OP-089 [Bee Biology]

Implementation of an extended SNP Panel for the genetic monitoring of honeybees and its application to A. mellifera mellifera and A. mellifera carnica samples across Europe

Jana Vanessa Huml[1,2,3], Richard Bernstein[4], Andreas Hoppe[4], Mairi E. Knight[3], Jonathan S. Ellis[3], Victoria G. Buswell[6], Dora Henriques5, M. Alice Pinto[5], Grace Mccormack[7], Aglagane Abdessamad[8], Asmaa Anwar[13], Alireza Arab[9], Dilek Muz[10], Mustafa Necati Muz[10], Marion Zammit Mangion[11], James Arruda Salome[12], Gabriele Soland Reckeweg[2]
[1]Mellifera.ch, 9533 Kirchberg, Switzerland
[2]Apigenix 2513 Twann, Switzerland
[3]University of Plymouth, PL4 8AA, Plymouth, United Kingdom
[4]LIB, Länderinstitut für Bienenkunde Hohen Neuendorf e.V., D-16540 Hohen Neuendorf, Germany
[5]CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[6]James Hutton Institute, Dundee DD2 5DA, United Kingdom
[7]University of Galway, H91 TK33, Ireland
[8]University Ibn Zohr, BP 32/S Agadir, Morocco
[9]University of Tehran, Department of Animal Science, Karaj, Iran
[10]Namık Kemal University, 59030 Süleymanpaşa/Tekirdağ, Turkey
[11]University of Malta, Msida MSD 2080, Malta
[12]Federal University of Santa Catarina, Santa Catarina 88034-700, Brazil
[13]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

A number of methods have been established for the routine genetic monitoring of honeybees. These mainly focus on the estimation of hybridization between subspecies, as introgression is a major threat to the genetic integrity of native subspecies, leading to the replacement of locally adapted forms and the loss of species' genetic diversity through homogenization. Given this focus, intra-subspecies diversity of markers is limited, making them less suitable for the estimation of other measures relevant in a conservation monitoring scheme, such as genetic diversity, inbreeding and effective population size.

In this project, we evaluate a new tool for the genetic monitoring of honeybees, a 70K SNP-chip based on Jones et al. (2021). This has the advantage of including markers identified in previous studies that reflect phenotypic differences, such as variation in immune response or associations with traits such as hygienic behaviour, honey production and varroa tolerance. It also includes previously published marker sets for the estimation of introgression. The inclusion of markers that show intra-subspecies variation of functional relevance, as well as the higher marker density compared to previous honeybee SNP-chips, makes this a promising tool including a wide range of applications. These are the estimation of genetic diversity, inbreeding and effective population size, parentage analysis and possible genomic selection in breeding programs. Further, the analysis of introgression was optimized to distinguish between six clusters comprising of the A-, O- and Y-Lineages, A. mellifera mellifera, A. mellifera carnica and A. mellifera ligustica, using a comprehensive database of 1270 reference samples, built considering a total of 20 subspecies and ~4600 samples across Europe, the Middle East and Africa.

Here, we report on the optimization and validation of these applications on the basis of the 70K SNP-chip and present results on ~4200 samples targeting A. m. mellifera and A. m. carnica across 20 European countries.

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LIB Länderinstitut für Bienenkunde

huml@mellifera.ch

Apigenix Institute for Beegenetics

UNIVERSITY OF PLYMOUTH

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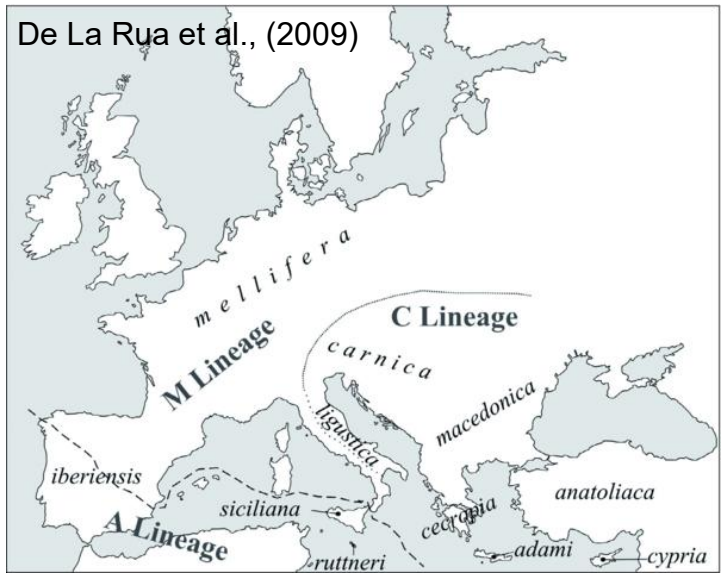
Lack of comprehensive tools for the routine genetic monitoring of honeybees

