Genetic improvement of biocontrol nematodes

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- **EPN are used in biocontrol of cryptic insect pest**
- **E-nema produces the species *Heterorhabditis bacteriophora*, *Steinernema feltiae* and *S. carpocapsae***
- **Also produced: yeasts, fungi and bacteria**
- **Currently 6 industry-scale tanks in production**
- **Capacity increase in 2017**
- **Downstream technology and drying facilities**

Biocontrol paradigma: Indigenous species/strains are best suited for biological control

Is there scientific evidence ? Yes, for the opposite!

In biocontrol new associations, not sharing a co-evolution, are superior to old associations (Hokkanen & Pimental, 1984 + 1989, Canadian Entomologist).

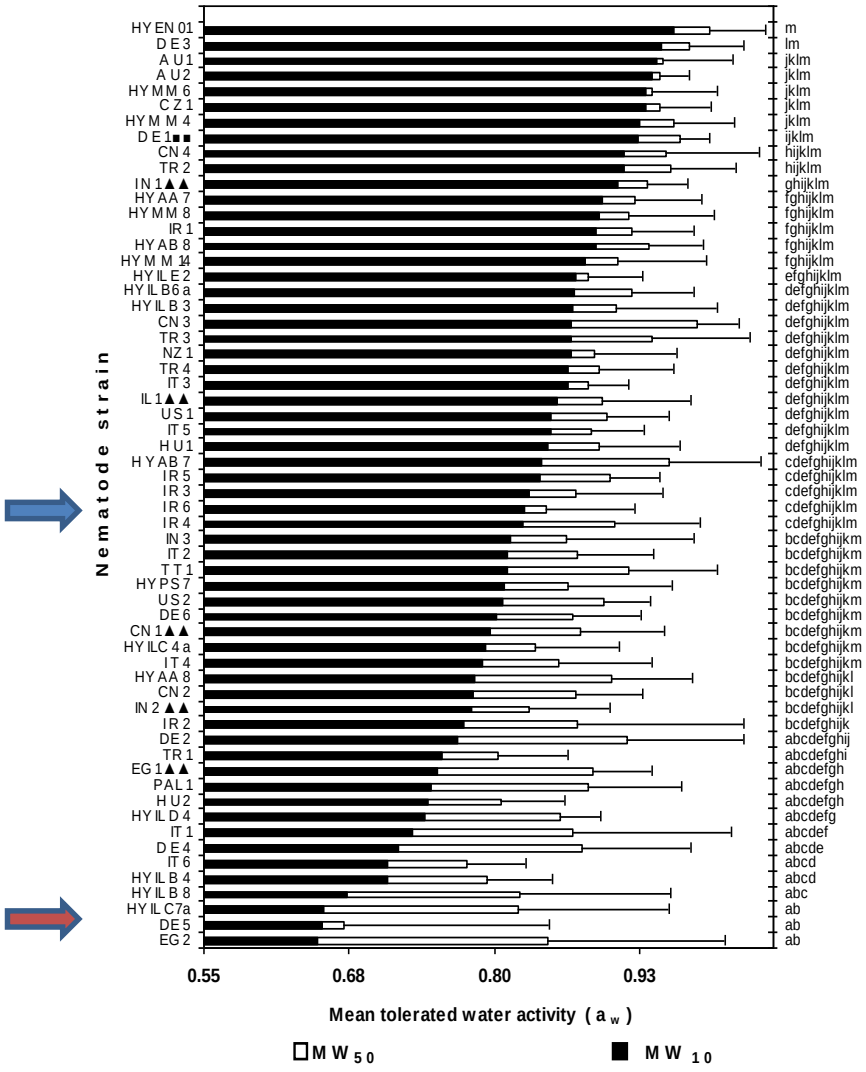
Host defences coevolve in response to attacks by parasites. The virulence of the parasite in such an association is decreasing and the resistance to parasite attack is increasing resulting in a relatively stable relationship tending to commensalism or mutualism.

New association approach is as safe as old associations in terms of environmental risks.

