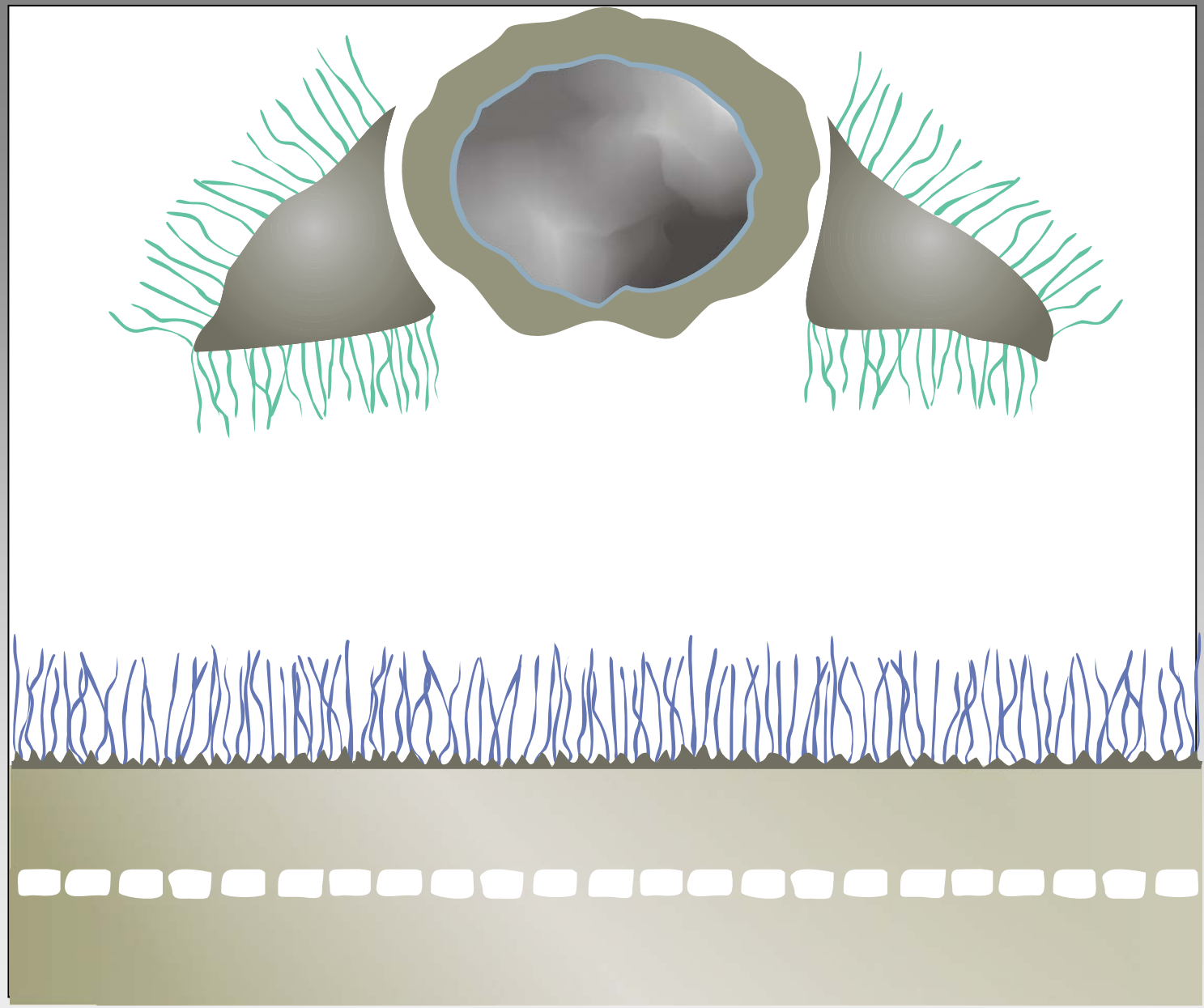






Davies, *Advances in Parasitology* In prep.

Spore attachment: *Velcro*-like mechanism



Davies, 2008, *Advances in Parasitology* in prep.

Reproductive mechanisms in major groups of Root-knot nematodes *Meloidogyne* spp.

Nematode spp.	Chromosome No.	Mode of reproduction
M. arenaria	30-46 polyploid	Mitotic parthenogenesis
M. incognita	41-46; polyploid	Mitotic parthenogenesis
M. javanica	42-48; polyploid	Mitotic parthenogenesis
M. hapla Race A	13-17; n	Amphimixis & meiotic parth.
M. hapla Race B	43-48; polyploid	Mitotic parthenogenesis

After Evans, AAF (1998) In: The Physiology and Biochemistry of Free-living and Plant-parasitic nematodes CABI (Eds RN Perry & DJ Wright)

Experimental design

M. incognita

Single egg mass 02-08-04

Routinely subcultured every 4-6 months

- 3 spore → 8 single egg mass populations → Tested against PP3 & RES147
- 26 spore → 8 single egg mass populations → Tested against PP3 & RES147

