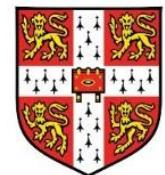


Potato cyst nematodes in the UK: understanding the problem and the search for new control measures

P. Thorpe, C. J. Lilley, E. G. J. Danchin, D. R. Laetsch, M. Da Rocha, C. Rancurel, N. E. Holroyd, J. A. Cotton, A. Szitenberg, E. Grenier, J. Montarry, B. Mimee, M. Duceppe, I. Boyes, J. M.C. Marvin, L. M. Jones, H. B. Yusup, J. Lafond-Lapalme, M. Esquibet, M. Sabeh, M. Rott, H. Overmars, A. Finkers-Tomczak, G. Smart, G. Koutsovoulos, V. Blok, S. Mantelin, P. J. A. Cock, W. Phillips, B. Henrissat, P. E. Urwin, M. Blaxter, J. T. Jones, K. Dybal, A. Reid, J. Pickup, K. Davies, E. Anderson, M. Back

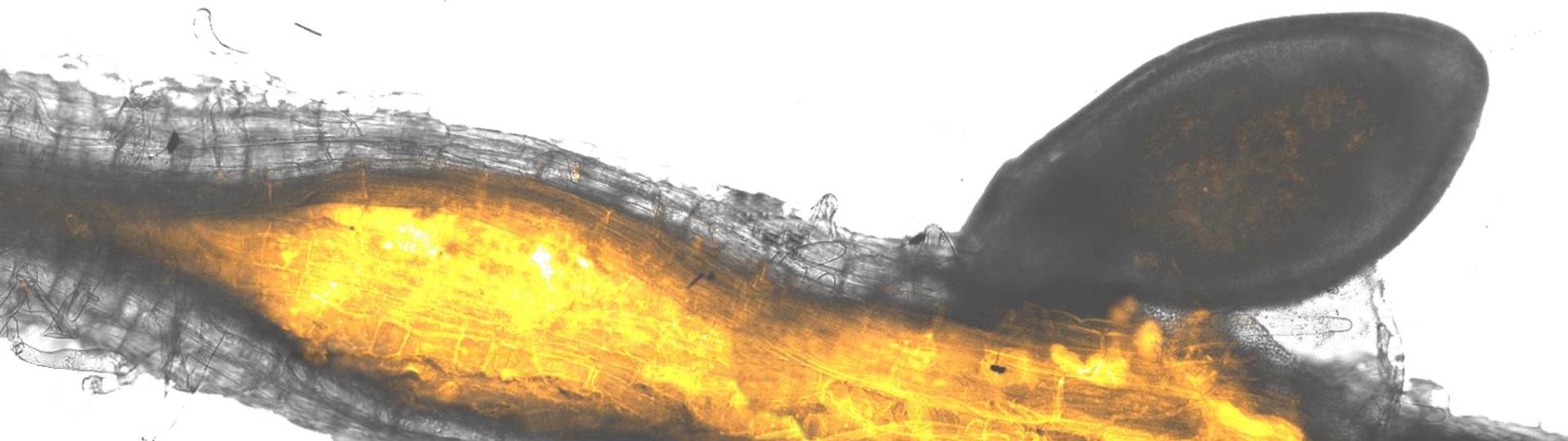
Sebastian Eves-van den Akker



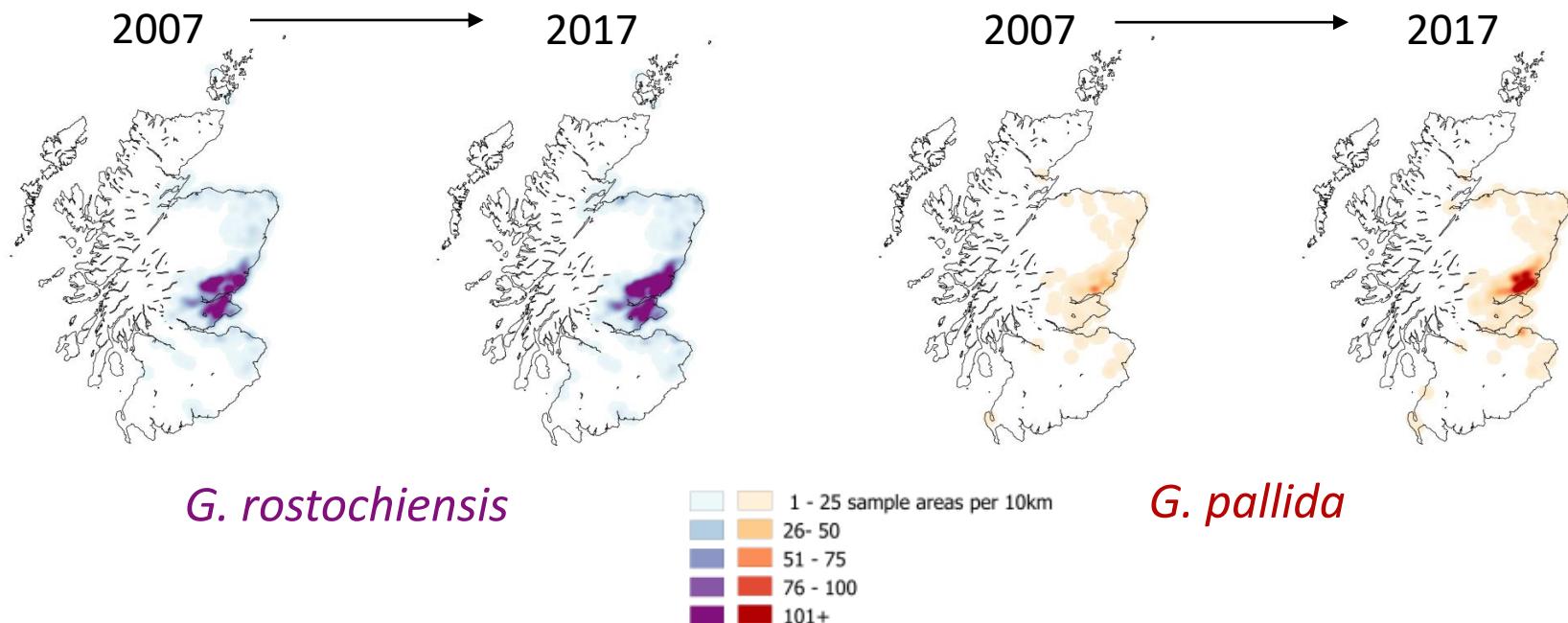
UNIVERSITY OF
CAMBRIDGE

Potato cyst nematode in the UK

- Native to South America - Europe acted as a secondary distribution point now in most potato growing regions of the world
- *Originario de América del Sur*
 - The diversity in the UK is only a fraction of what is present in South America
 - *La diversidad en el Reino Unido es una fracción de la diversidad en América del Sur*
 - Potato cyst nematodes are spreading
 - *Ellos están extendiendo*

