Exploring the possibilities to use archived pest aphids for molecular analysis of long-term aphid-parasitoid-endosymbiont interactions

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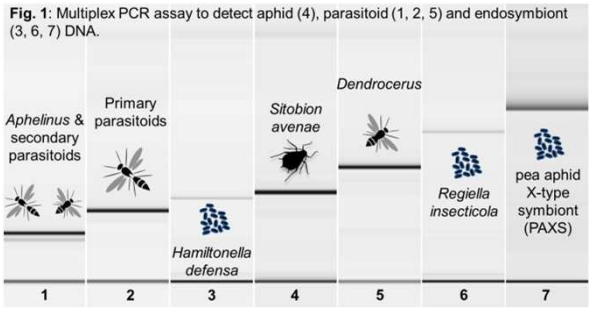
Background

- Detailed knowledge about aphid-parasitoid-endosymbiont interactions is important to better understand aphid control by parasitoids. Aphids collected by suction traps over several decades in Europe would provide the ideal sample set to molecularly examine these interactions on a long-term basis.
- However, it is unknown if such aphids, which were stored for multiple years, can provide amplifiable DNA of aphids, parasitoids and endosymbionts. This question is addressed in our project.

Material & Methods

1. DNA of 119 archived aphids, which were collected either in the 20th (1977-1980) or in the 21st (2001-2009) century within the Rothamsted Insect Survey (UK), was extracted using Biosprint 96 and a Chelex-based method.
2. Aphids were molecularly identified to species level using PCR assays and DNA barcodes.
3. All samples were also subjected to an aphid-parasitoid-endosymbiont multiplex PCR assay to detect DNA of parasitoids and three endosymbiont species (Fig. 1).

Fig. 1: Multiplex PCR assay to detect aphid (4), parasitoid (1, 2, 5) and endosymbiont (3, 6, 7) DNA.



Results

1. All samples from the 21st century contained amplifiable DNA while this was true for only four samples from the 20th century (Fig. 2).
2. Out of the 21st century samples, sequencing was successful in 97% (n=33) and 100% (n=35) of Biosprint- and Chelex-extracted samples, respectively, and revealed 24 aphid species. Additionally, four out of twelve 20th century samples (33%) were successfully sequenced providing sequences of non-aphid insects, birds, and microorganisms (Fig. 3).

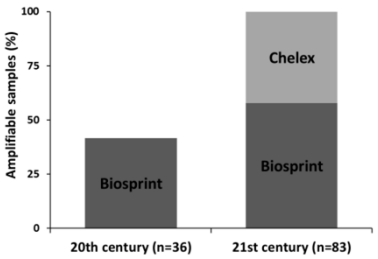


Fig. 2 DNA amplification success using universal invertebrate primers C1-J-1859 (Simon *et al.* 1994) and HCO-2198 (Folmer *et al.* 1994) in aphids collected in 20th (1977–1980) and 21st (2001–2009) century via suction traps.

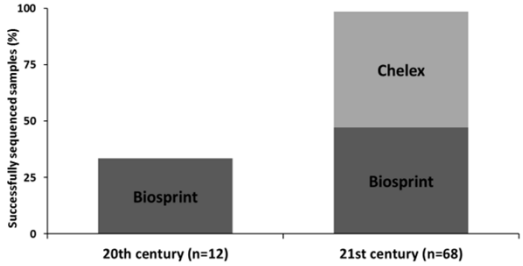


Fig. 3 Successfully sequenced samples using PCR products amplified by general invertebrate primers. PCR products were amplified from aphid DNA extracts generated by Biosprint 96 and the Chelex extraction protocol. The aphids were collected in 20th (1977–1980) and 21st (2001–2009) century via suction traps.

3. Using diagnostic PCR assays on the 21st century samples, 17 were identified as cereal aphids, including seven *Sitobion avenae*, four *Rhopalosiphum padi* and six *Metopolophium dirhodum*. Subjecting these samples to the aphid-parasitoid-endosymbiont assay, one sample tested positive for DNA of primary parasitoids and 19 samples for DNA of secondary endosymbionts, including 14 for *Hamiltonella defensa*, two for *Regiella insecticola* and two for PAXS.

Simon *et al.* Annals of the Entomological Society of America 87, 651-701, 1994
Folmer *et al.* Molecular Marine Biology and Biotechnology 3, 294-299, 1994

Conclusions

Aphid samples collected in the 21st century are a suitable source of molecular information for studying aphid-parasitoid-endosymbiont interactions on a long-term basis. on the contrary, older samples from the 20th century, do only rarely allow retrieving usable DNA which might be due to inappropriate storing methods and/or the long time storage.

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