M. hapla VW8 & VW9

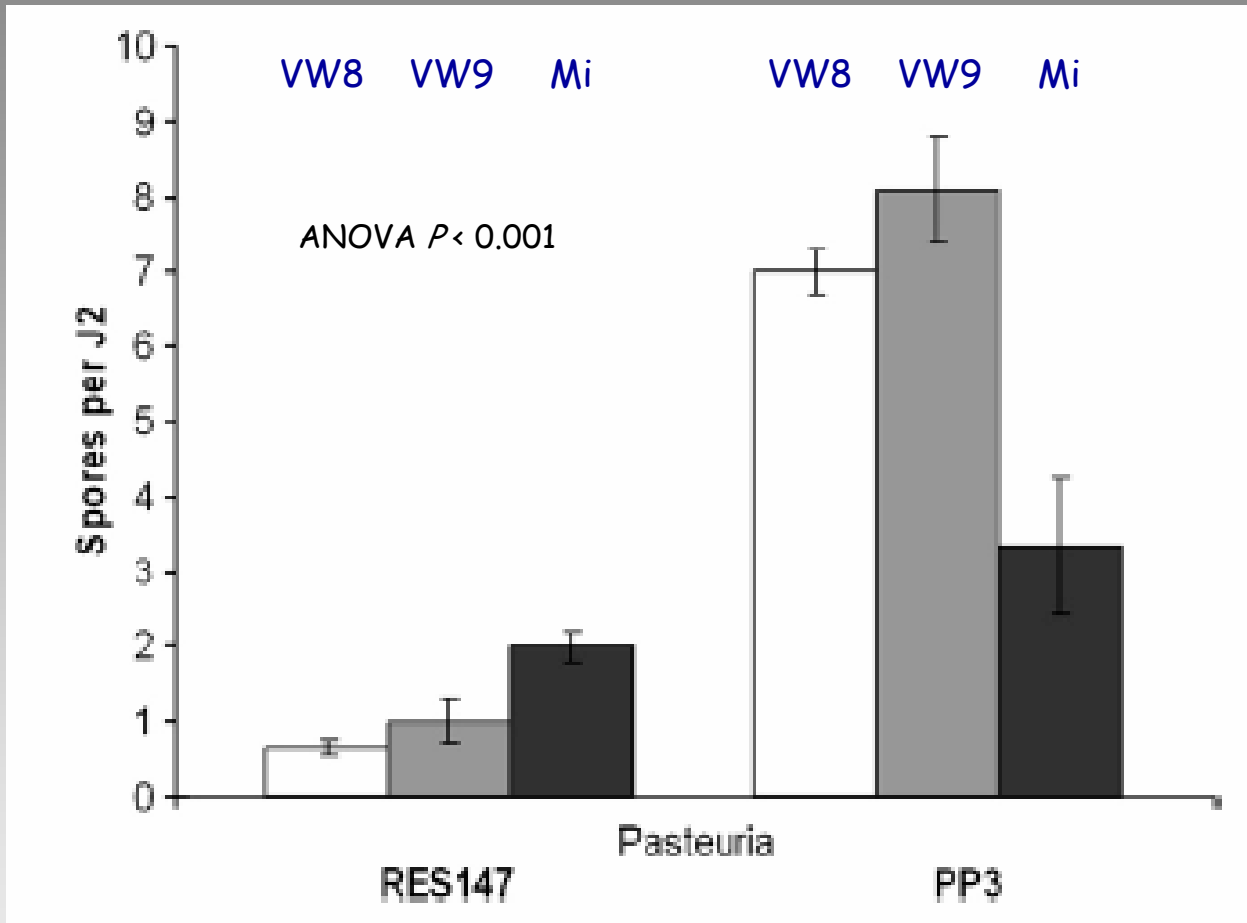
Single egg mass

18 generations of single egg mass

- VW8 single juveniles → 2 single egg masses from 2 single J2 populations → Tested against PP3 & RES147
- VW9 single juveniles → 2 single egg masses from 3 single J2 populations → Tested against PP3 & RES147

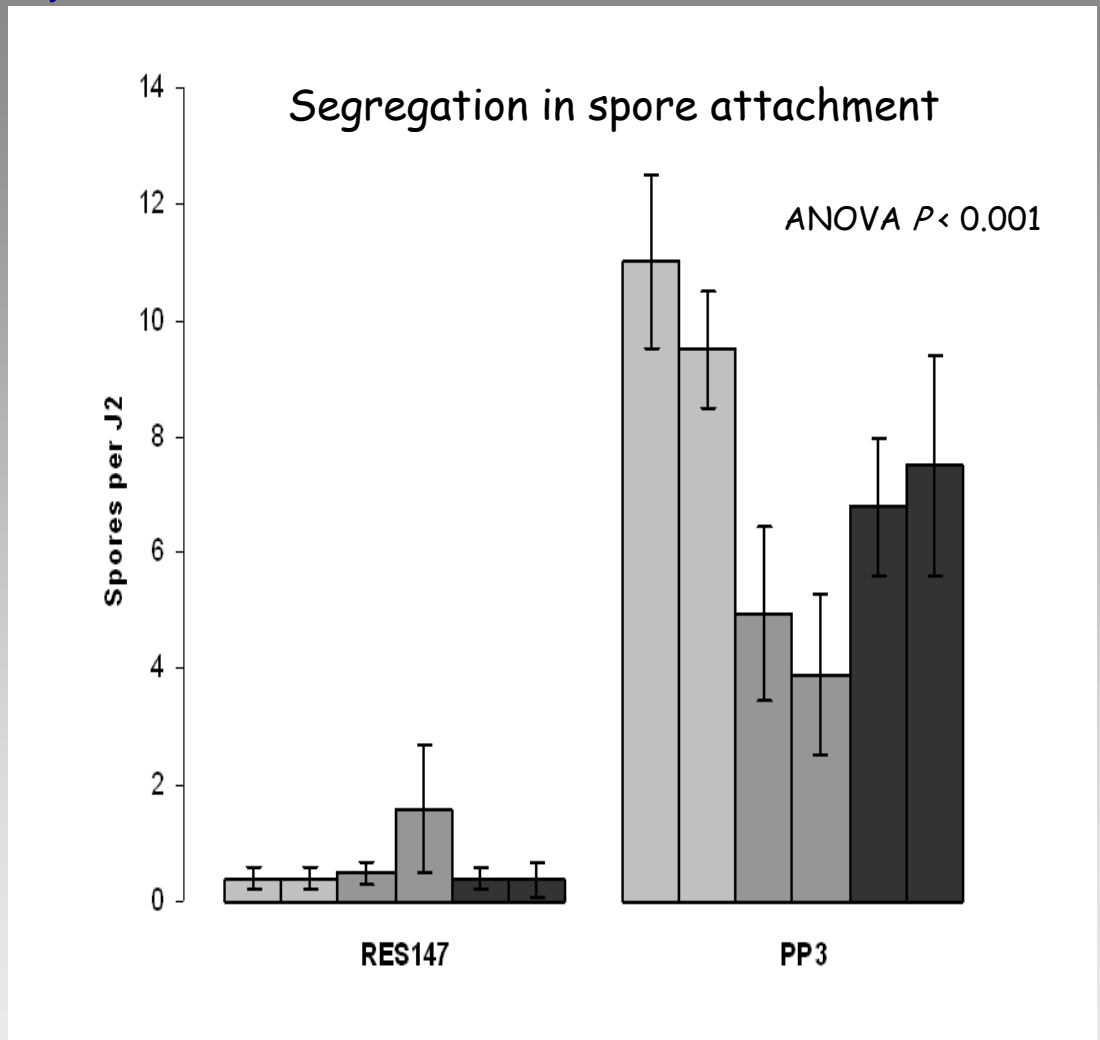
Davies, Rowe & Williamson 2008 *Int. J. Parasit.*, 38, 851 - 859

