Bucking the Trend: The Causes and Consequences of a Colonising Pollinator

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1) Background

* Evidence for severe declines of many bumblebee species have been documented in the UK and internationally (1), in contrast the Tree Bumblebee (*Bombus hypnorum*) has expanded its range and increased in abundance (2).
* Previously a Continental European species, it was first recorded in the UK in 2001 (3) and has since expanded across much of the country (2).
* *B. hypnorum* colonisers may negatively impact native ecosystems in a myriad of ways including; resource competition and competition for nest sites with native species, co-introduction of pathogens and pollination of exotic plants (4).
* However, the species is already a part of the European bumblebee fauna, therefore it may fall into an empty available niche and have negligible impacts on native communities in the UK.

2) Aims of the Project

* **Investigate the genetic attributes which may have facilitated the rapid colonisation of the UK by *B.hypnorum* through;**

The use of genetic tools to assess the route and nature of the colonisation

Comparatively assessing the genetic diversity across the colonising wave

Analysing genetic signatures of adaptation in the colonising wave

* **Investigate the potential for competitive foraging interactions with native species**
* **Assess whether parasitic release was an influencing mechanism of the colonisation**

3) Sampling Strategy

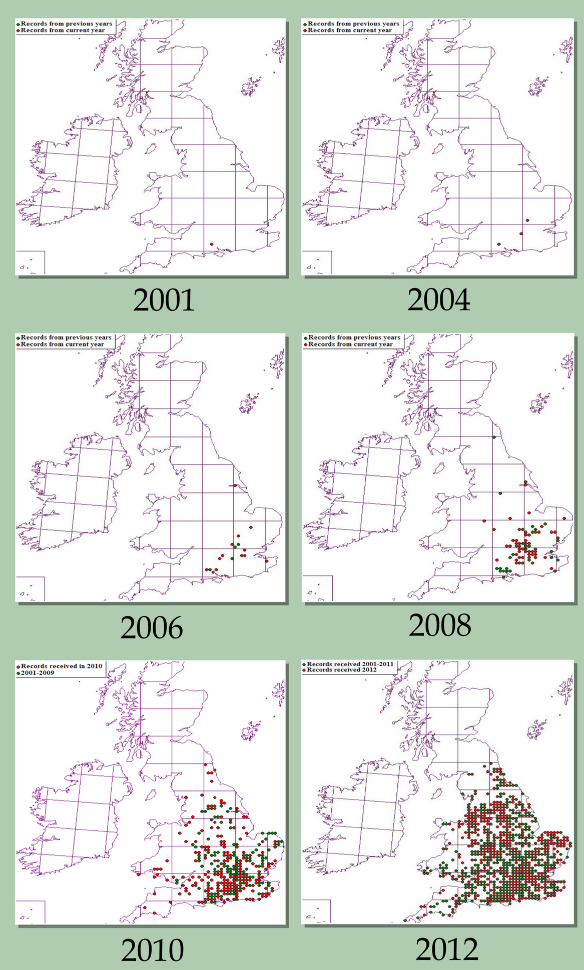


Figure 1. The distribution of *B.hypnorum* across the UK from the initial record in 2001 to 2012 (2).

The sampling strategy aimed to capture diversity across as much of the colonising range as possible.

Considering that *B.hypnorum* has expanded to colonise an extensive proportion of the UK in only 12 years (figure 1), sampling encompassed the extent of the distribution in the UK, including the region of the first sighting to the most recent accounts at the very periphery of their expanding range (figure 2).

Figure 2. UK sampling sites depicted by the asterisks; blue representing the 1st colonising wave, green the 2nd, red the 3rd and purple the 4th. Their range for sightings in 2005, 2009 and 2011 depicted by the blue, green and red lines respectively for comparison (2).

Conspecifics on the continent provide a base-line comparison of genetic diversity and parasite load.

During the summer of 2013 and 2014 *B.hypnorum* workers were collected from 11 locations across the UK (figure 2, also illustrating the dynamics of the expansion) and 6 locations along the Continental European Coastline (figure 3, overlaying the species' distribution in Western Europe).