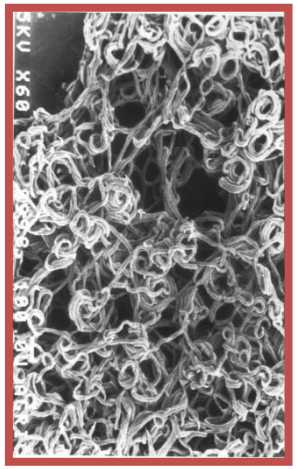
EPN, for several reasons, are exceptionally safe.

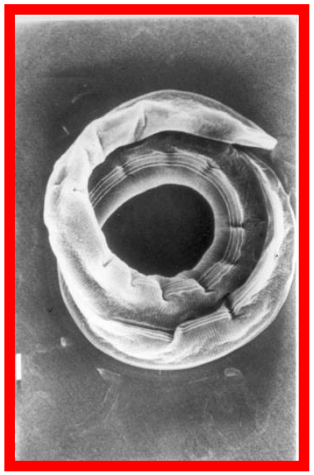
Our approach: Use of classical genetics to improve beneficial traits of EPN

But what about adaptation to local climatic conditions?



Phenotyping strains for desiccation tolerance. Tolerated water activity after adaptation (Mukuka et al., 2010, Nematology)



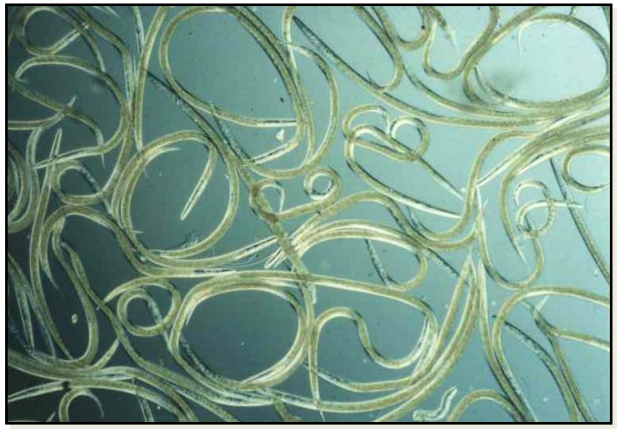


From Germany

From Negev dessert

Why is the nematode *Heterorhabditis bacteriophora* an excellent target for improvement by breeding?

- It has a 7 day life cycle (Addis et al. 2016)
- Can be propagated in vitro in small and large scale (Ehlers, 2001)
- Dauer juveniles can be stored in liquid nitrogen
- We can cross, because they produce males and females (Johnigk & Ehlers, 1999)
- We can also produce highly homozygous inbred line because they also reproduce through self-fertile hermaphrodites (Johnigk & Ehlers, 1999)
- They are closely related to the model nematode *Caenorhabditis elegans*, we thus can use genetic information available for this species (Sommer, 2015)