Endospore attachment of *Pasteuria* populations RES147 & PP3 to *Meloidogyne hapla* strains VW8 & VW9 and *M. incognita*



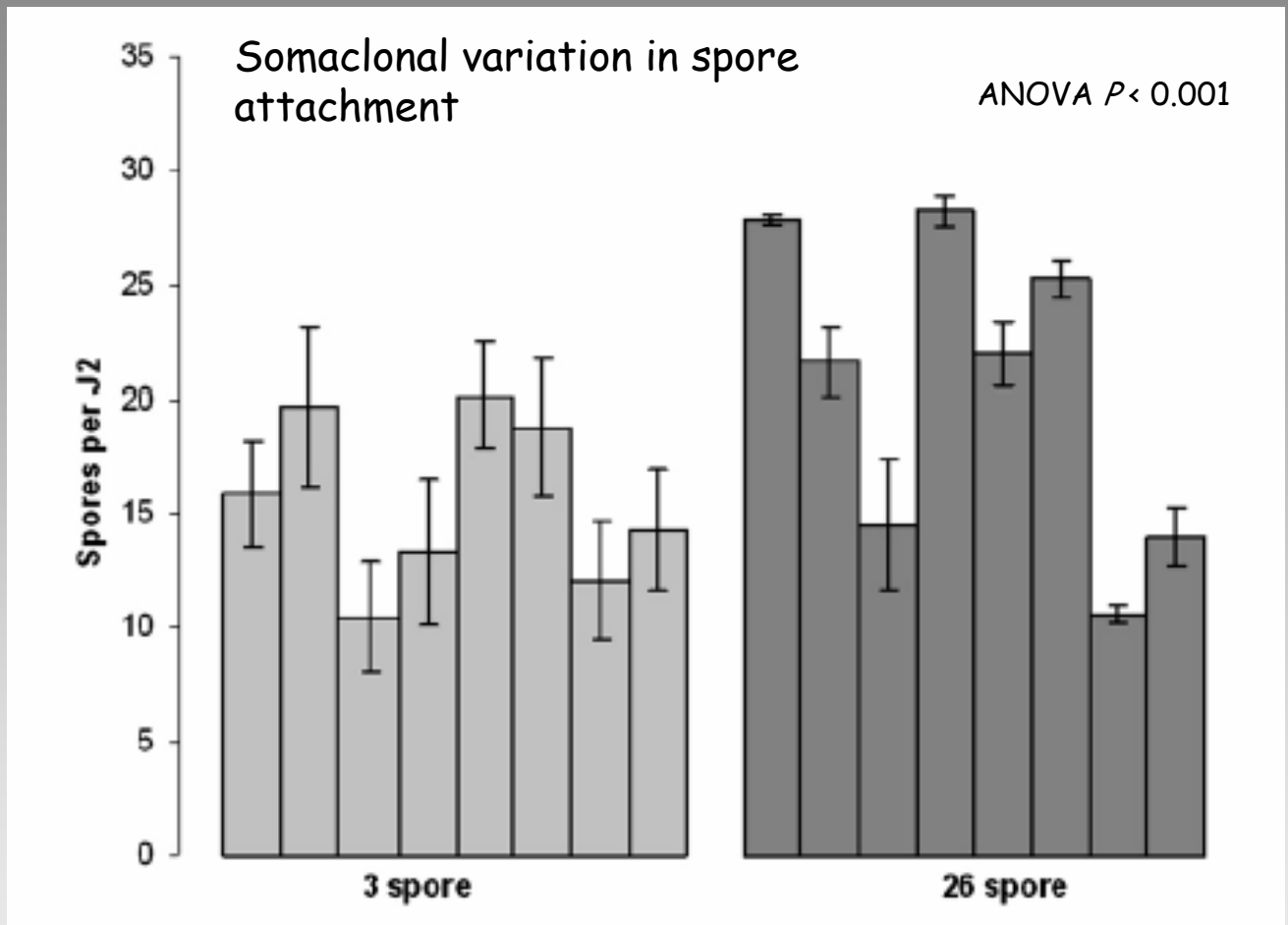
Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

Spores, RES147 & PP3, attaching to single juvenile descent lines arising of *M. hapla* VW9K1 (light grey), VW9K2 (dark grey), VW9K3 (black)



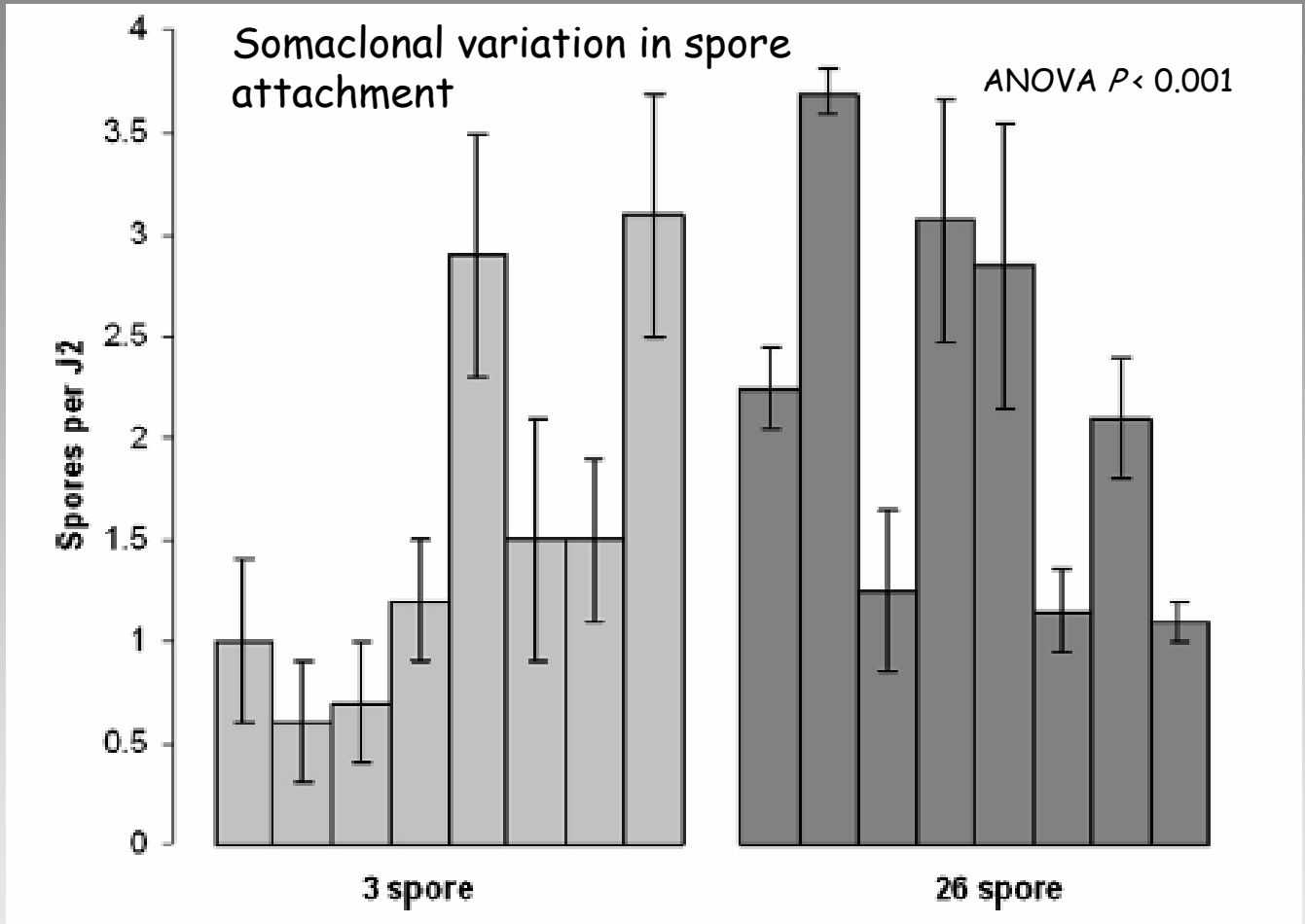
Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