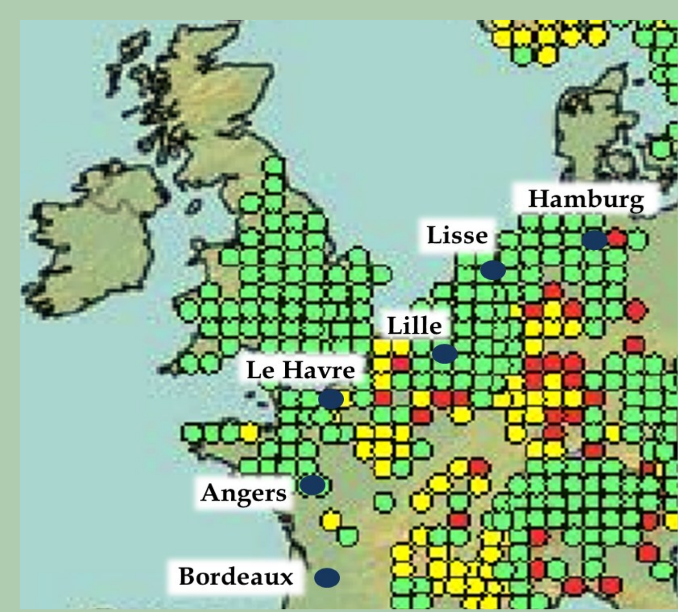


Figure 3. *B.hypnorum* distribution in Europe from the Atlas of European Bees (5). The sample sites are in navy. *(Updated in 2012. The green dots represent sightings post 1990, the yellow sightings between 1950 and 1990 and in red are sightings pre 1950).*

4) Next-Generation Sequencing

We are conducting the genetic analysis in house on the PGM Benchtop Sequencer using a modification of the RADseq method (6) derived from our in-silico digest calculations (see figure 4).

* **The Pilot Project** will address the issue of coverage; the number of copies of each fragment. We aim to directly compare the coverage recommendations between pooled and individual approaches to inform the design of subsequent experiments.
* **The Origin and Colonisation Dynamics** will be investigated by comparing phylogeographic signals to detect the founders' region of origin and possibly the colonisation route(s) **and** assessing population genetic parameters across the entirety of the colonising range.
* **Signatures of Selection;** specifically enacting on populations at the periphery of the expansion will be investigated to potentially identify candidate genes of selective significance and their related areas of functional importance.

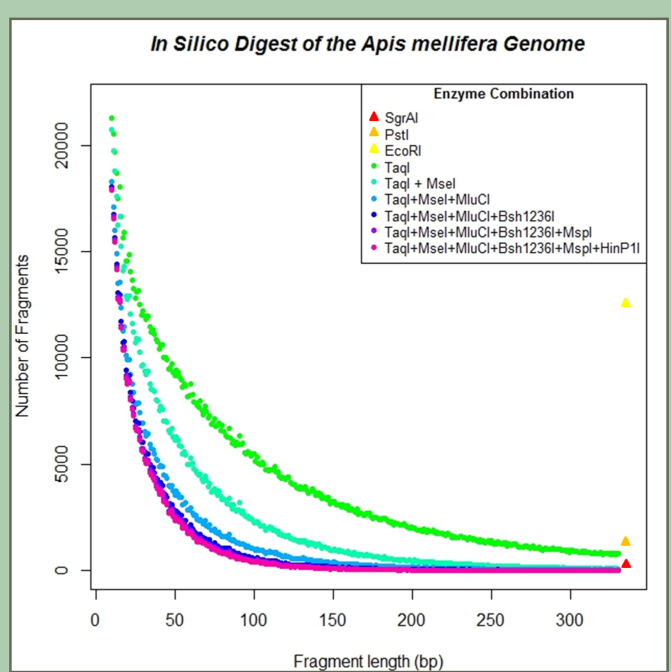
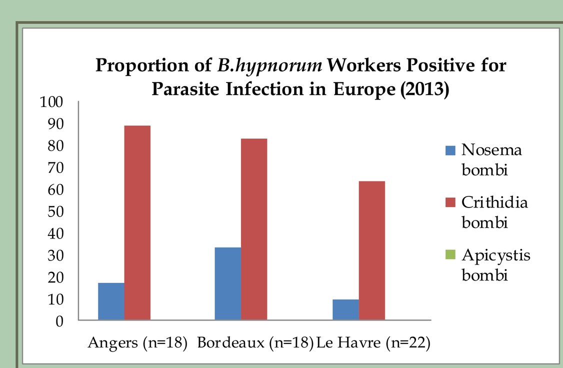


Figure 4. The relationship between chosen restriction enzyme and remaining fragment number. The reduction possible with additional digestions of varying enzyme combinations as used in the RESTseq method (7) are depicted by dots. The total number of fragments ranging between 50-300bp resulting from digestions with single rare-cutter enzymes are depicted with triangles for comparison.
In a single run 5 pooled populations could be sequenced when digested with EcoRI, this is true for Apis melliffera and Bombus terrestris.