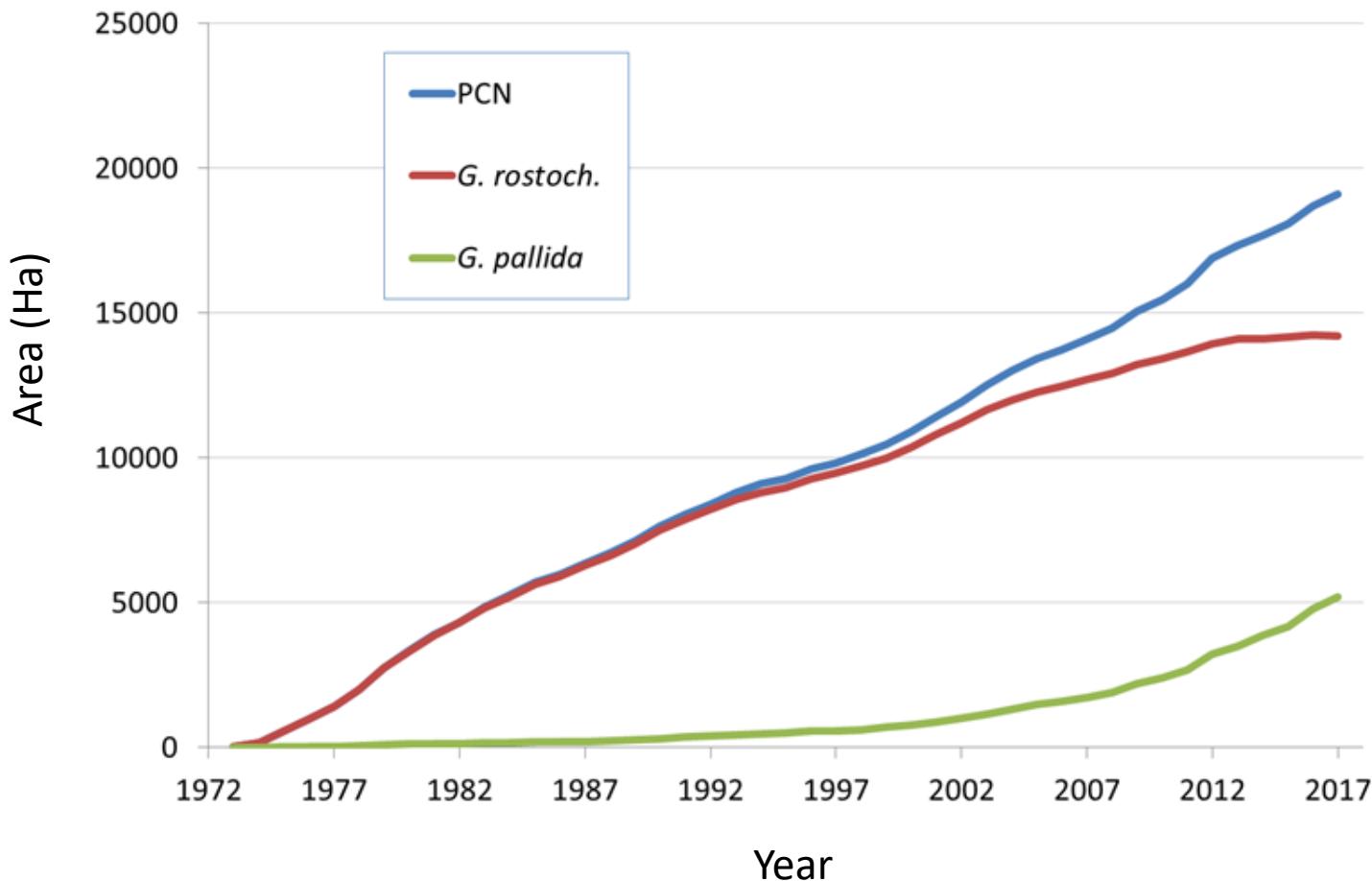


Potato cyst nematode in Scotland



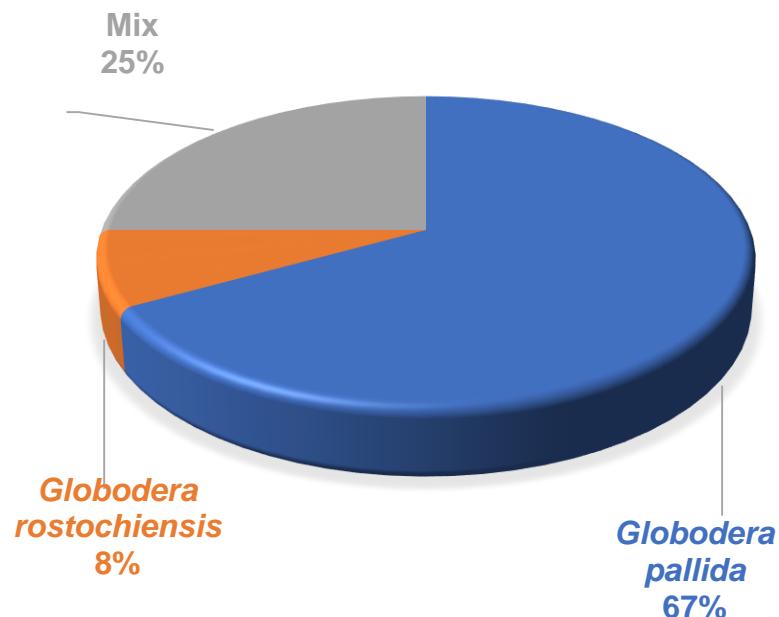
G. rostoch. 2010 (Ha)	G. rostoch. 2017 (Ha)	G. rostoch. inc	G. pallida 2010 (Ha)	G. pallida 2017 (Ha)	G. pallida inc
13,453	14,217	<u>6%</u>	2,411	5,214	<u>116%</u>