Endospore attachment of *Pasteuria* population PP3 to single juvenile descent lines of *Meloidogyne incognita*



Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

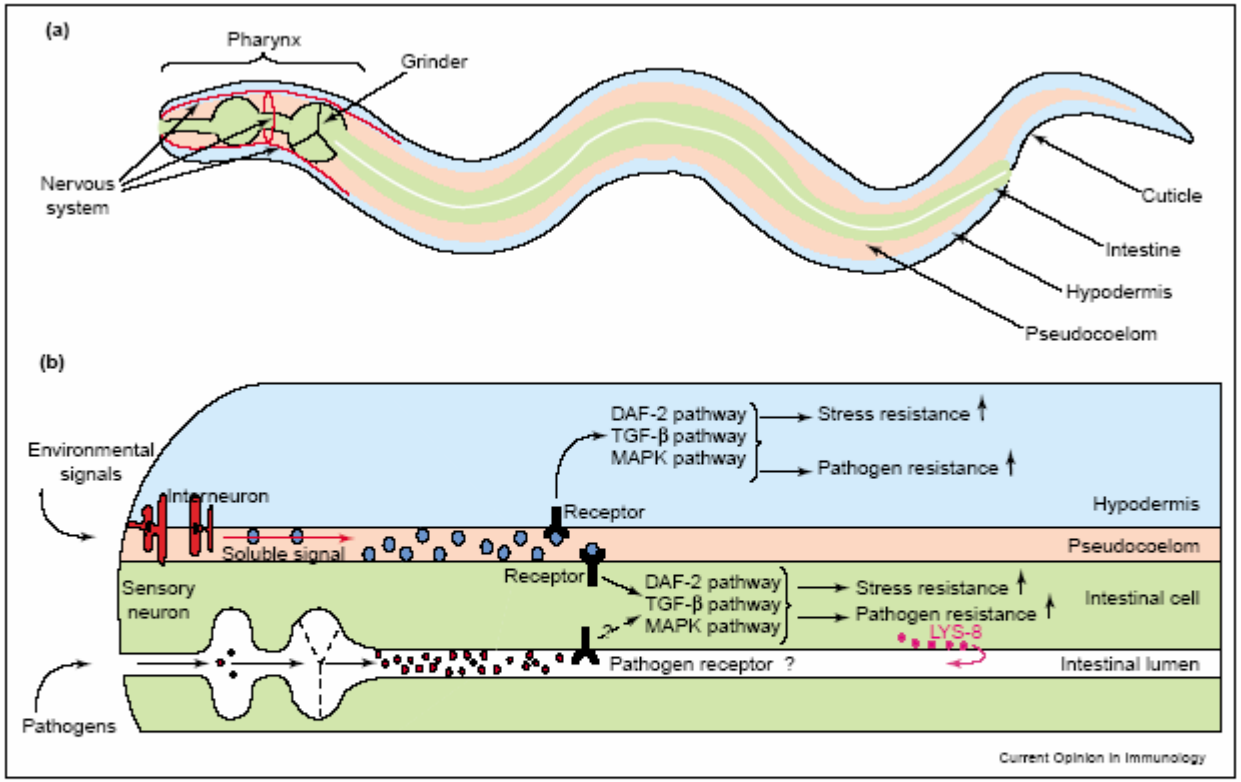
Endospore attachment of *Pasteuria* population RES147 to *Meloidogyne incognita*



Davies, Rowe & Williamson 2008 *Int. J. Parasit.*,38, 851 - 859

DAF-2, IGF & innate immunity in *worms*

Figure 2



Antimicrobial defences in *C. elegans*. **(a)** Basic anatomy of *C. elegans*. Of particular note are the physical barriers, the grinder that mechanically disrupts the bacteria that form a worm's normal diet, and the cuticle that envelopes the animal, both of which protect the worm from microbial aggression, and the pseudocoelom, a fluid-filled cavity that separates the intestinal cells from the hypodermis. **(b)** A model for the cellular basis of innate immunity in *C. elegans*. The presence of pathogens in the environment is perceived via the sensory neurons, which generate a signal that is transmitted to target tissues via the pseudocoelom. Supporting such an idea is the fact that, in contrast to their ligands, which are secreted factors expressed in the nervous system, the different proteins involved in the DAF-2 and DBL-1 signalling cascades (see Figure 1) are expressed in the intestine and hypodermis, as are putative antimicrobial proteins, such as LYS-8 and R09B5.3. It is possible that the establishment of an infection in the intestinal lumen also plays a role in triggering a defence reaction, via an as yet uncharacterised mechanism. It is hypothesised that antimicrobial proteins and peptides are secreted into the intestine, via specialised vesicular traffic, as illustrated for LYS-8.

