This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement number 817622.

EUROPEAN DISTRIBUTION OF GENETIC VARIANTS ASSOCIATED WITH *VARROA* DRONE BROOD RESISTANCE IN HONEY BEES

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Background

Already since the early spread of *Varroa destructor* on *A. mellifera*, numerous natural surviving colonies have been described in which suppression of mite reproduction was or is a prominent defensive trait. In 2019, B. J. G. Broeckx et al., (2019) published a model in which eight single nucleotide polymorphisms (SNPs) in seven different *Apis mellifera* genes were associated with drone brood resistance (DBR, i.e. mite non-reproduction in drone brood) in one Dutch hybrid *Varroa destructor* resistant/sensitive colony (1). The eight variants model predicted 88% of the drone phenotypes correctly and identified six risk variants and two protective variants. The researchers hypothesized that some SNPs (found in different transporters) might cause a lower brood pheromone production by the drone pupae, while another SNP in the dynein beta chain (a cytoskeletal motor protein involved in retrograde transport in insect olfactory neurons) could cause better sensing of these reduced pheromone concentrations by the brood-caring worker bees (1).

Objective

The objective of this study was to determine the allelic frequencies of these eight SNPs associated with DBR in pooled worker bee samples from different honey bee colonies across the European continent to evaluate the potential for sustainable beekeeping and –breeding in Europe through the use of marker-assisted selection (MAS).

Materials & Methods

A total of 366 *A. mellifera* colonies across 14 participating European countries have been sampled for adult worker bees during the autumn of 2022 (**Fig. 1**). The sampling strategy comprised countries located along the longitudinal axes of the continent as well as countries spread along its latitudinal axes. Per colony, gDNA was prepared from pooled worker bees. For each gDNA sample to be analyzed, eight qPCR genotyping assays with dual-labeled probes were performed according to the protocols described by Bouuaert et al., (2021) (2). Calibration curves were constructed by pooling proportions of volumes of individual thorax gDNA equal to the percentages of Wt and Vt alleles in the calibration curve samples. In each qPCR assay with dual-labeled probes for Vt allele frequency analysis in the European pooled bee samples, calibration curve standards of 0%, 20%, 40%, 50%, 60%, 80% and 100% Vt allele were run in duplicate. For each genetic variant, the percentage of Vt allele in a pooled worker gDNA sample was determined by regression analysis of the end-RFU value of the Vt allele's fluorophore in the sample vs. the intraplate calibration curve. Data analysis was done with the Bio-Rad CFX Manager 3.1 Software.

