Bacterial Bleeding Canker of Horse Chestnut

-unraveling its secrets through genomics

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The disease

Newly emerging disease occurring in Belgium, The Netherlands, Germany and Britain

Epidemic started in 2002/03

Symptoms now affect thousands of horse chestnuts in Britain

Causes lethal cankers on stem and branches
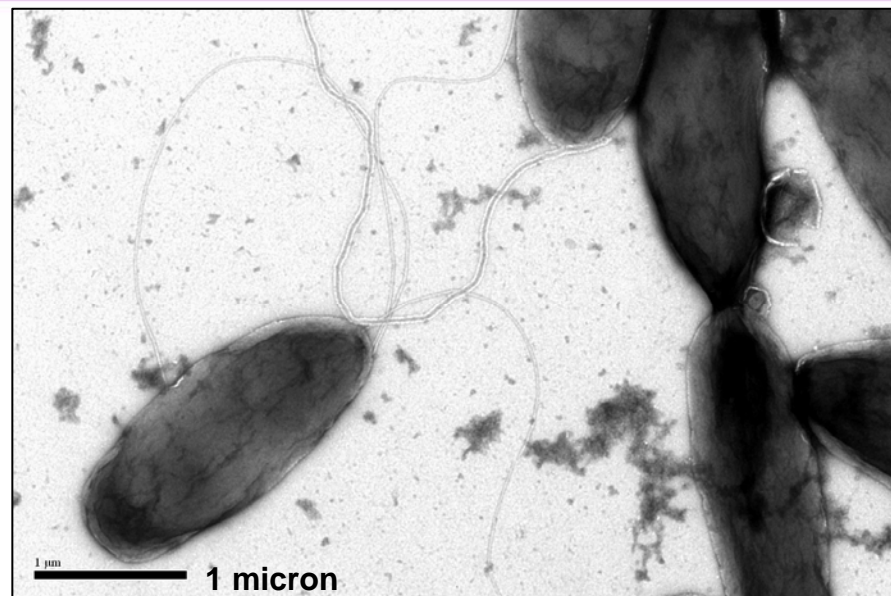
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Causal agent

Caused by *Pseudomonas syringae* pv. *aesculi* (*Pae*)

Rod-shaped bacterium with flagella

Only previously known to occur on **leaves** of Indian horse chestnut in India

Contrast with symptoms on woody parts of European horse chestnut

How did this pathogen get to Britain?

1 micron

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- Bacterial diseases represent an increasing threat to Britain's forest, woodland and amenity trees

- The *Pae* epidemic provides an ideal opportunity to develop a system for tackling newly emerging tree diseases

- We have used state-of-the-art molecular tools in our study of *Pae*

- These new tools can be employed to deal more rapidly with future biosecurity threats

Studies in 2008-2009

In 2008-2009 we……

1. Developed a molecular based (real-time PCR) assay to detect *Pae* in diseased horse chestnut trees

2. Determined how *Pae* infects woody parts by studying naturally infected horse chestnut trees

3. Sequenced the genome of *Pae* to gain insights into its evolution and biology

Development of detection method for *Pae*

Staff at NRS (and Norway) developed a 'quantitative real-time PCR assay' to detect *Pae*

Real-time PCR assay uses a unique primer pair which targets and amplifies a region of DNA specific *only* to *Pae*

CAAAGACGAGCGCAGCGGGAAGGAAGAGCTGTTCAAATACGAAGGCGGTT
GCGTGCCTTCGTTGAATACCTGAACACCAACAAGACGCCGGTCAATCAGGT
TCCACTTCAATATTCAACGCGACGACGGCATTGGTGTCGAGATTGCGCTG

Assay tested for specificity on;