www.sciencedirect.com **Current Opinion in Immunology** 2004, 16:4–9

Anne Millet and Jonathan Ewbank 2004 Curr. Opin. Immunol. 16, 4-9

An evolutionary conserved phosphorylation cascade involving the insulin/insulin-like growth factor (IGF) receptor is well characterised in *C. elegans*

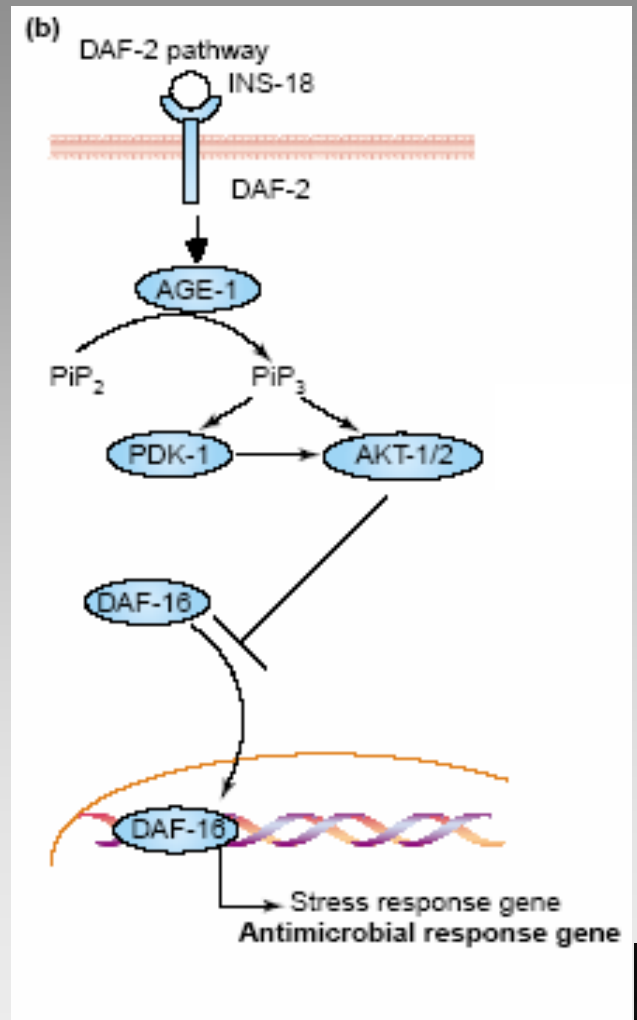
Activation of phosphorylation pathway begins with binding of insulin-like ligand to DAF-2 receptor (38 present in Ce only a few characterised)

DAF-2 activates AGE-1 which converts phosphatidylinositol biphosphate to a triphosphate

PiP_3 binds to the AKT-1/AKT2 complex that phosphorylates the Forkhead transcription factor DAF-16

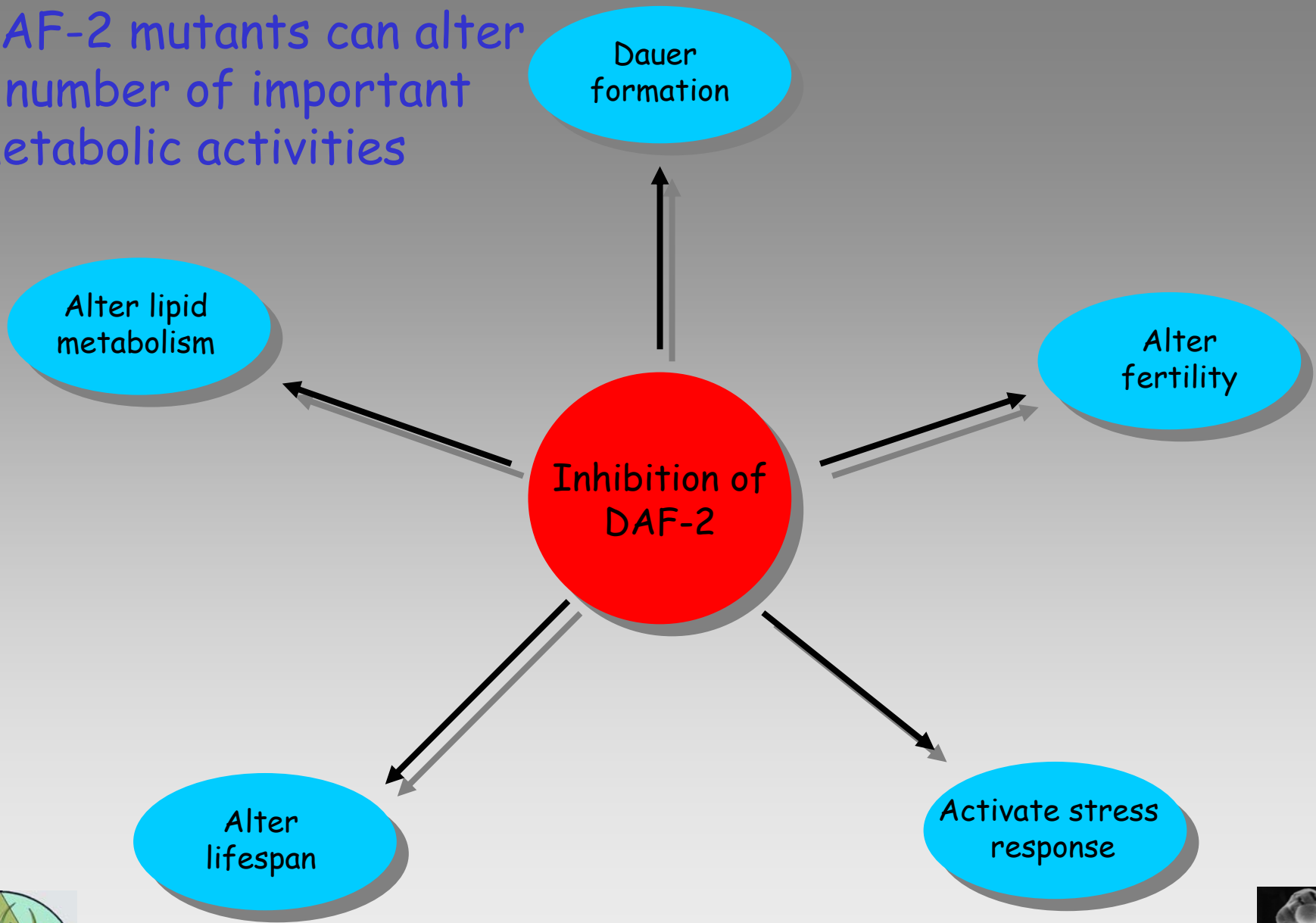
DAF-16+P cannot be translocated to nucleus to activate DAF-2 pathways