Potato cyst nematode in Scotland



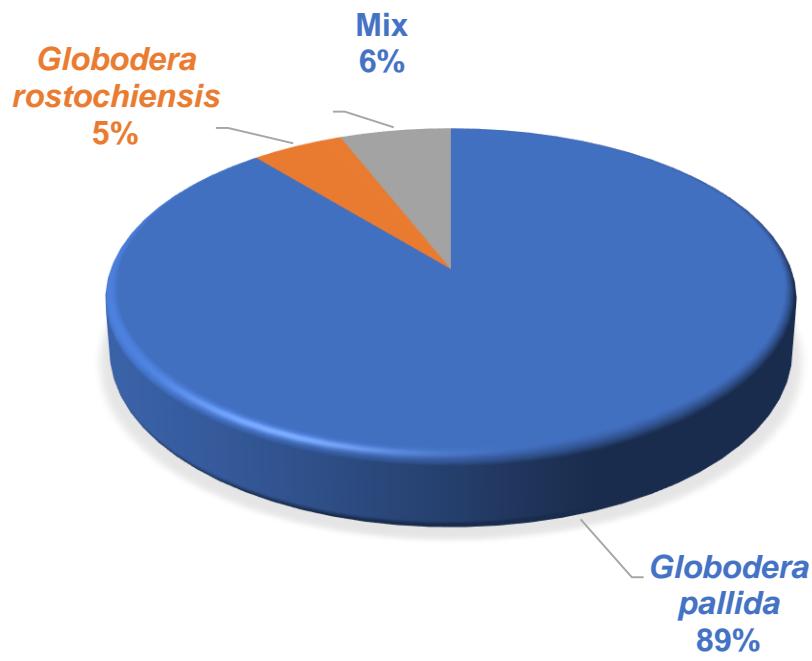
Potato cyst nematode in England

2000



Minnis *et al.*, 2002

2016



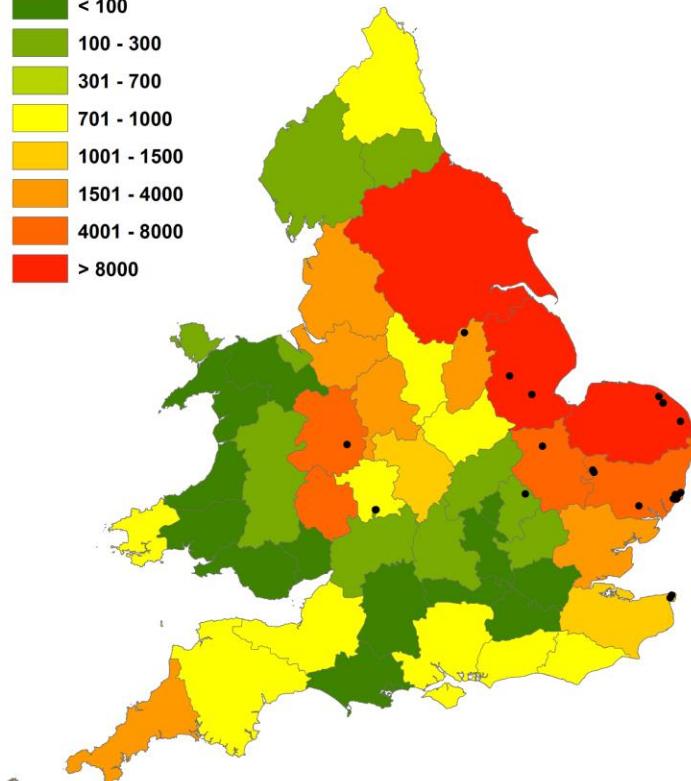
Dybal *et al.*, Unpublished

K. Dybal M. Back

Potato cyst nematode in England

Planted area (ha)

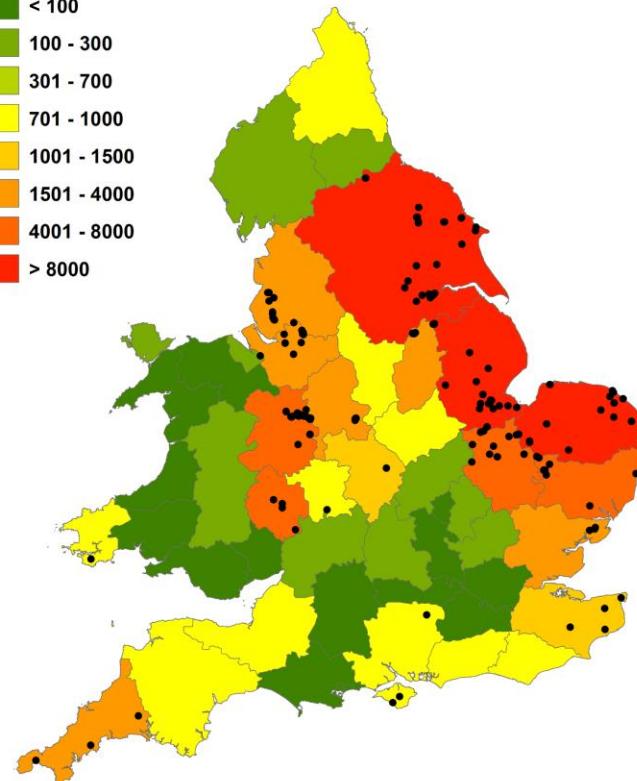
- < 100
- 100 - 300
- 301 - 700
- 701 - 1000
- 1001 - 1500
- 1501 - 4000
- 4001 - 8000
- > 8000



G. rostochiensis

Planted area (ha)

- < 100
- 100 - 300
- 301 - 700
- 701 - 1000
- 1001 - 1500
- 1501 - 4000
- 4001 - 8000
- > 8000



G. pallida

1) Understanding the problem

Entendiendo el problema

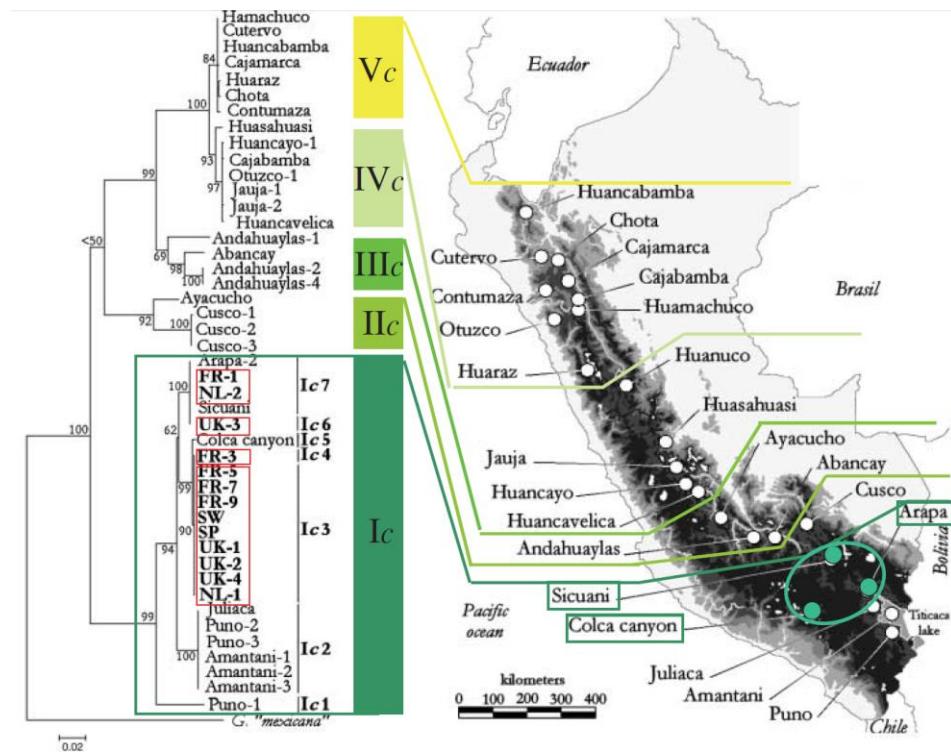
2) The search for new control measures

la búsqueda de una solución

i) DNA marker

Mitochondrial gene, Cytochrome B (CytB):

- UK *G. pallida* similar to South American populations.
Poblaciones del Reino Unido similares a las poblaciones de América del Sur
- 3 genotypes of *G. pallida*
3 genotipos de G. pallida
- 1 genotype of *G. rostochiensis*
1 genotipos de G. rostochiensis



Plantard et al 2008

ii) National DNA collection



SASA: annual pre-plant tests of seed and ware potato land across Scotland
pruebas anuales de granjas de papa