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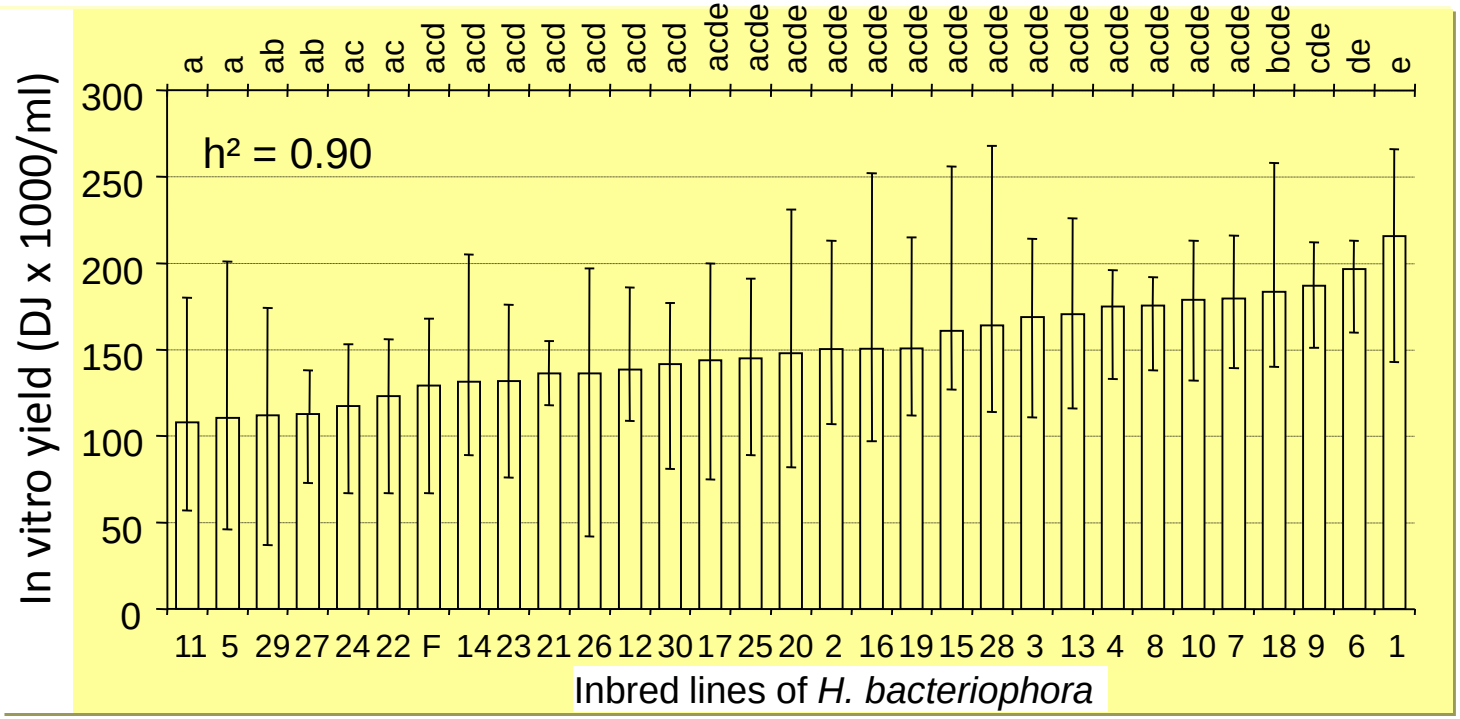

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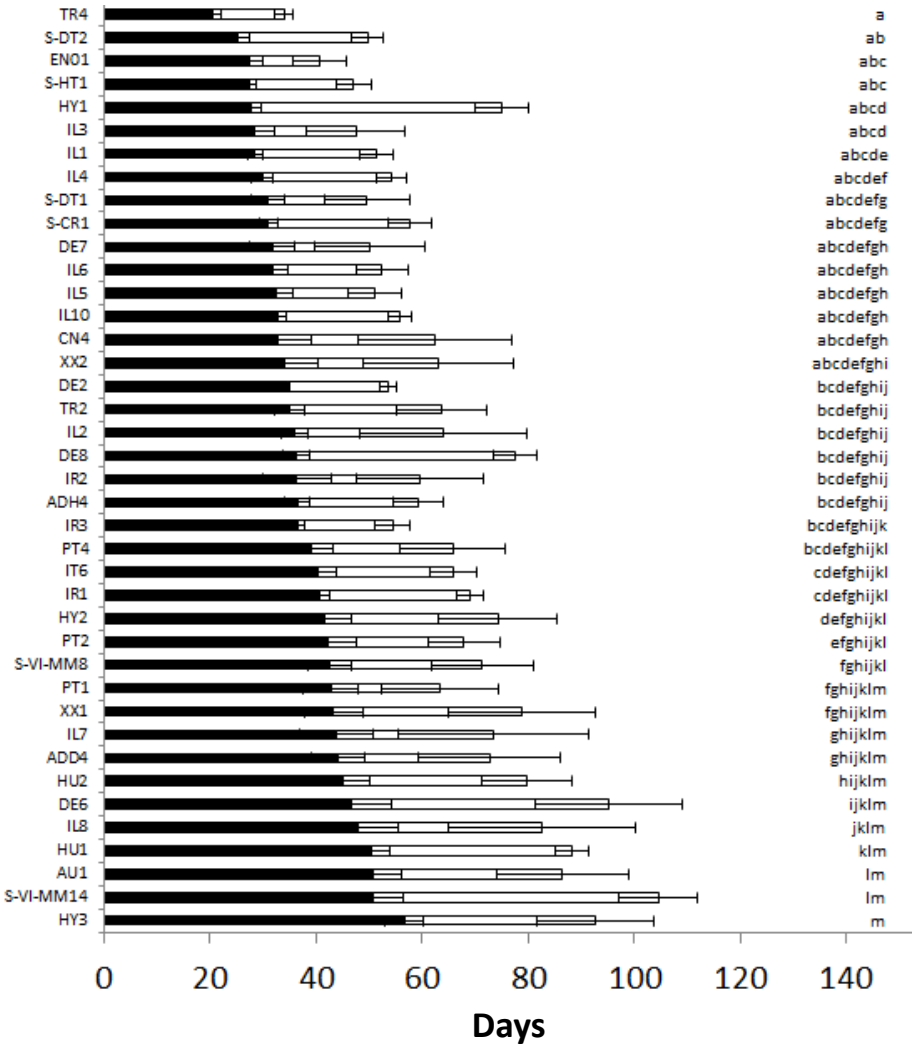
Most strains and inbred lines have been phenotyped for