9 strains *Pae*

17 other strains of *P. syringae*

11 other *Pseudomonas* (non-pathogenic) species

14 other species of bacteria found on horse chestnut trees
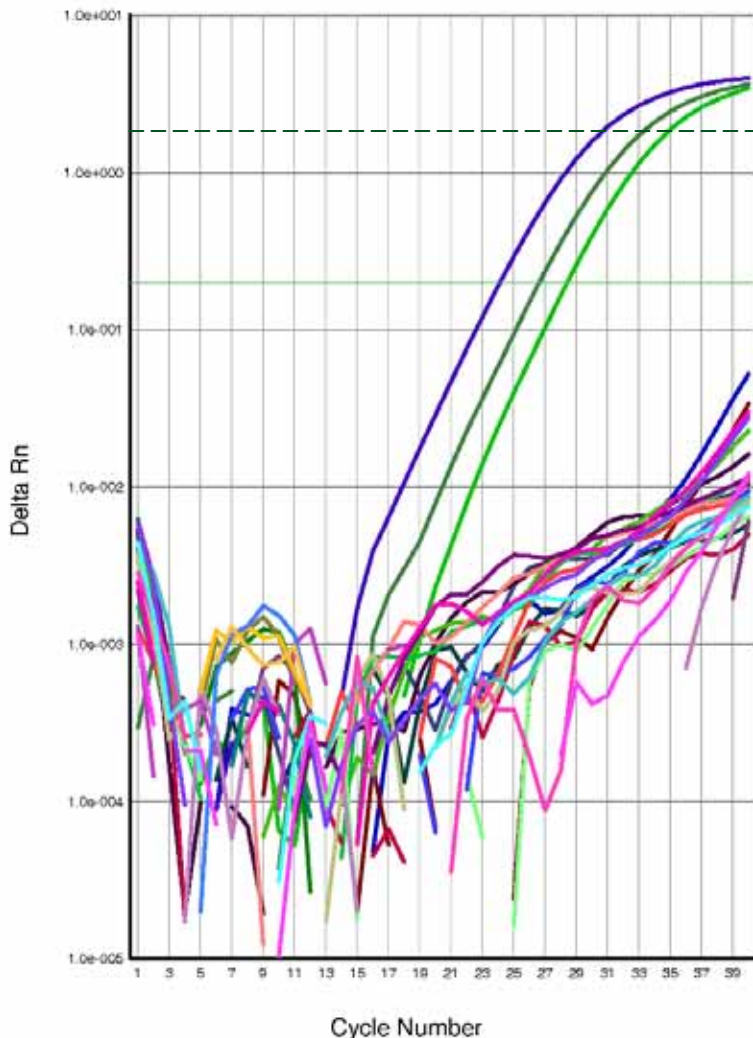
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Quantitative real-time PCR assay

Fluorescence is detected by real-time PCR machine – *only in the presence of Pae*

Can detect *Pae* directly from diseased horse chestnut tissues

The amount of DNA is also measured - Assay detects as little as 1 pg DNA

Fast, specific, sensitive!

Green, S., Laue, B., Fossdal, C.G., A'Hara, S., Cottrell, J. 2009. Infection of horse chestnut (*Aesculus hippocastanum*) by *Pseudomonas syringae* pv. *aesculi* and its detection by quantitative real-time PCR. <u>Plant Pathology</u> 58, 731-744.

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Applications for real-time PCR :

(The first) Rapid detection of *Pae* in horse chestnut – now routinely used in Tree Health Advisory Service at NRS