DAF-16 can enter nucleus
L1 and L2 activates dauer formation
L3 – adults activates stress response and anti-microbial genes



(Millet & Ewbank, 2004)

DAF-2 mutants can alter a number of important metabolic activities



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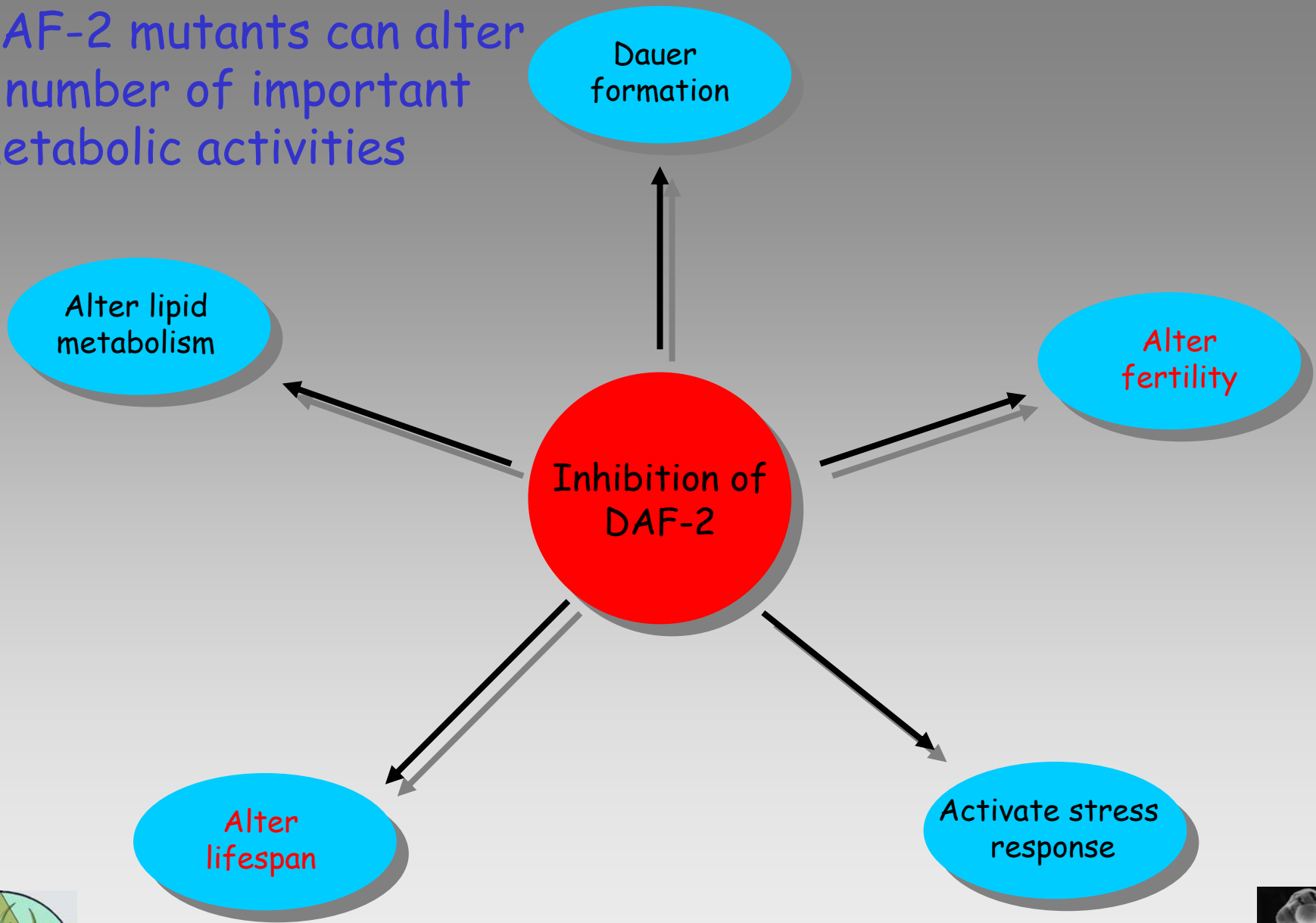
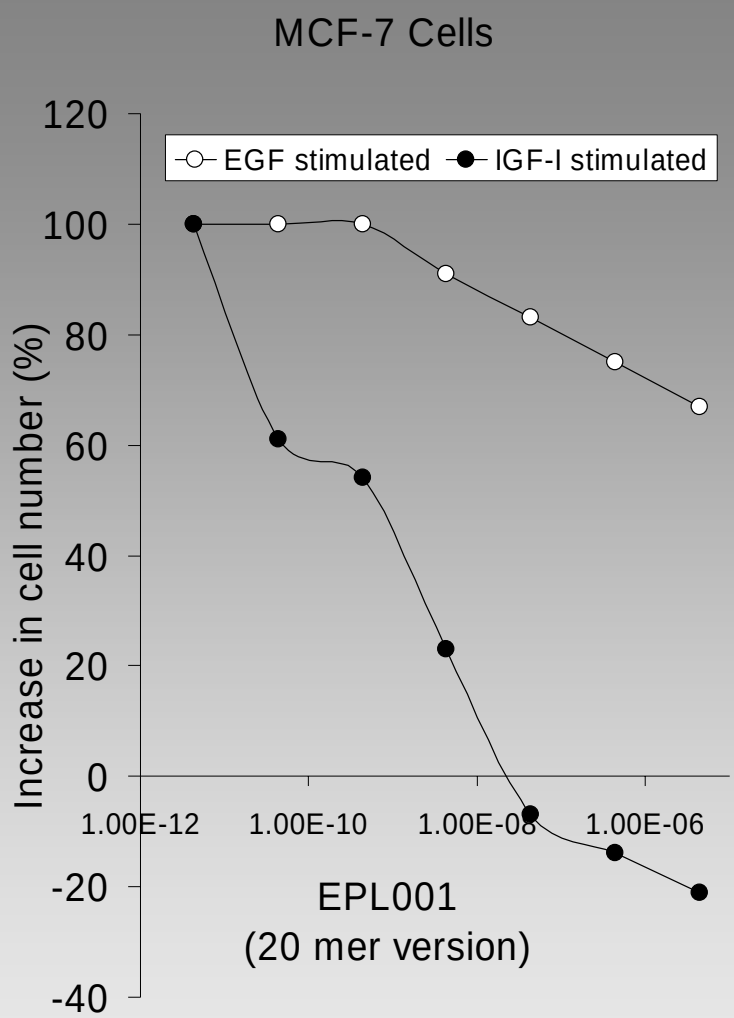