Proof of concept

Prueba de concepto

i) DNA marker

ii) National DNA collection

Idea:

“Type” ~1000 DNA samples in a single semi-quantitative experiment
genotipo 1000 muestras en un experimento

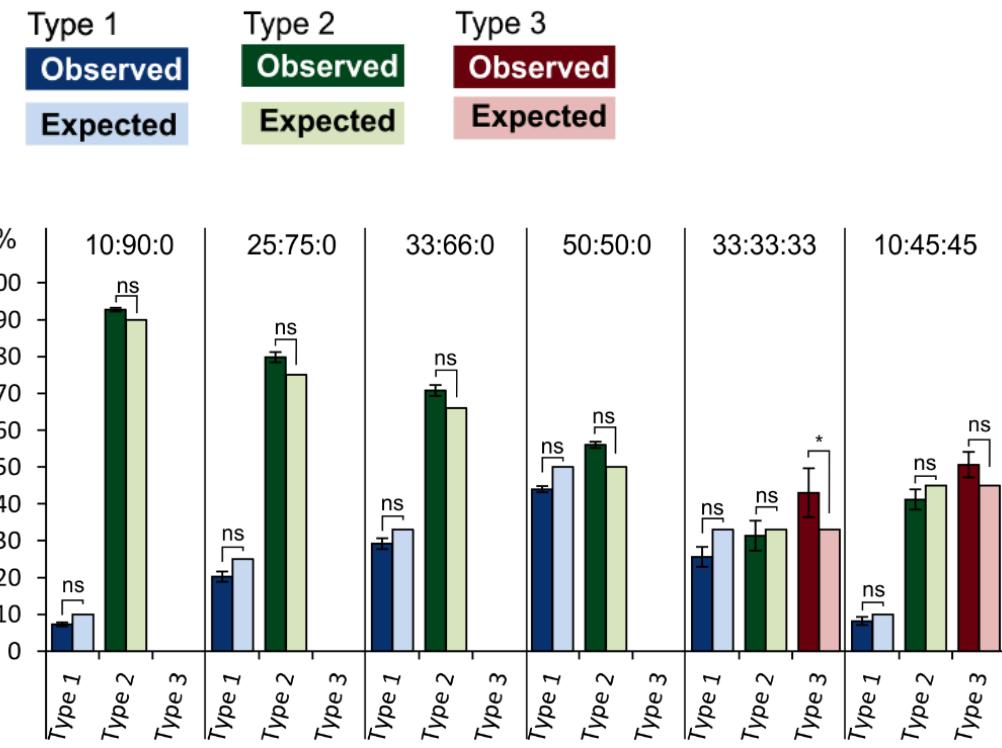
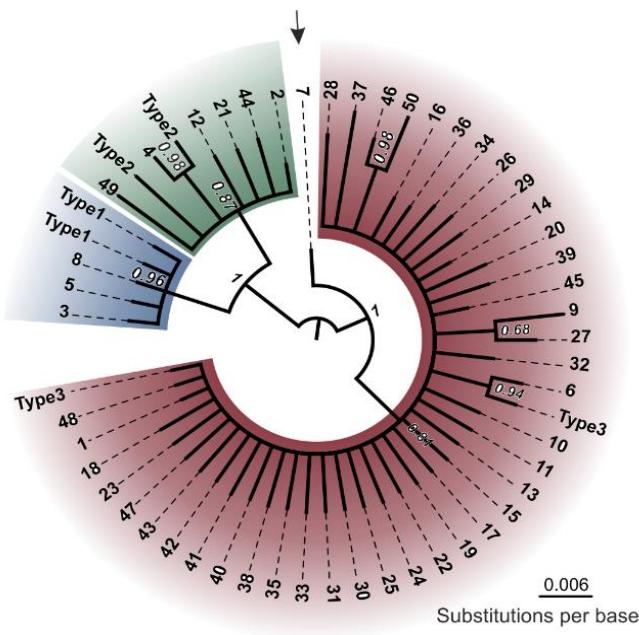
Method:

- PCR
- Sequence
- Analyse

Did it work? Yes

¿Funcionó?

- We identified the 3 known types
Identificamos los 3 genotipos conocidos
- For control samples with a “known” mix of DNA, the ratio of reads = the ratio of types
proporción de “reads” = la proporción de genotipo