Monitor survival and spread of *Pae* in soil, water and tree debris at various times of the year
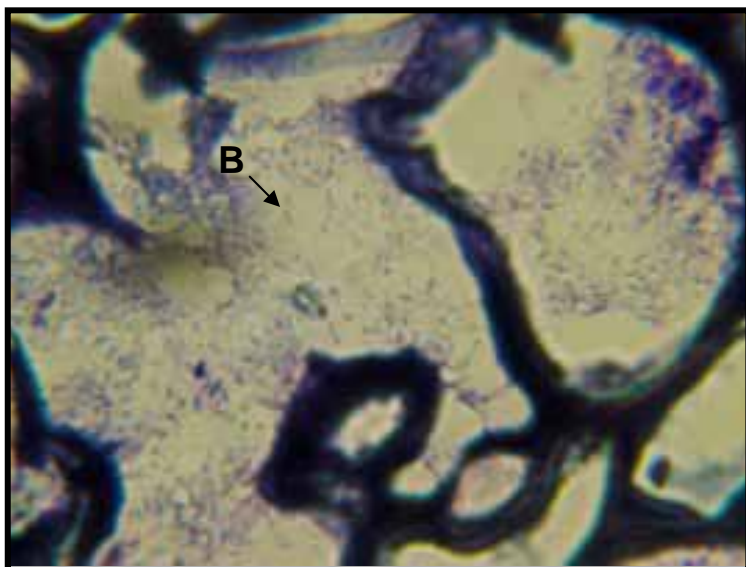
Used in studies on infection processes of *Pae*

Infection

Pae infects branches *via* **lenticels**, leaf scars, nodes and other natural bark weaknesses

i.e. high number of potential infection sites on woody parts
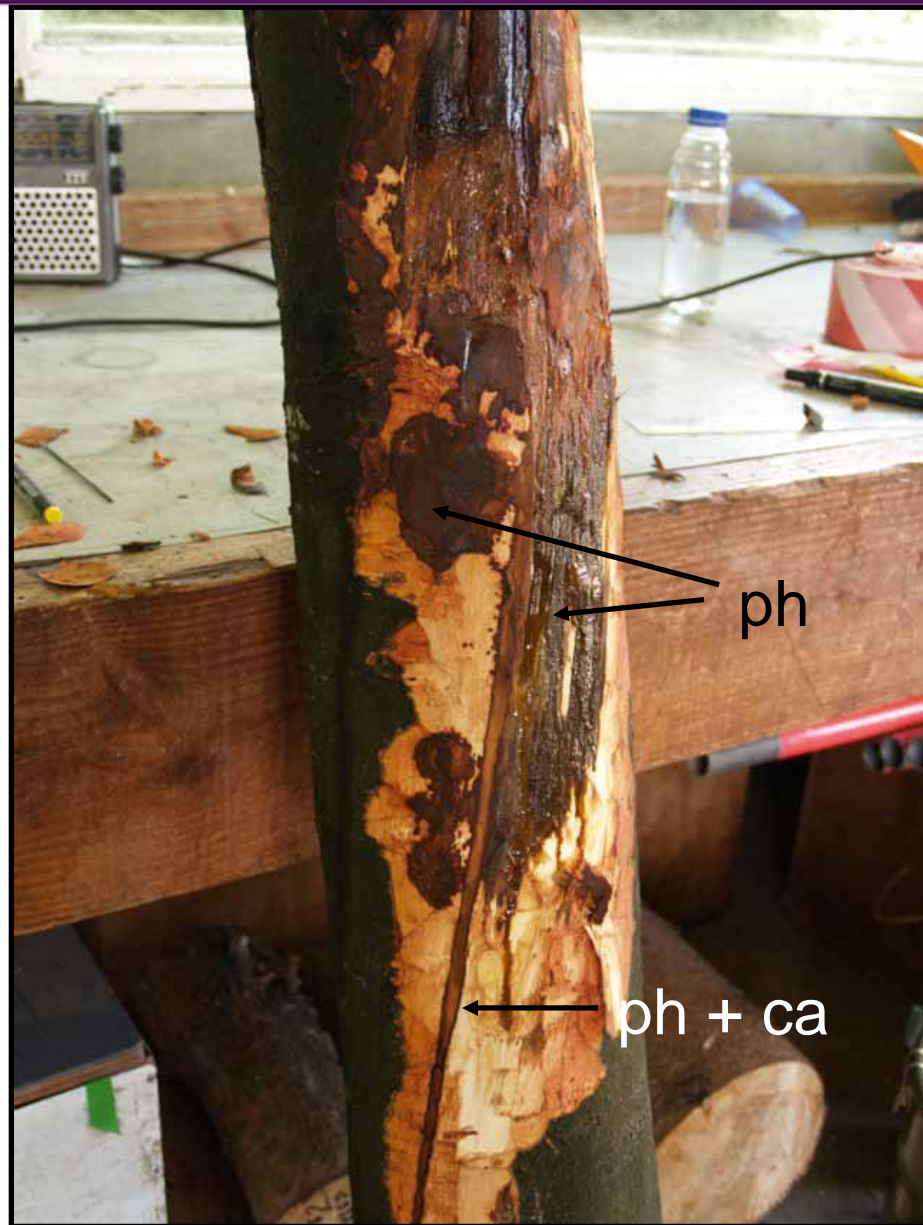
Lesions develop during host's dormant season