EPL001 is peptide that inhibits IGF:

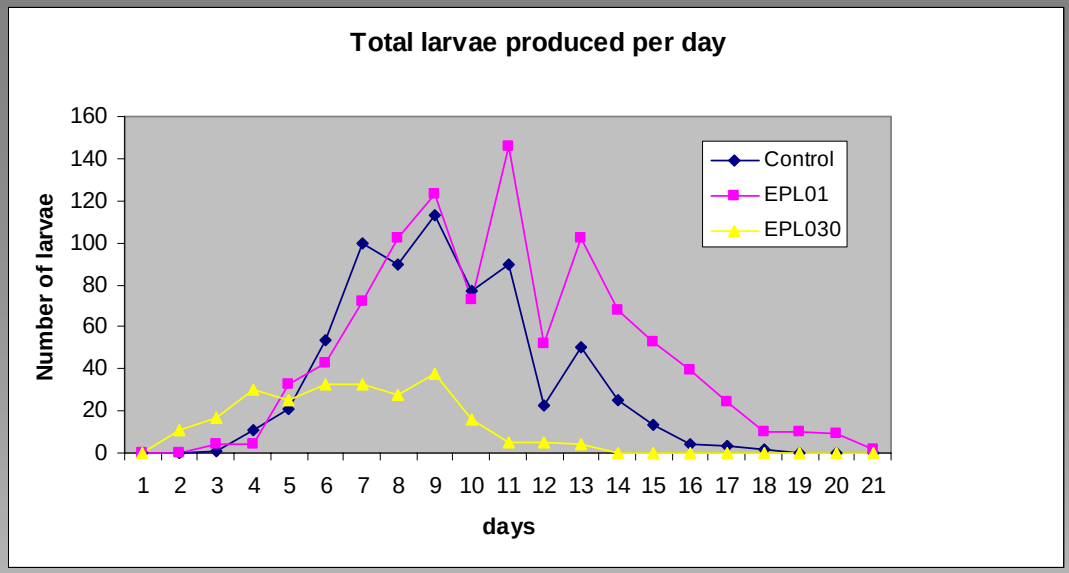
72 hours after application EPL001 inhibits

Epidermal Growth Factor (EGF)
&
Insulin Growth Factor (IGF)

stimulated
MCF-7 cells
(breast carcinoma cell line)



John Haylor *et al.*, in prep.