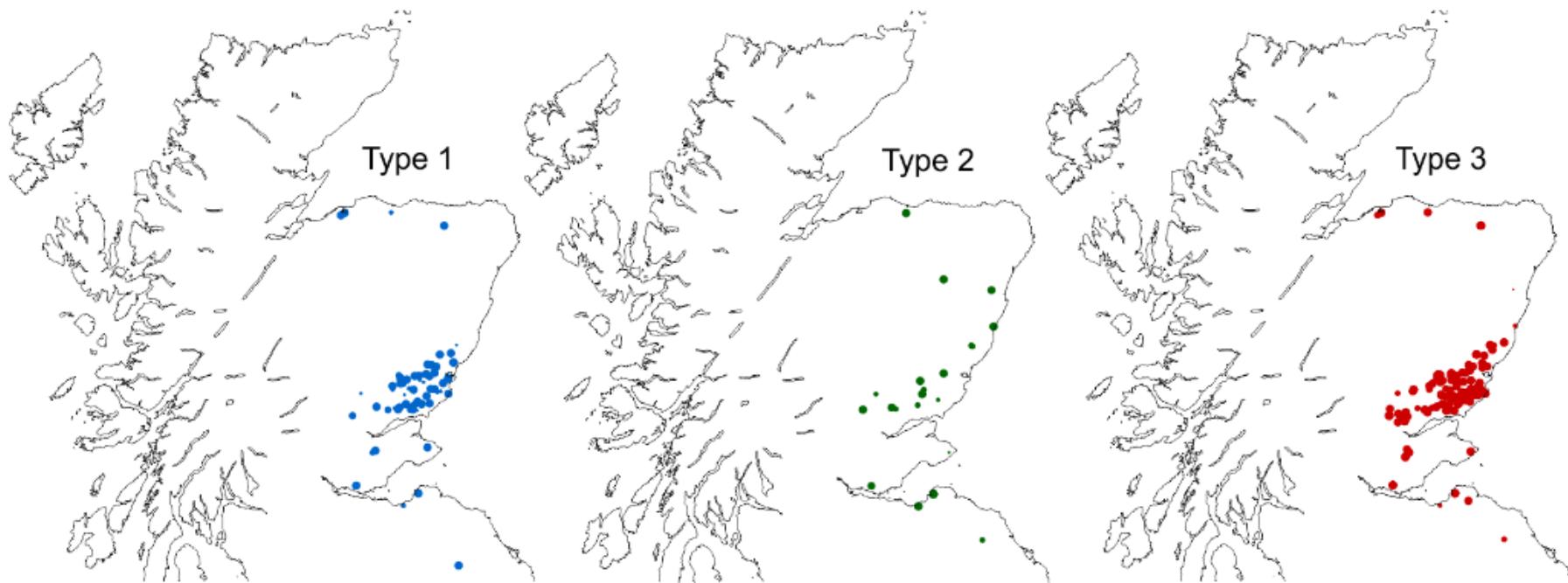


Country-scale diversity of *G. pallida*

Diversidad a escala de país

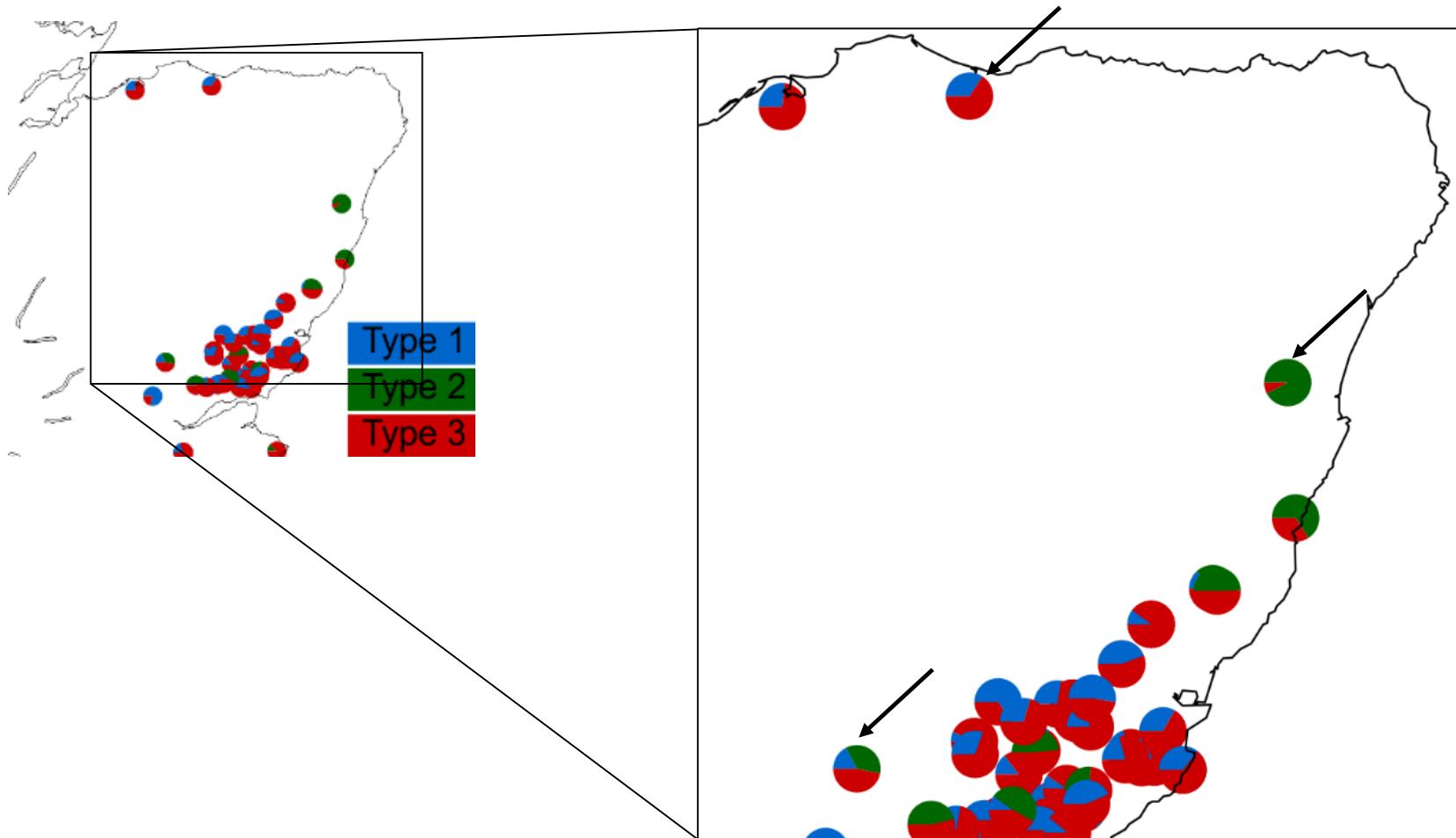
Most fields contain a single mitotype, one fifth contain a mix of mitotypes, and less than 3 % contain all three mitotypes.

La mayoría de los campos contienen un genotipo. 20% contiene una mezcla de genotipo, <3% contiene los tres genotipos.



Country-scale diversity of *G. pallida*

Diversidad a escala de país

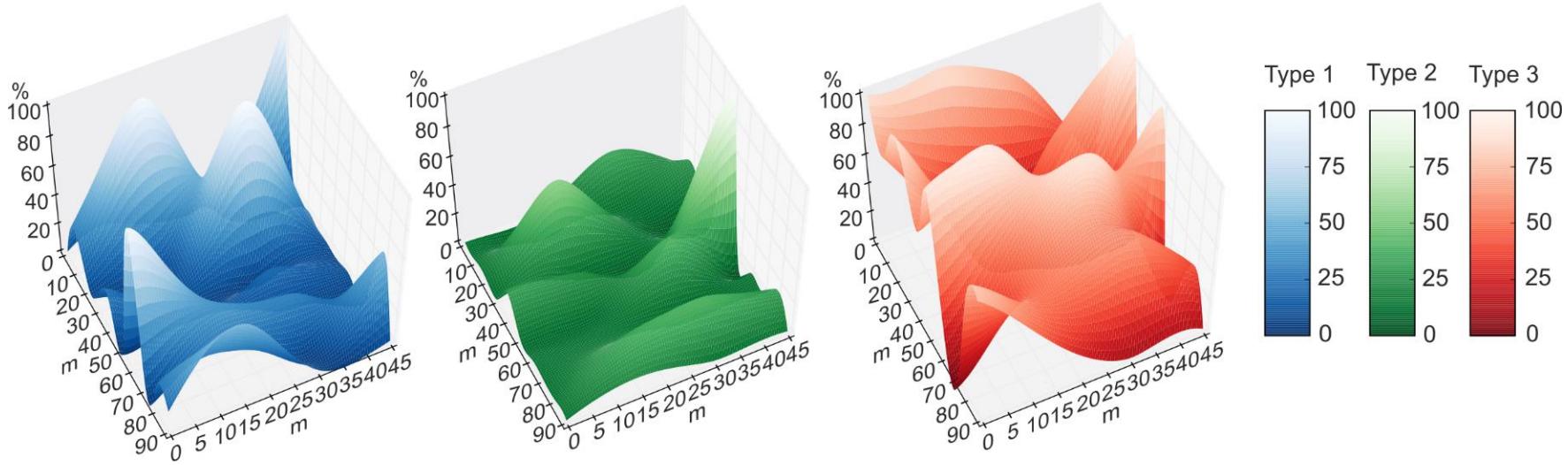
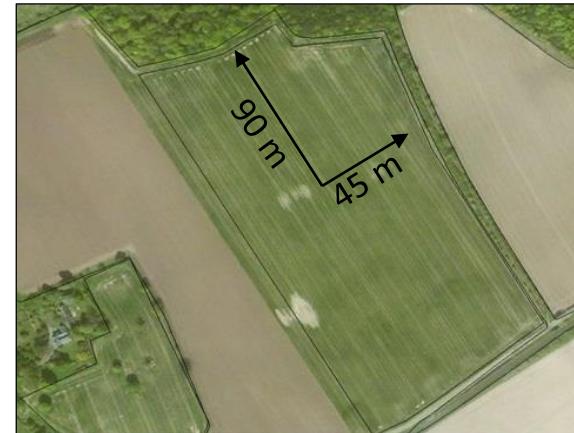