10 μm

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Infection

Pae infects cortex, phloem and cambium

No evidence for infection or spread in xylem

Can spread very rapidly within a tree in phloem and cambium

ph

ph + ca

Summary of Infection

Pae invades the woody parts of horse chestnut at multiple sites that are exposed to wind and rain, i.e. aerial inoculum

Pae colonises the cortex, phloem and cambium causing extensive, and eventually continuous lesions within the tree

(Characterisation of the infection of horse chestnut (*Aesculus hippocastanum*) by *Pseudomonas syringae* pv. *aesculi,* H. Steele, B.E. Laue, G.A. MacAskill, S.J. Hendry and S. Green, in review)
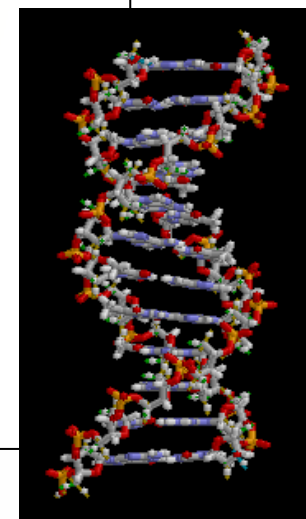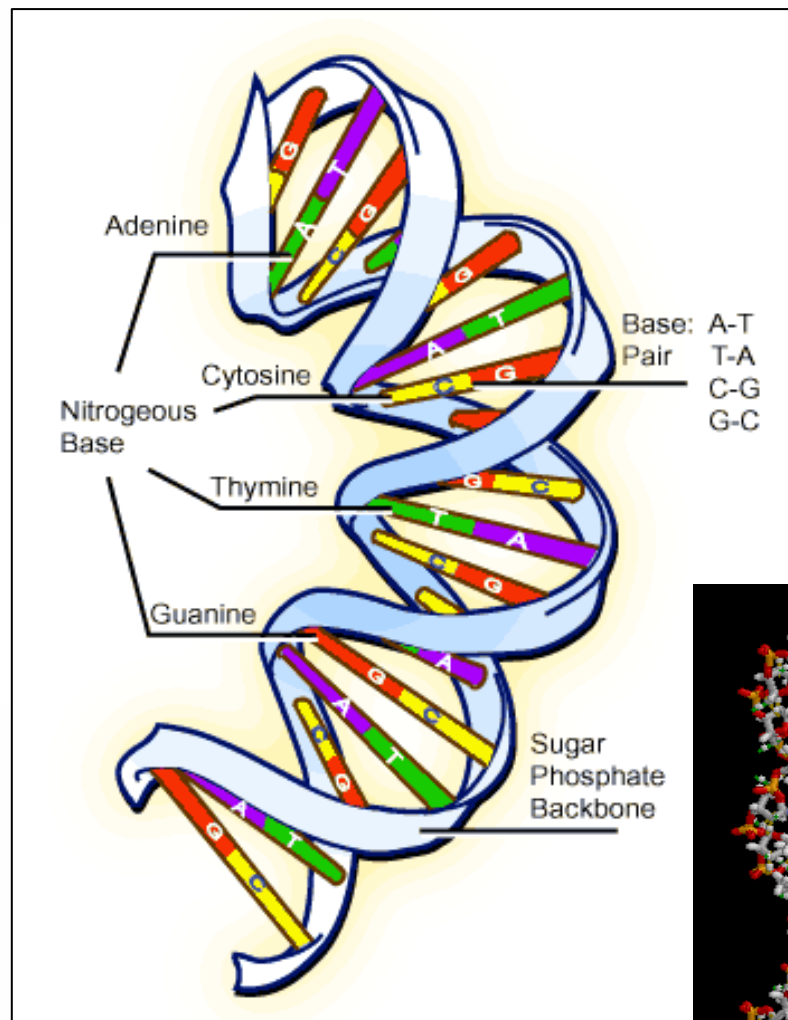
Genome sequencing *Pae*