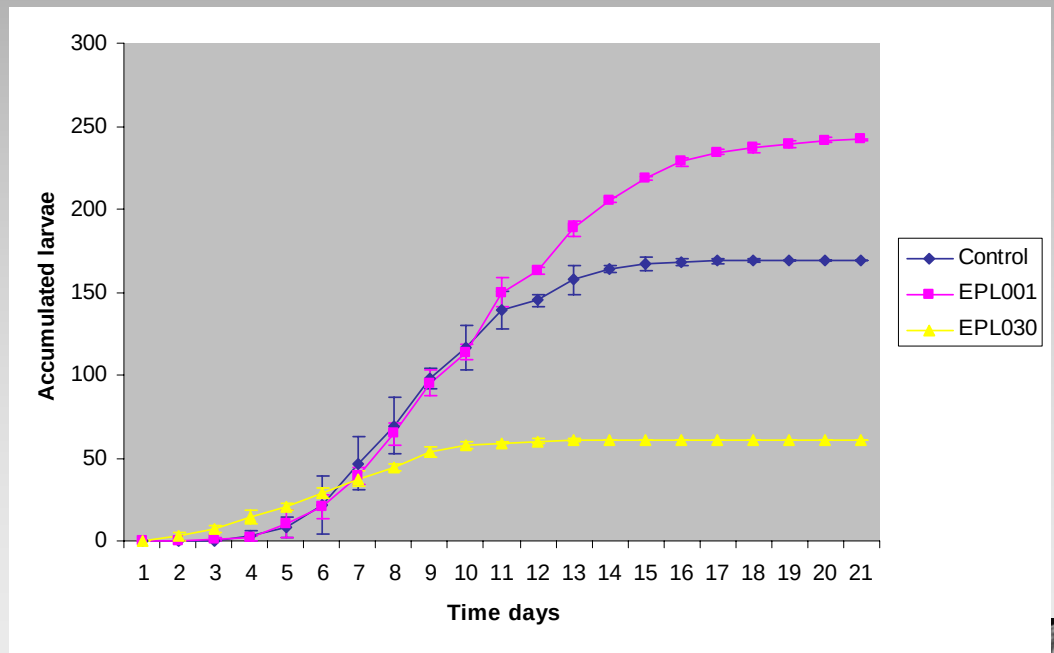


Manipulation of life-span and fecundity in *C. elegans*

Mean larvae per adult

Control	EPL001	EPL030
17	24	6
	+43%	-64%

ANOVA $P < 0.05$

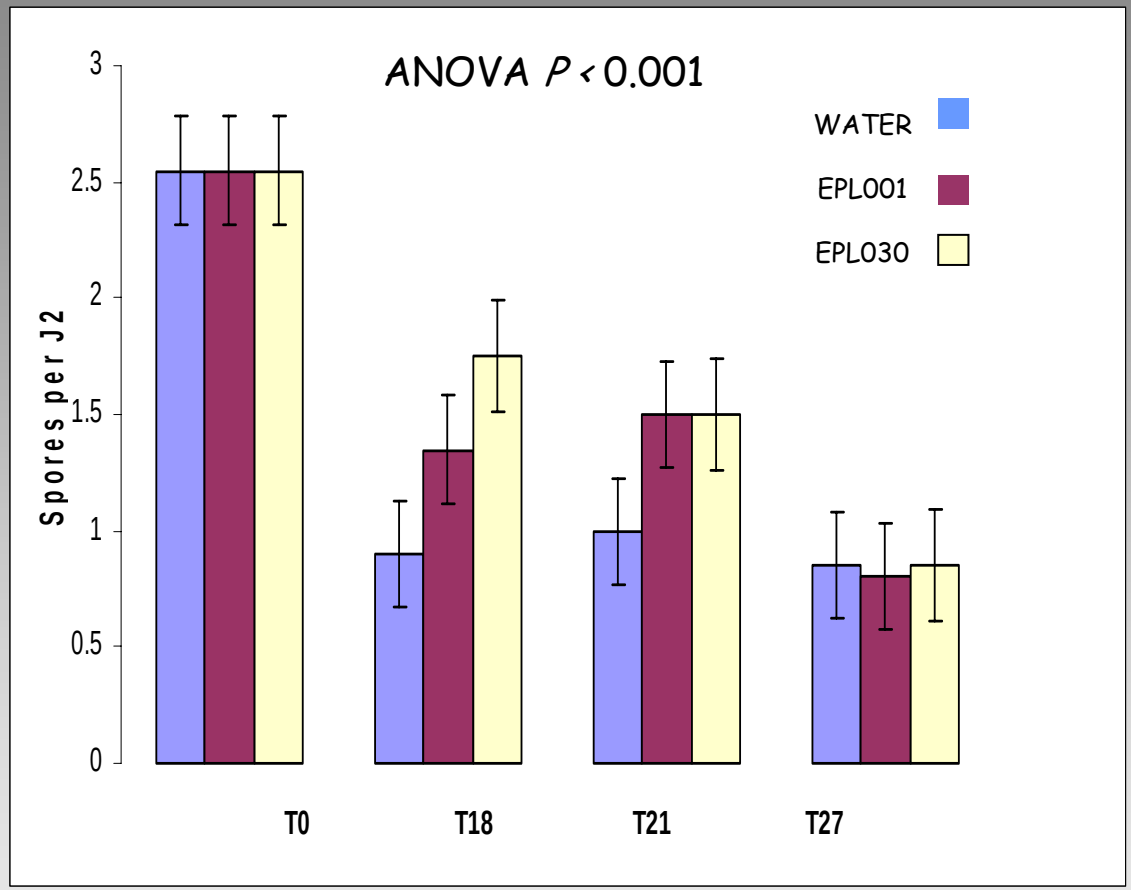


Davies and Hart (2008) *Nematology* 10, 103-112

The effects of EPL001 and EPL030 on the attachment of *Pasteuria* to root-knot J2s

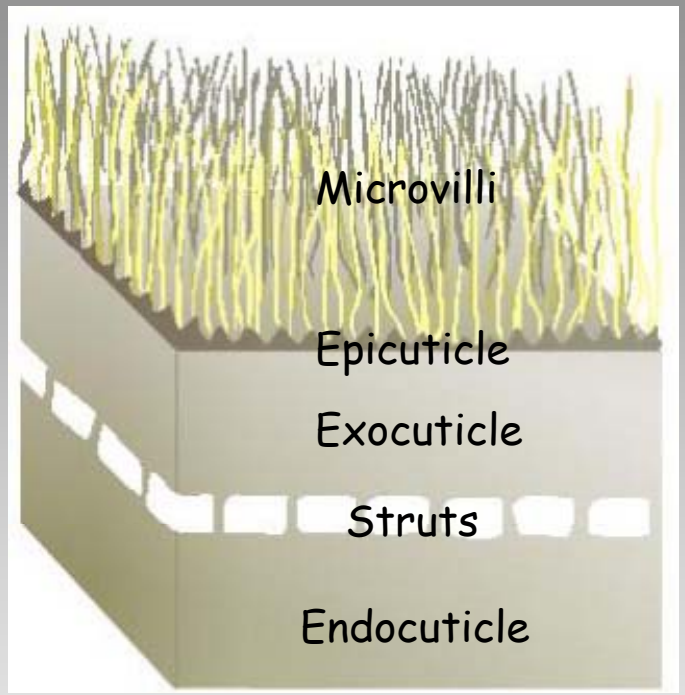
- *Meloidogyne incognita* allowed to hatch in water
- Treat with 1μM of EPL001 and EPL030 (water control)
- At 0, 18, 21 and 27 hrs wash (x3) water
- Endospore (strain RES147) attachment test by centrifugation
- Count endospores adhering to the cuticle

The effects of EPL001 and EPL030 on the attachment of *Pasteuria* to root-knot J2s

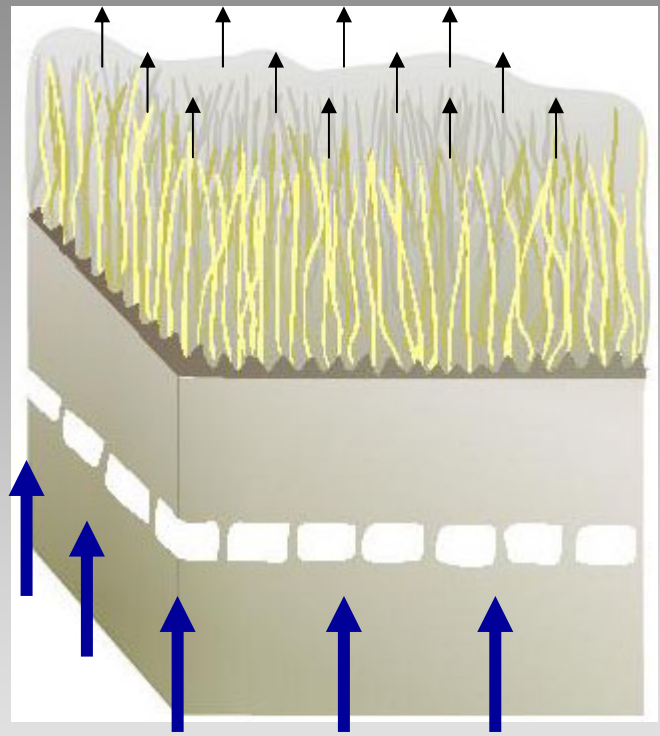


Davies unpublished

Modulation of surface coat through EPL001 and EPL030



Excretory/secretory products



Young → Old

Time line

DAF-2 modulation of E/S products

Davies unpublished

Summary and conclusions

1) A *Velcro*-like model of endospore attachment is proposed whereby Collagen-like fibres on the spore interact with microvilli on the surface of the nematode cuticle

2) Endospore attachment to the cuticle is host specific

3) Segregation in spore attachment occurred in single juvenile descent lines of *M. hapla*

4) Somaclonal variation could be observed in spore attachment to single juvenile descent lines of *M. incognita*

5) Peptides EPL001 and EPL030 affect endospore attachment potentially through the DAF-2 signalling pathway

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John Haylor

ENDOCRINE PHARMACAUTUCALS
John Hart

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