Friend or Foe: *Arsenophonus* in Honey Bee Health

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1 Introduction

The etiology of the honey bee decline, and phenomena such as colony collapse disorder (CCD), remains unknown. Yet there is a consensus, that the increased incidence of disease agents is a key contributor.

A symbiotic bacterium, *Arsenophonus*, has recently been identified in colonies expressing poor performance[1].

This work aims to determine the causes of reduced health in *Arsenophonus* positive colonies and examine the foundations of the *Arsenophonus* – honey bee interaction. We also hope to address the lack of reliable diagnostic tools for forecasting bee health, by exploring if the *Arsenophonus* status of a hive could constitute a soft marker for hive health predictions. Our research takes a multidisciplinary approach and works closely with the National Bee Unit at Fera.

2 *Arsenophonus* – Insect Interactions

The genus *Arsenophonus* is associated with a diversity of insect hosts and symbioses, ranging from parasitic male-killing to coevolving mutualists[2]. *Arsenophonus* symbiont roles have been well characterised in parasitoid wasps, whitefly, triatomine bugs and louse flies.

But bees have a very different lifestyle, with a complex colony structure and 'superorganism' status, little can be inferred from previous studies.

A US study reported hives affected by colony collapse disorder (CCD) showed a higher increase in *Arsenophonus* bacteria, relative to other species[3]. *Arsenophonus* continues to be found in honey bees across the globe[3,4,5,6], but the nature of its interaction has not been further characterised.

3 Hypotheses

1. *Arsenophonus* is pathogenic and the causative agent of poor health in infected colonies.
2. *Arsenophonus* amplifies only as a consequence of colonies weakened by other factors.
3. *Arsenophonus* may be a protective symbiont, thus associated with regions of poor colony health.

Symbiont OR Pathogen ?

Evidence from the Genome

- A pathogenicity or symbiosis island with leucine rich repeat (LRR) regions and sequence similarity to *Photorhabdus luminescens* makes caterpilllars floppy (MCF) gene. MCF is associated with toxin induced death and apoptosis in insect haemocytes[7].
- Type IV secretion system elements, synthase genes and other 'virulence factors' are also evident. *Relics of a past lifestyle or still functional?*

Genes required for both pathogenic and symbiotic lifestyles are often synonymous, making it difficult to infer the effect of *Arsenophonus* on honey bees using genomic data alone.

4 Prevalence in UK honey bees?

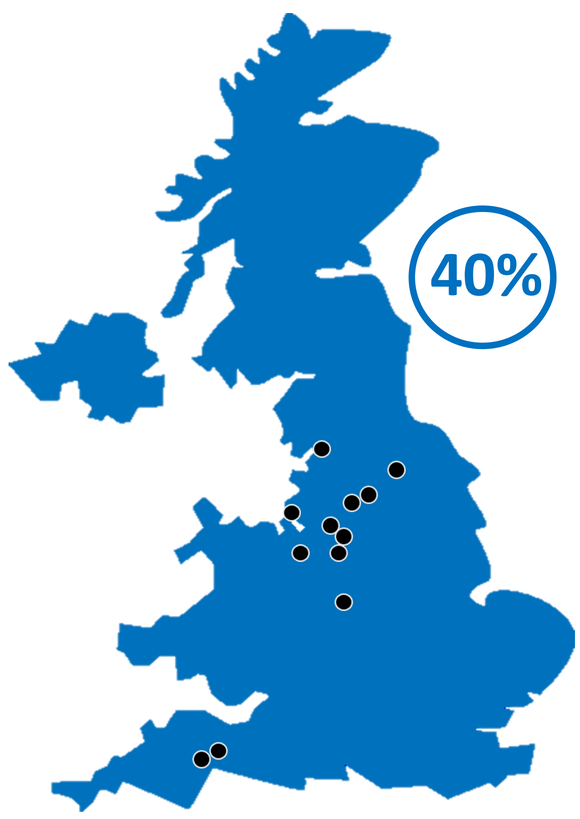

Figure 1. Distribution of colonies screened across the UK
The number of colonies associated with each location varies from 1-20, with multiple hives being screened within Yorkshire, Merseyside, Greater Manchester and Devon areas. *Arsenophonus* was present at all locations, with the exception of Merseyside (6 colonies screened).

A total of 55 colonies from across the UK have been screened for *Arsenophonus,* 22 of these tested positive using PCR assays. Giving *Arsenophonus* a prevalence of 40% in sampled honey bees (95% confidence intervals of 27% to 54%).

No spatial pattern in *Arsenophonus* presence was evident, with at least one colony testing positive from almost every area.

Method: Chelex resin DNA extractions were performed on pooled samples of honey bee worker legs from each colony. Host and symbiont DNA was amplified via PCR to allow detection of *Arsenophonus.*

5 Development of an MLST

To begin to elucidate the diversity of *Arsenophonus spp.* in honey bees we have developed a Multi Locus Sequence Typing (MLST) scheme, targeting six housekeeping genes and a further two genes of interest.

Sequenced fragments are aligned to the respective orthologs in *Arsenophonus* recovered from honey bees in Switzerland (Institute of Bee Health, Bern).

Preliminary data supports a single sequence type of *Arsenophonus* circulating in bees in the UK and Switzerland.

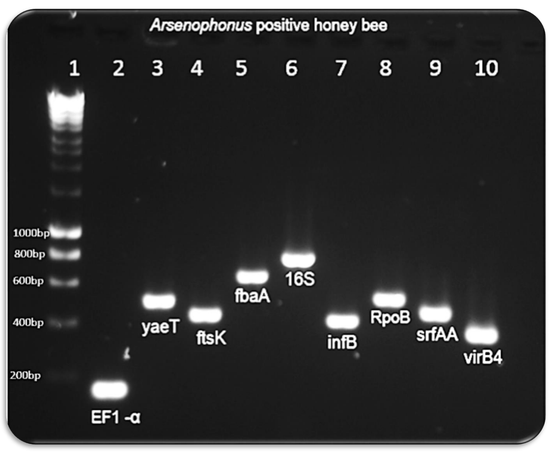

Figure 2. PCR detection of *Arsenophonus* from a honey bee
Amplification of fragments is visible for host control (lane 2), all six MLST genes (lanes 3 – 8) and two further virulence associated genes (lanes 9 - 10). A 1kb ladder (lane 1) is used for size reference and template DNA pertains to a honey bee sample from Exeter.

Future Direction

Isolate *Arsenophonus* for culture, if successful this will provide a platform for infection challenge experiments, transmission studies and an improved genome sequence.

References

[1] Giles Budge, Fera, pers comm.
[2] Novakova *et al, BMC Microbiol*, 2009
[3] Cornman *et al*, PLoS One, 2012
[4] Babendreier *et al*, *ART*, 2006
[5]Aizenberg-Gershtein *et al,* PLoS One, 2013
[6] Corby-Harris *et al*, PLoS One, 2014
[7] Daborn *et al*, Proc Natl Acad Sci ,2002

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