What does **genomics** mean?

'**Genomics'** is the study of the genomes of organisms.

In modern molecular biology, the **genome** is the entirety of an organism's hereditary information encoded in DNA

Genomics involves determining the entire DNA sequence of organisms and fine-scale genetic mapping

What did we do in the *Pae* genome sequence project ?

1. We sequenced the entire genome of a *Pae* strain from Pitlochry in 2009 and determined genome size and number of genes

2. We also sequenced genomes for *three* other *Pae* strains – one from India, one from Glasgow, one from southern England

3. We compared the genomes of the Indian and the 3 British strains to gain information on their evolutionary relationships

4. We identified genes unique to *Pae* which may represent specific adaptations for infection of a woody host

(in collaboration with GenePool Genomics Facility at the University of Edinburgh and the Sainsbury's Laboratory in Norwich)
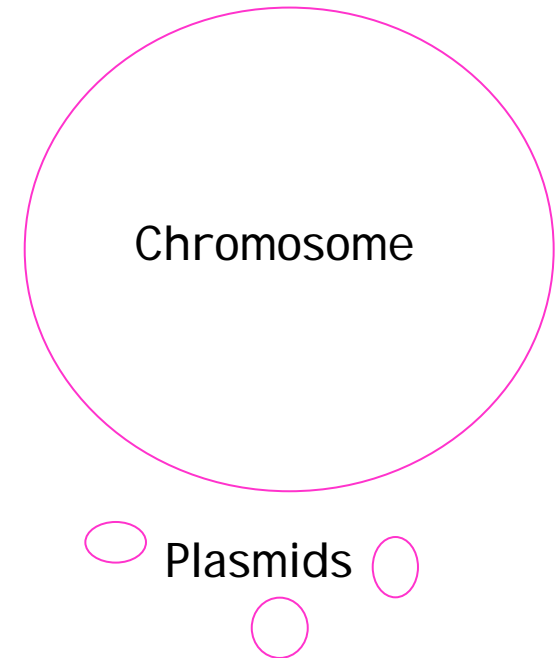
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Results: Genome composition

The *Pae* genome has:

5,926,327 nucleotides - ATCG's

Arranged in one large circular chromosome with several small plasmids

5, 621 predicted genes

Chromosome

Plasmids

Evolutionary relationships among *Pae* strains

The three British *Pae* strains are genetically almost identical and probably originate from a single, recent introduction into Britain

The three British *Pae* strains are genetically similar (95%) to the Indian *Pae* strain, indicating that they share a common ancestor