5) Parasite Analysis

Samples were molecularly screened with parasite specific primers for the three most common parasites of bumblebees: *Nosema Bombi*, *Crithidia Bombi* and *Apicystis Bombi*. Apidae primers were used to confirm that each extraction had worked and presence was confirmed by the detection of a band at the expected fragment length. *This method does not indicate extent of infection only presence/absence.*

Apicysis bombi is a low prevalence parasite and therefore the low proportion of positive detections are to be expected. Crithidia bombi is present in high proportions in all locations. However, Nosema bombi appears to be in higher proportions at the UK sites.



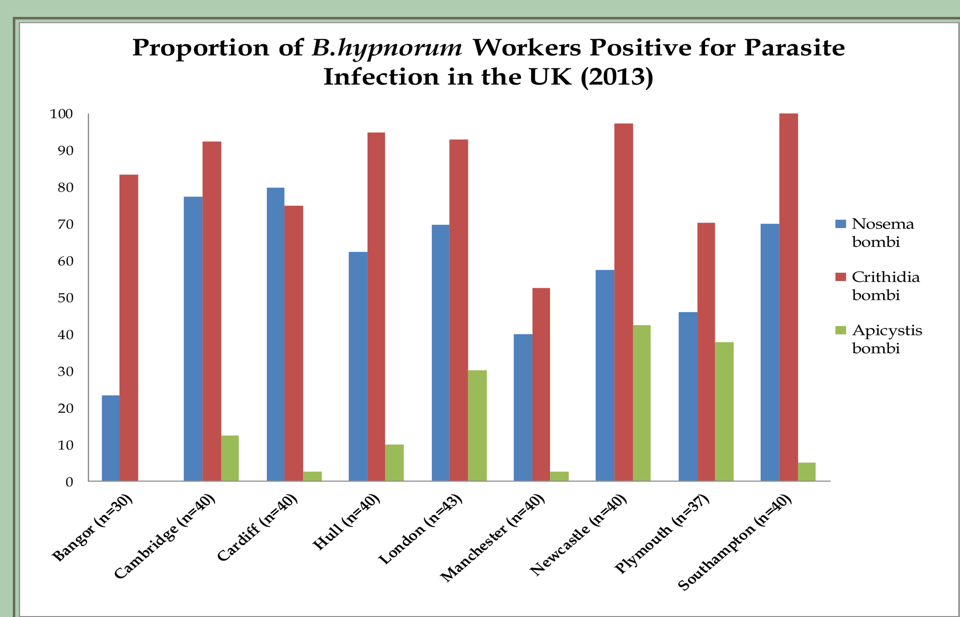


Figure 5 (above) and Figure 6 (left). Parasite load for *B.hypnorum* workers sampled across the UK and France. *Please note that the samples sizes for each population are not equal.*

The proportion of positive parasite detections appear to be higher in B.hypnorum than in workers of other UK species (S. Rustage PhD Unpublished). The same trend has also been found in queens (8).

6) Conclusions and Future Directions

* A colonising population may experience release from its natural enemies, facilitating its success (9). *B.hypnorum* appears to be expanding despite suffering a higher proportion of parasites than its congeners and tentatively its conspecifics on the continent. Another aspect of this species' ecology may have been sufficiently advantageous to overcome this, allowing it to expand successfully.
* The sequencing analysis will help answer a key question concerning *B.hypnorum's* colonisation of the UK ,'where did it come from?' and paint a comprehensive picture of the dynamics of the expansion potentially including the detection of colonisation route(s), assessment of primary population genetic parameters (e.g. evidence for a bottleneck) and identification of genetic regions of selective importance.

The findings may have implications for the conservation of UK bumblebees and be informative in light of pollinator distribution shifts and the potential for similar colonisation events to occur as a consequence of climate change.

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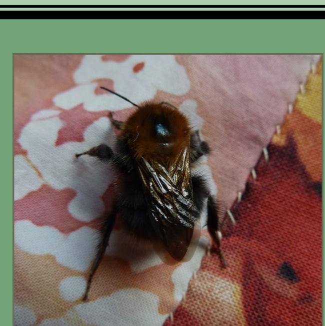

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