- **desiccation and heat tolerance**
- **longevity and persistence**



Mean Time Survived (MTS) in 40 *Heterorhabditis bacteriophora* wild type strains at 25°C, Error bars: SD from three independent trials (p≤005)

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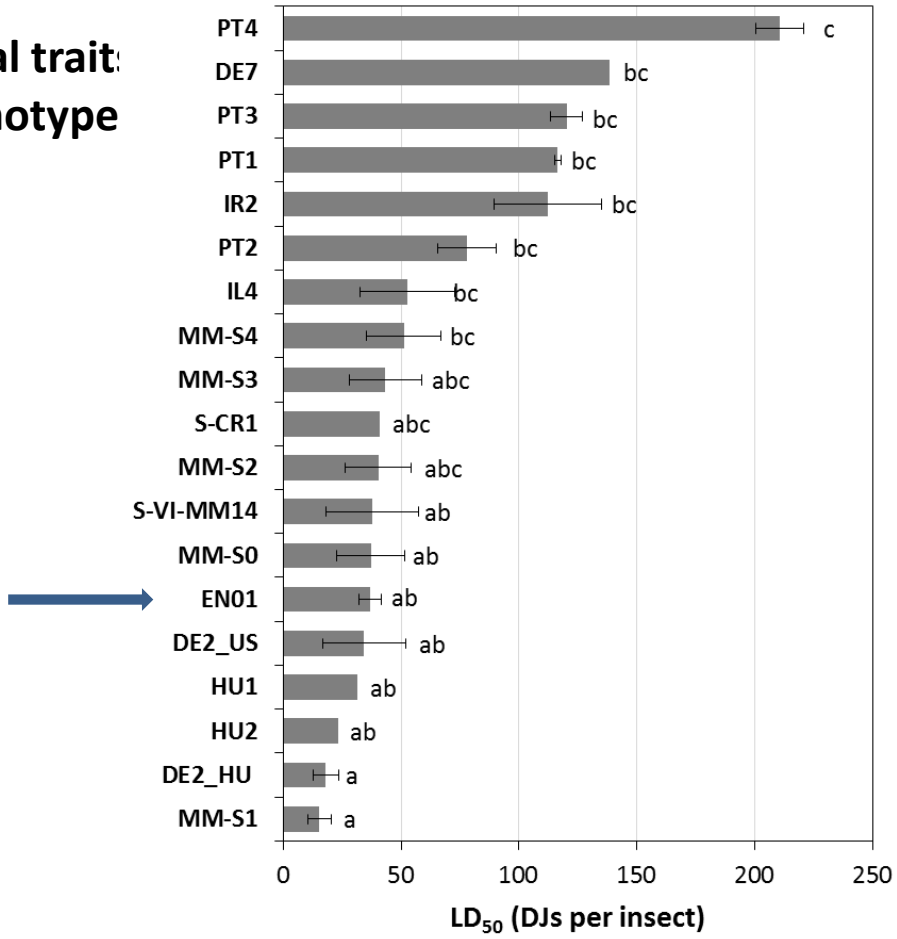

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- **desiccation and heat tolerance**
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- **virulence against 2 insects**
- **other traits can be investigated**