One *Pae* strain
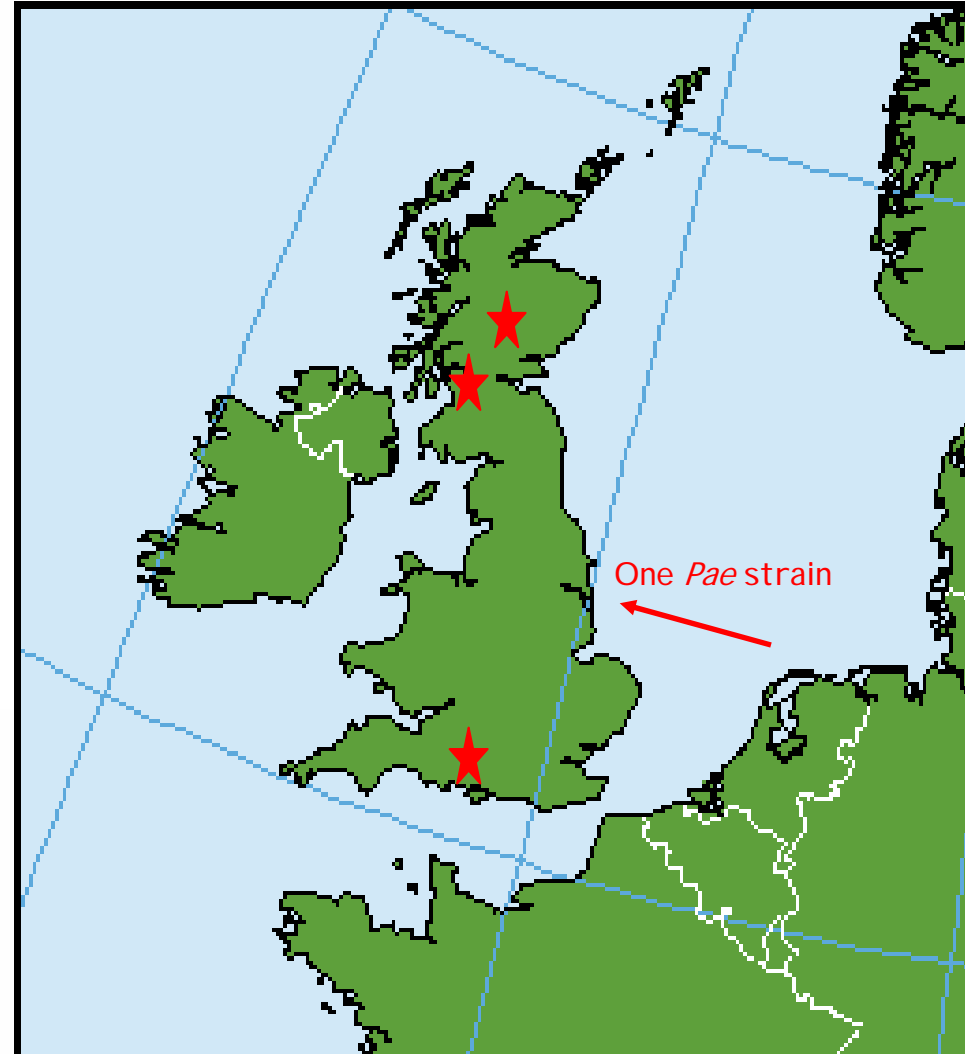
Evolutionary relationships between *Pae* and other pathovars

There are about **50** different pathovars of *Pseudomonas syringae* on a range of plant hosts, including herbaceous plants and trees

Pathovar (genomes are complete)	Plant Host
Ps syringae	snap bean
Ps tomato	tomato
Ps oryzae	rice
Ps tabaci	tobacco
Ps phaseolicola	bean
Ps glycinia	soybean
Ps pisi	pea
Ps thea	tea
Ps mori	mulberry
Ps morsprunorum	cherry

*Pae* is the first bacterial tree pathogen to be sequenced
We compared the genome of *Pae* with other pathovars of herbaceous plants.

