Manipulating the secondary symbionts of the potato aphid, *Macrosiphum euphorbiae*

Clarke, H.[1, 2], Karley, A.J.[1], Cullen, D. W.[1], Hubbard, S.F.[1, 2,3]
[1]Scottish Crop Research Institute, UK, [2]University of Dundee, UK, [3]University of St Andrews, UK

Background

Most aphid species possess the obligate primary bacterial endosymbiont, *Buchnera aphidicola*, which provides the aphid with essential amino acids lacking from their phloem sap diet. Many species of aphid can also harbour facultative secondary endosymbiotic bacteria. In the pea aphid three of these symbionts, *Serratia symbiotica*, *Hamiltonella defensa*, and *Regiella insecticola*, have been shown to confer thermal tolerance and increased resistance to natural predators and pathogens, respectively, to their aphid host. However, little is known about the role of these secondary symbionts in other aphid species, particularly in agriculturally important pest species such as the potato aphid, *Macrosiphum euphorbiae*.

'The potato aphid, *Macrosiphum euphorbiae*'

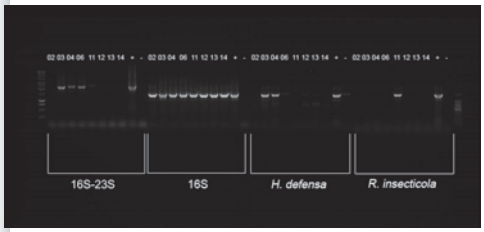
Aims of the project

The project aims to determine the impact of secondary symbionts on the performance of *M. euphorbiae*, particularly in response to hymenopteran parasitoids This will be addressed using genetically identical aphid lines that differ only in their symbiont status, created by introducing or eliminating the facultative symbionts *H. defensa* and *R. insecticola* into or from asexually reproducing potato aphids.

Methods being used

Diagnostic PCR

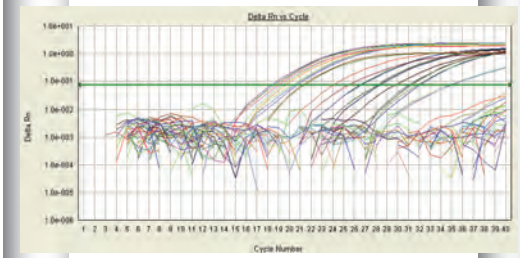
Clonal lines of *M. euphorbiae* were screened for the three main symbionts studied in the closely-related pea aphid, *Acyrthosiphon pisum*, using published primers (Douglas *et al*., 2006). Specificity validation has involved the cloning and sequencing of PCR products, and the design of fluorescent *in situ* hybridisation probes to verify the endosymbiotic nature of these bacteria. Further screening will also investigate any association of phages with the symbiont bacteria.



Diagnostic PCR electrophoresis gel image

Real-time quantitative PCR

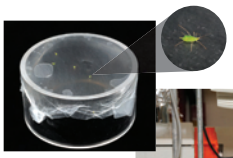
Primers were designed to quantify the genome copy numbers of both the primary symbiont, *Buchnera*, and the secondary symbionts by qPCR. The results will be normalised to single-copy genes such as *ef1*-α amplified from the aphid host tissue, and the relative densities of the primary and secondary symbionts will be compared between aphid lines.



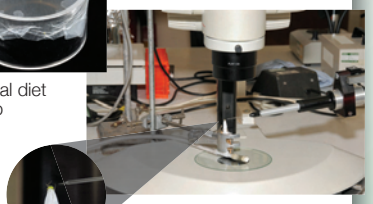
Trial qPCR amplification plot

Curing and artificial infection

Infected *M. euphorbiae* lines are being cured using antibiotics administered either orally or by microinjection. The antibiotics have been selected to eliminate secondary symbionts without eliminating the obligate primary symbiont *Buchnera*. Conversely, uninfected aphid lines are being injected with haemolymph extracted from aphids harbouring known secondary bacteria to create newly infected lineages.



Artificial diet set-up



Microinjection apparatus

Future work

Fitness experiments

Aphid performance parameters such as survival, development, relative growth rate and fecundity will be measured in cured and infected *M. euphorbiae* lines to determine any fitness costs or benefits associated with harbouring the secondary symbionts.

Resistance to parasitoid wasps

M. euphorbiae lines both with and without secondary bacteria will be exposed to the parasitoid wasp *Aphidius ervi* to ascertain whether these symbionts alter aphid susceptibility to parasitoid attack. Reciprocal crosses will also be conducted to show whether prior exposure to the secondary symbionts alters the survival of the wasp larvae and therefore of the aphid host.

Reference
Douglas, A., François, C. and Minto, L. (2006a). Facultative 'secondary' bacterial symbionts and the nutrition of the pea aphid, *Acyrthosiphon pisum*. *Physiological Entomology*, Vol. 31, pp. 262-269.

Acknowledgement
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