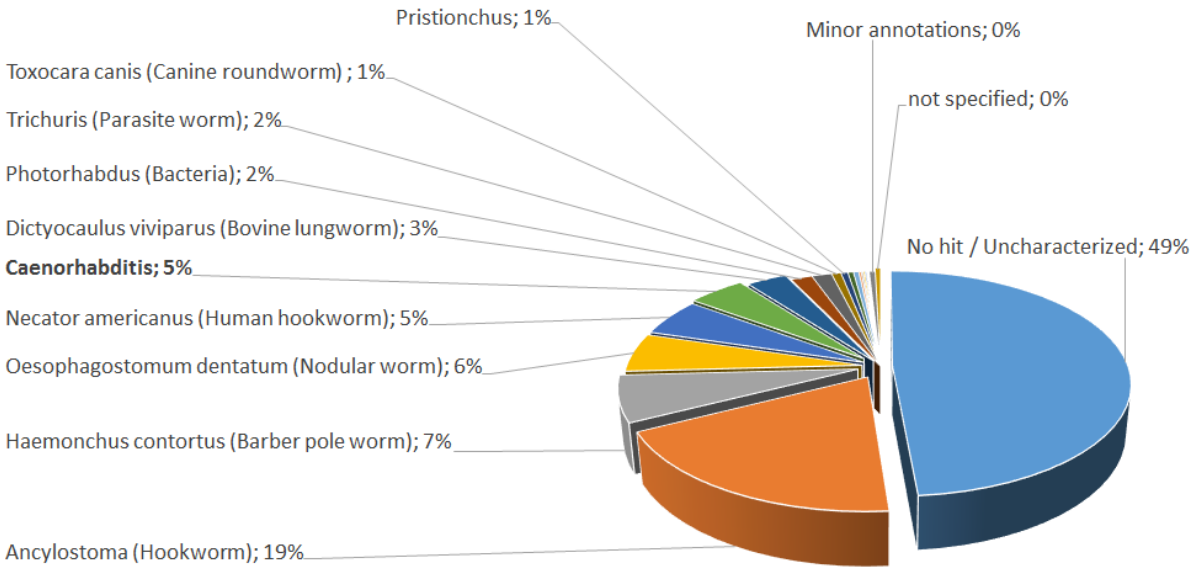
Crosses have been produced
Hybrids have been selected for improved performance
Genotypes and expression profiles are analysed

PT1-Il1	HU2-IL1	Cross	sel.cross
0,35013298	3,16874585	1,32824802	2,92014669
0,48040393	2,28244389	1,50212312	1,4813038
-1,8630191	1,5849625	0,70182051	1,33518419
-0,97021653	1,5849625	-0,32460223	1,74064125
-1,3434489	1,45220729	0,60721531	1,05933414
0,27434081	1,44655991	0,4327418	1,15095886
-0,25169219	1,43873854	-0,06566607	1,01422462
0,31385866	1,38947579	0,31259023	1,00220427
0,36725209	1,35777329	0,87174551	1,10184382
0,35826994	1,34712865	0,87760337	1,92014669
0,26784999	1,33291948	0,64193333	1,16028762
0,14219861	1,3169813	0,74080766	1,32192809
0,25118469	1,24416087	1,65047034	1,6460571
-0,07763741	1,23208148	0,28678301	1,55019708
0,254884	1,22380919	0,59591425	1,324129
0,49138839	1,2037586	0,41071676	1,33518419
0,36259211	1,19671729	0,58193785	1,42406541
-0,04874433	1,16588666	0,52686524	1,13022667
0,40581897	1,1646307	-0,14684139	1,80829858
0,23770464	1,1325656	0,45977437	1,64429664
-0,362434	1,10152995	-0,70450308	1,13727485
0,40811809	1,08356472	-0,42371037	1,33518419
0,10841155	1,0119806	0,41434401	1,19276424
-0,49966619	1,01100249	0,74363581	1,51724818

What will we do?

- **We will search for genetic markers related to beneficial traits**
- **A marker-associated breeding programme will result in nematode strains with improved biocontrol characters**

Annotation of 22,000 *H. bacteriophora* transcripts derived from the HU2-IL1 and PT1-IL1 lines under stress



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BIOCOMES

e-nema

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