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Evolutionary relationships among *Pae* and other pathovars

By comparing conserved genes we found that *Pae* clusters with other pathovars on woody hosts

This indicates evolutionary adaptations for infection of the woody parts of the tree

Pae must have acquired new genes for infection of the woody parts of horse chestnut

0.02

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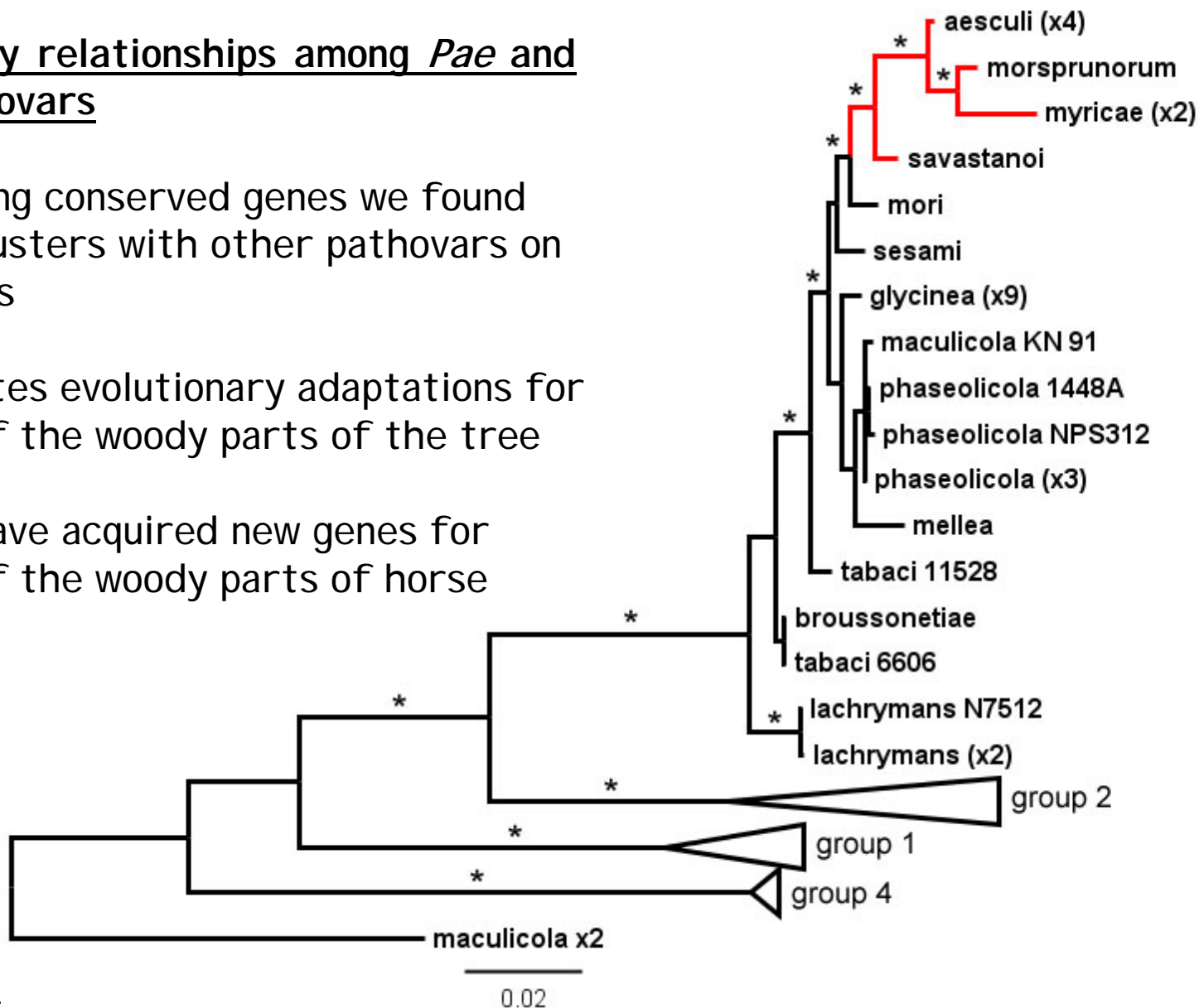
'<u>Woody-specific genes</u>'?