Field-scale diversity of *G. pallida*

Diversidad a escala de campo

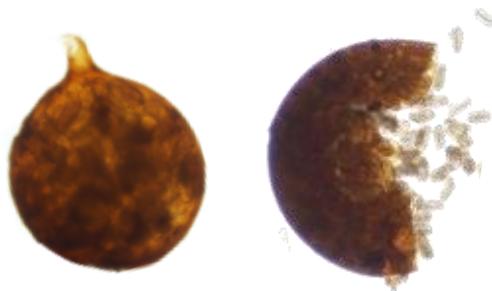
Complex underlying landscape of diversity

Paisaje complejo de diversidad

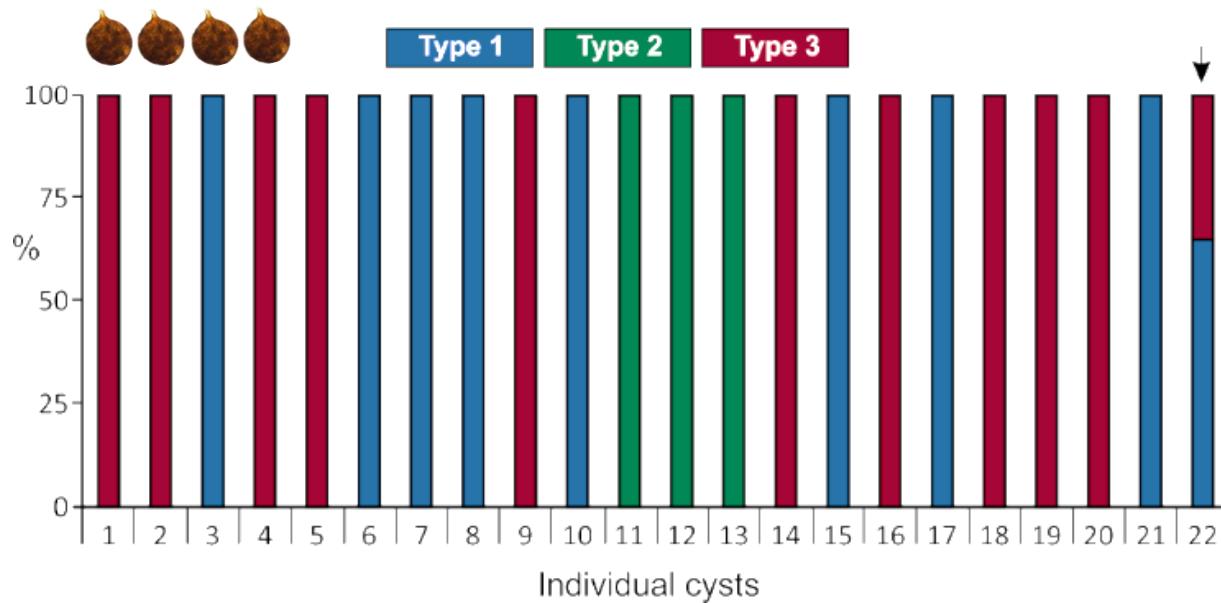


Individual-scale diversity of *G. pallida*

Diversidad a escala de individual



More support in favour of “hybrid” cysts.
Evidencia de quistes “híbridos”.



Take home messages

Conclusiones

- We have demonstrated a proof of principle
Prueba de principio
- We can address multiple questions simultaneously
Responder preguntas múltiples
- We can expanded beyond 1000 fields
Podemos hacer más

1) Understanding the problem

Entendiendo el problema

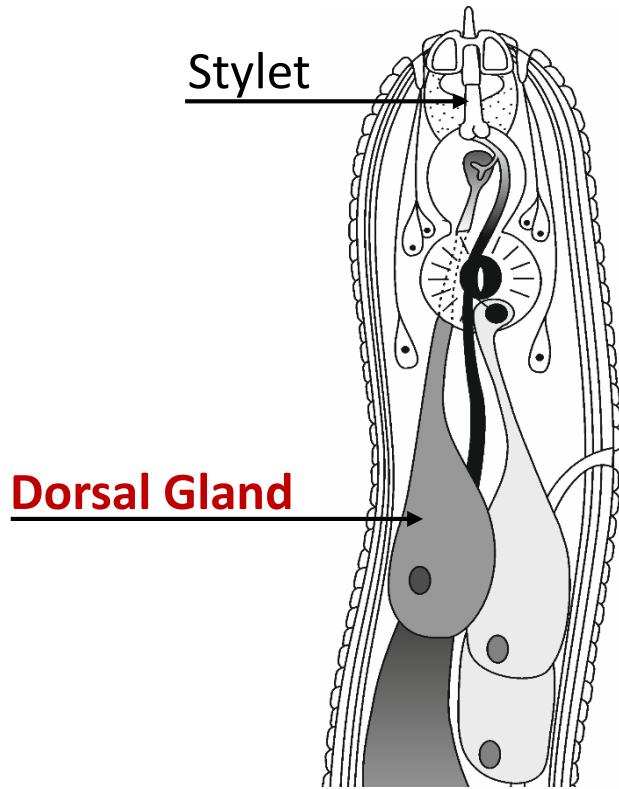
2) The search for new control measures

la búsqueda de una solución

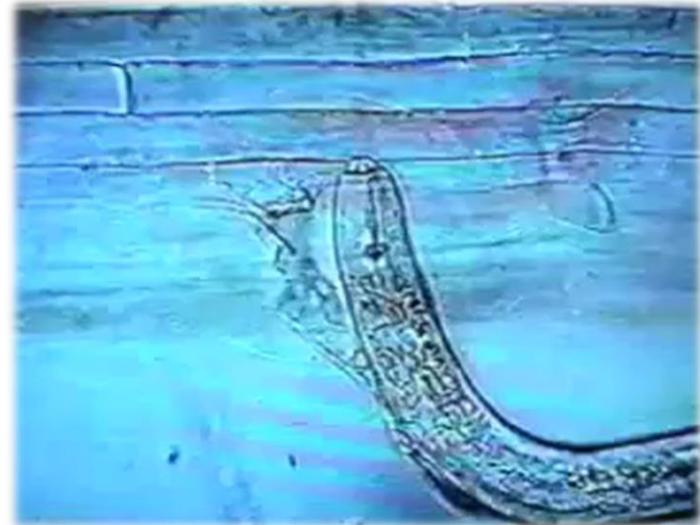
The “toolbox”: gland cells

La caja de herramientas

- Dedicated effector-producing tissues (gland cells)
Tejidos dedicados que producen efectores