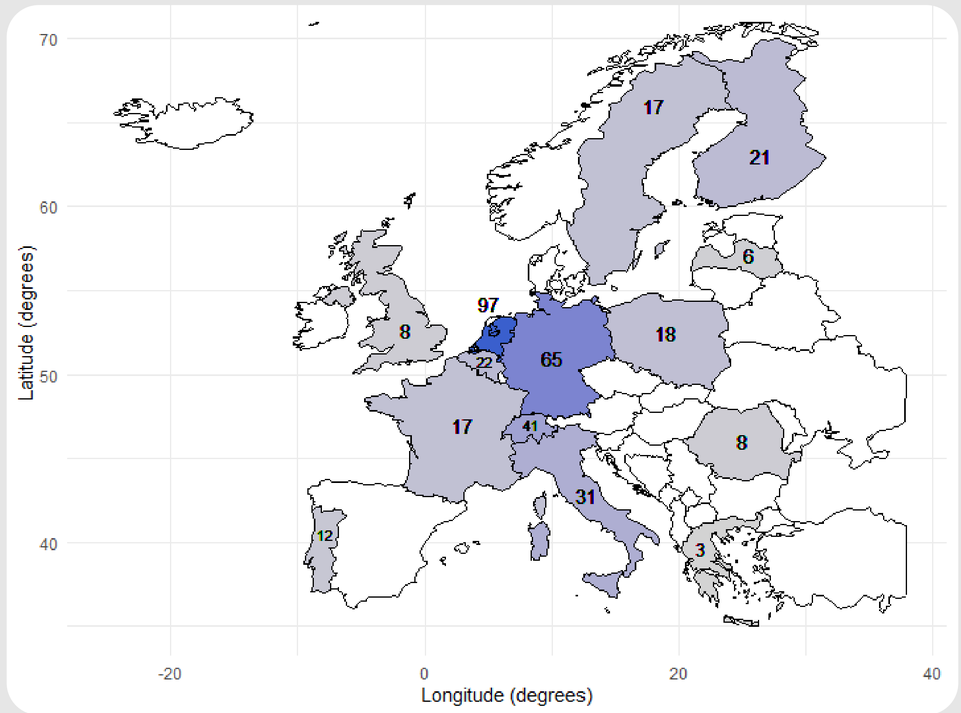
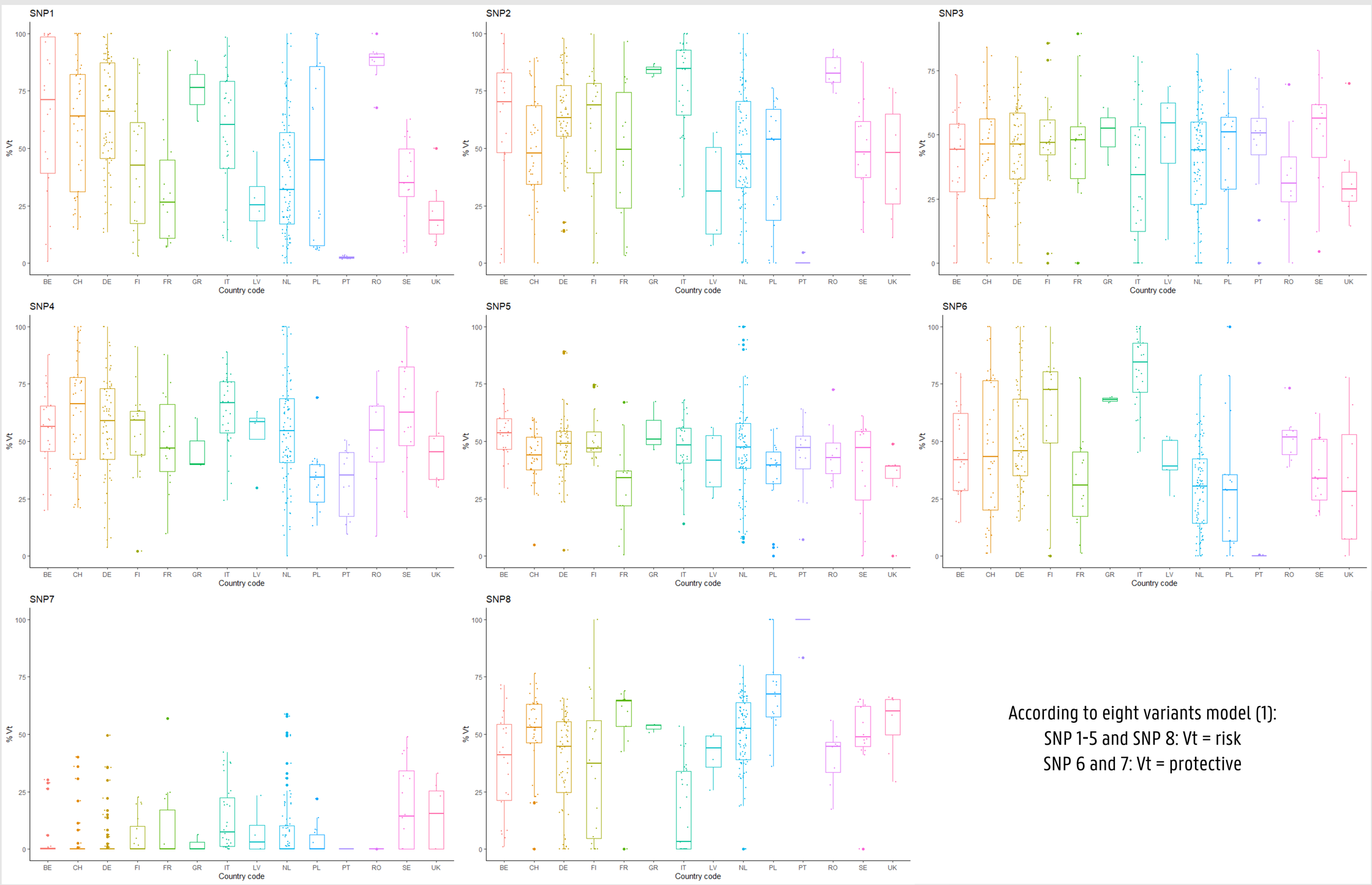


Fig. 1 Number of sampled colonies per country

Results



According to eight variants model (1):
SNP 1-5 and SNP 8: Vt = risk
SNP 6 and 7: Vt = protective

Conclusion

For SNP 3, no differences in distributions of %Vt allele in worker pools were found between the screened countries (Kruskal-Wallis H; $p > 0{,}05$). For all remaining SNPs, some countries showed significant differences in distributions of %Vt allele in worker pools compared to others (Kruskal-Wallis H; $p < 0{,}05$). More detailed results (Post-hoc country-pairwise Mann-Whitney U Tests) are available, but not shown on this poster. The obtained percentages of Vt allele in the worker pools will be linked to the known *A. mellifera* races of the samples in future data analysis.

References

(1) Broeckx, B., De Smet, L., Blacquière, T. et al. Honey Bee Predisposition of Resistance to Ubiquitous Mite Infestations. Scientific Reports 9 (2019). https://doi.org/10.1038/s41598-019-44254-8
(2) Claeys Boúúaert, D., Van Poucke, M., De Smet, L. et al. qPCR assays with dual-labeled probes for genotyping honey bee variants associated with varroa resistance. BMC Veterinary Research 17, 179 (2021). https://doi.org/10.1186/s12917-021-02886-x

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