Are there genes unique to *Pae* which may represent specific adaptations for infection of a **woody** parts of horse chestnut?

Compare the *Pae* sequences to the available genomes of other *Pseudomonas syringae* pathovars which infect **soft** tissues

We identified a number of genes and genetic pathways unique to *Pae* which may enable the infection of trees

These included;

1. Genetic pathway for degradation of aromatic compounds derived from lignin and other plant-derived phenolics

Benzoate catabolism
Hypothetical proteins
Homology to other *P. syringae*
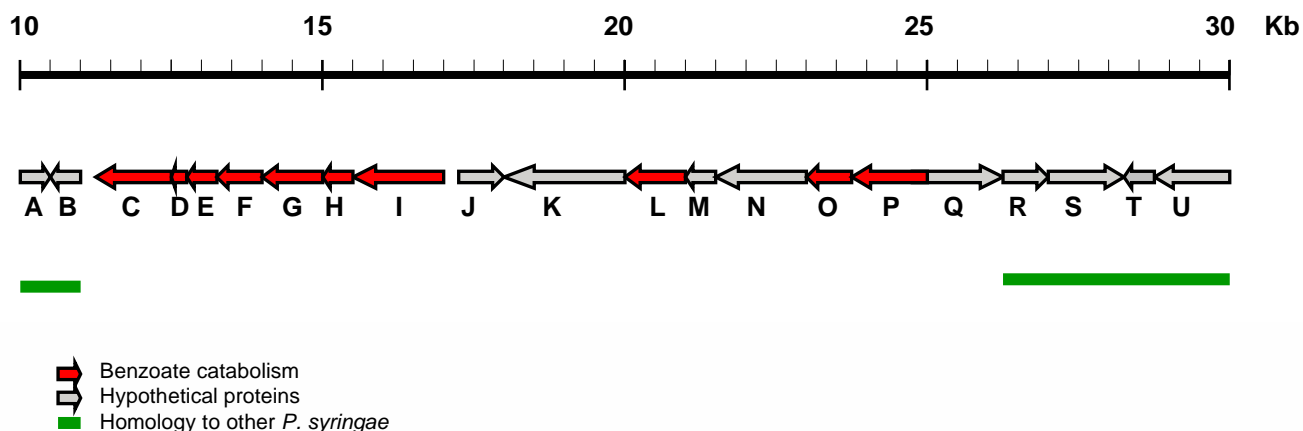
2. Genes for enterobactin synthesis, fatty acid biosynthesis, NO metabolism, toxin pathway, host attachment, sucrose uptake

Summary

1. British strains of *Pae* most likely originate from a single, recent introduction into Britain, and may have descended from an Indian strain.

2. *Pae* is most closely related to other *Pseudomonas syringae* pathovars of **woody** hosts.

3. The tree-infecting pathovars of *Pseudomonas syringae* appear to have evolved from pathovars on herbaceous hosts

4. *Pae* has acquired a suite of 'unique' genes and genetic pathways which may enable it to infect and live in the woody parts

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Current and future work

1. Confirm the origin of the British *Pae* strains by genetic comparisons with newly acquired *Pae* strains from India (with help from CABI Bioscience)

2. Survey the genetic variability of a broad range of *Pae* strains from Britain and continental Europe. Determine whether the European strains were the source of the British strains

3. Determine the routes of transmission of *Pae* and its ability to survive and spread in soil and water

4. Evaluate the role of the *Pae*-unique genes in pathogenicity on trees

5. Study the evolutionary adaptations of other *Pseudomonas syringae* pathogens of woody hosts

               BBSRC-CASE PhD studentship will commence in 2010
               (in collaboration with P. Sharp at The University of Edinburgh)