Globodera rostochiensis

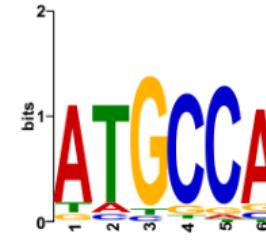
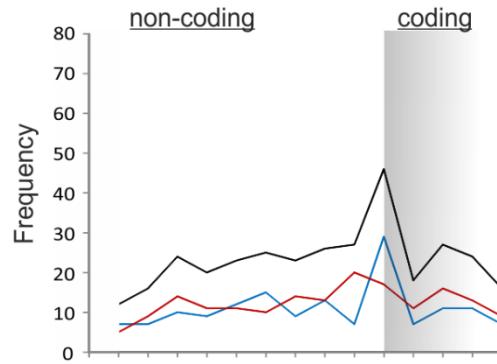
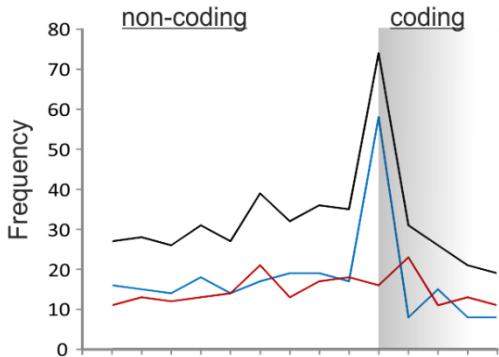
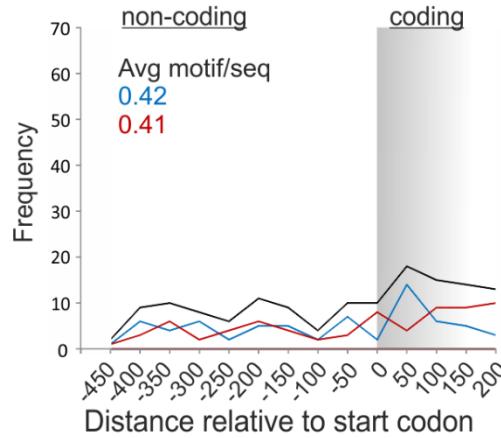
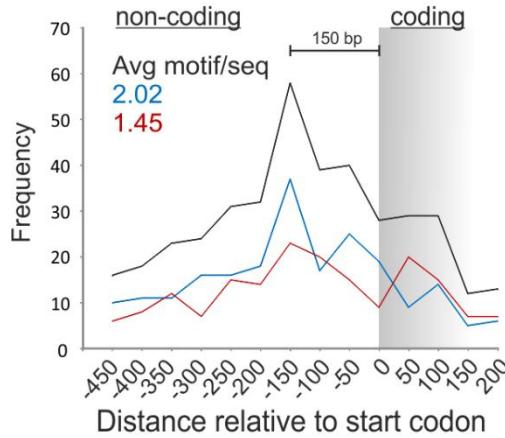


The DOG box

Compare promoter regions

Comparar regiones promotoras

Dorsal gland effectors VS Random non-effectors

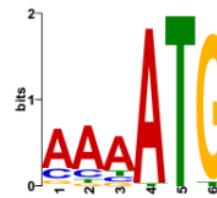


DOrsal Gland
box (DOG box)

75%(avg 3.5/promoter)

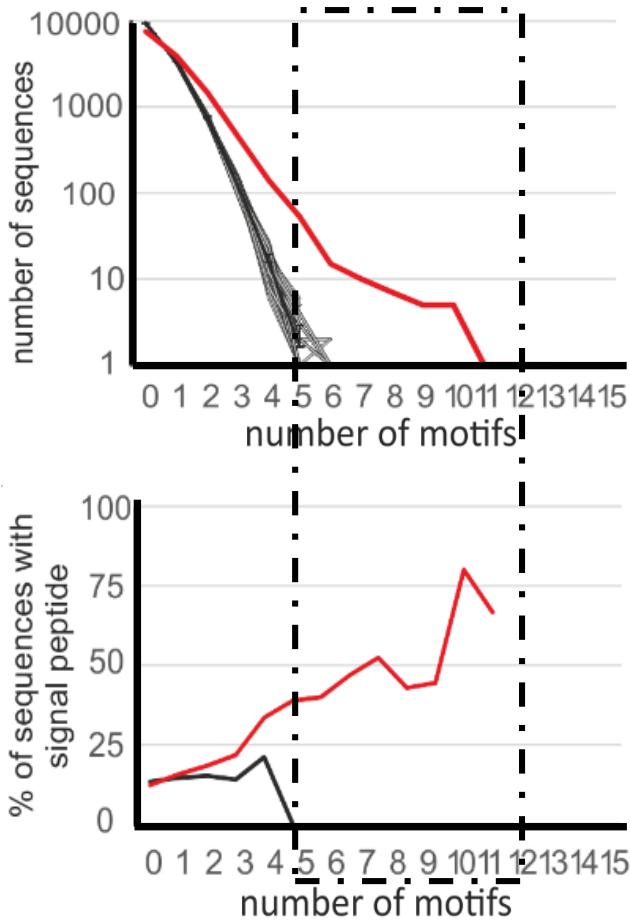
- Forward strand
- Reverse strand
- Both strands

Kozak-like motif



The DOG box

Globodera rostochiensis



The DOG box

Opens the “Toolbox”

Abre la "Caja de herramientas"



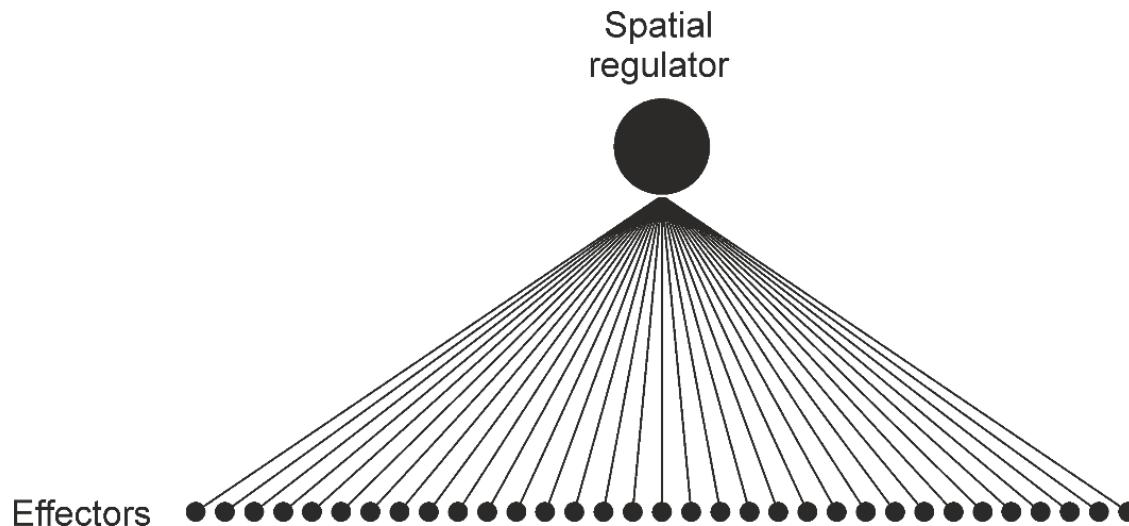
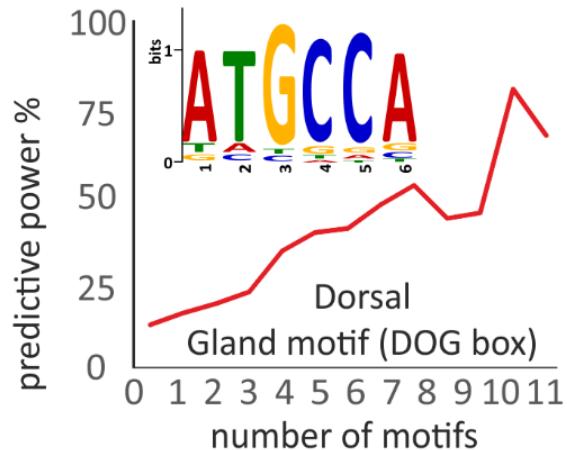
Implies the existence of a “Reader”

Implica la existencia de un "lector"



The regulation of Parasitism

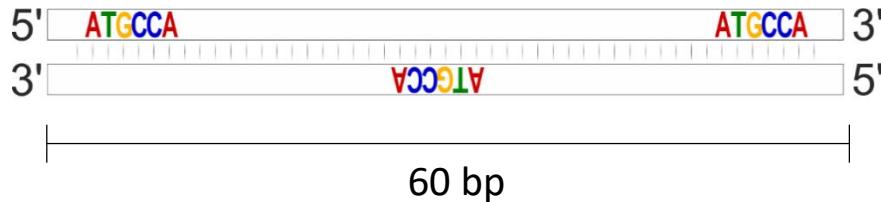
Regulación del parasitismo



The search for the “reader”

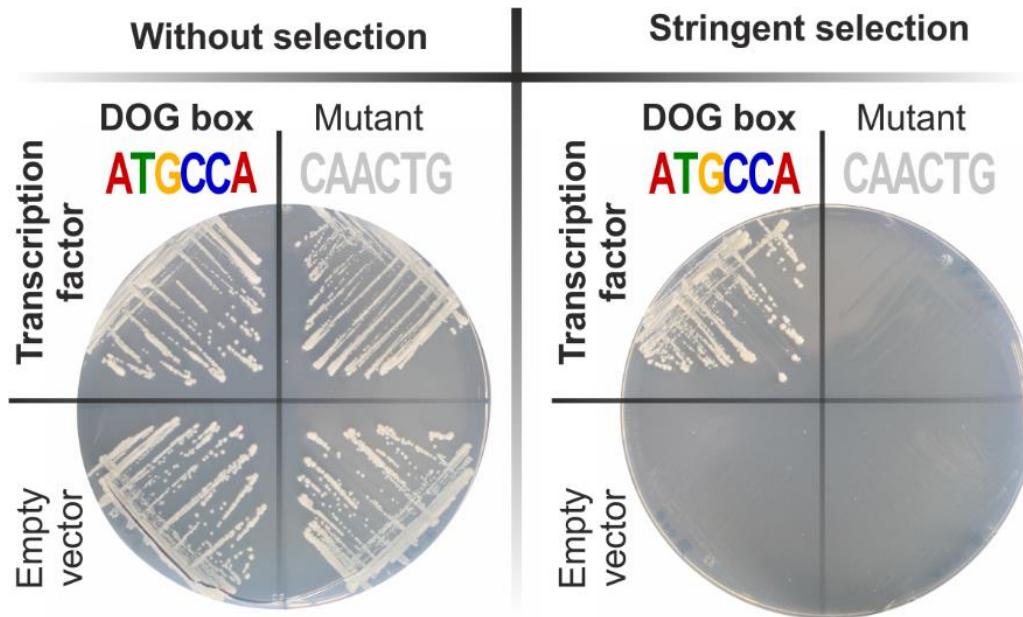
La búsqueda del "lector"

DOG effector promoter



VS

Y1H library

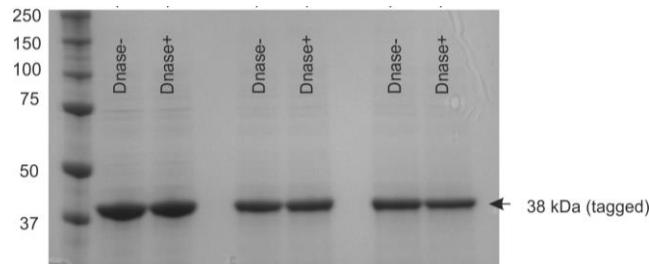


The search for the “reader”

La búsqueda del "lector"

Purify the reader protein

Purifica la proteína

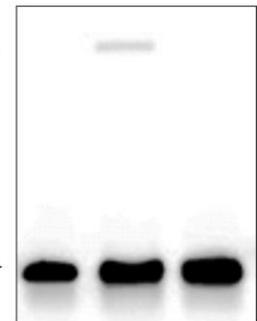


Confirm interaction with DOG box

Confirmar interacción

"Hot" DOG box (1x)	+	+	+
DOG box "reader" protein	-	+	+
"Cold" DOG box (100x)	-	-	+

DNA:Protein complex →



Confirm specificity of the interaction

Confirmar especificidad

competition with unlabeled
DOG box

excess "cold" oligo
protein
0.2 ng "hot" oligo

- - 3X 6X 12X 25X 50X

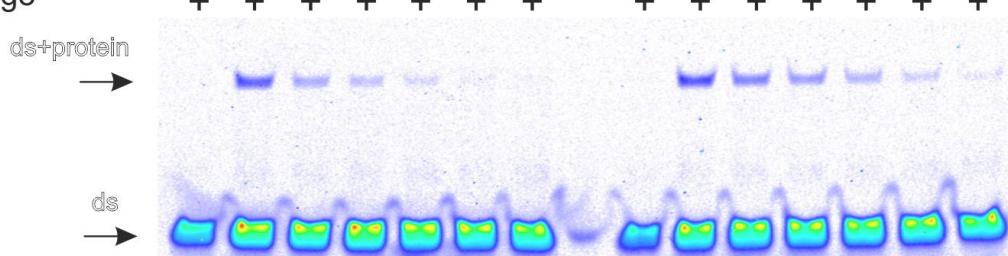
competition with unlabeled
mutant DOG box

- - 3X 6X 12X 25X 50X

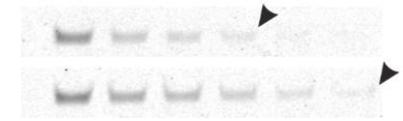
ds+protein



ds



DNA →





Potato Cyst Nematodes – **The DOG box**
– Dorsal gland only



Root-knot nematodes – **MiDOG box – Dorsal gland only**



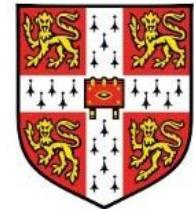
Pine wilt nematodes – **STATAWAARS**
– Dorsal and Subventral glands



Acknowledgements

Thank you for your time

Gracias



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Collaborators and friends



UNIVERSITY OF LEEDS