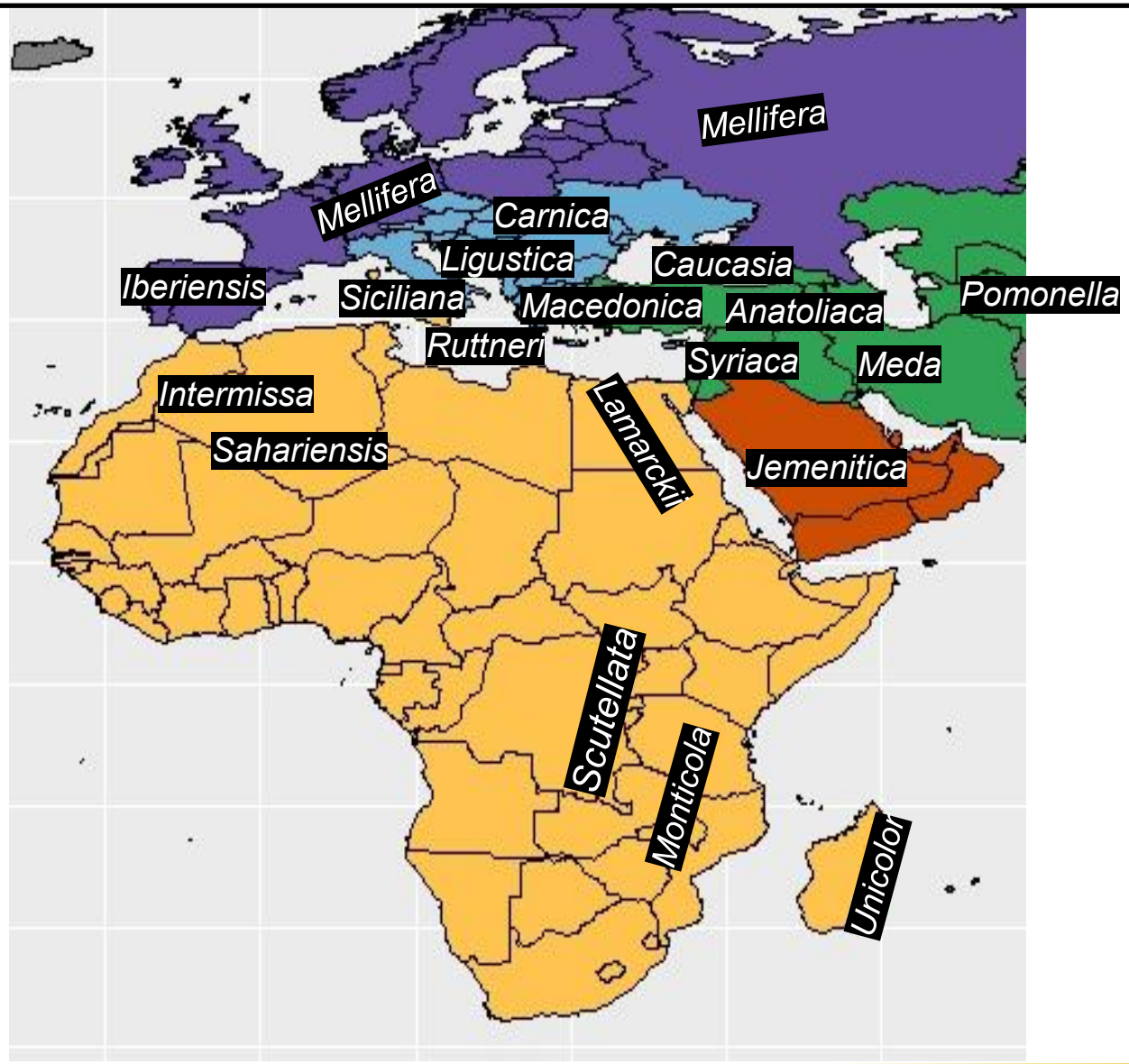
- Designed for single application, most commonly taxonomic testing



- Mitochondrial DNA lineage assignment (Garnery et al., 1992)
- Subspecies classification (Momeni et al., 2021)
- Quantification of C and M lineage introgression (Henriques et al., 2018)

- Lack of comprehensive test including all evolutionary lineages to quantify introgression (e.g. from Buckfast hybrids)
- Lack of hybrid test integrated with other applications

Sampling using Illumina Honeybee2021 70K SNP-chip



A-lineage

O-lineage

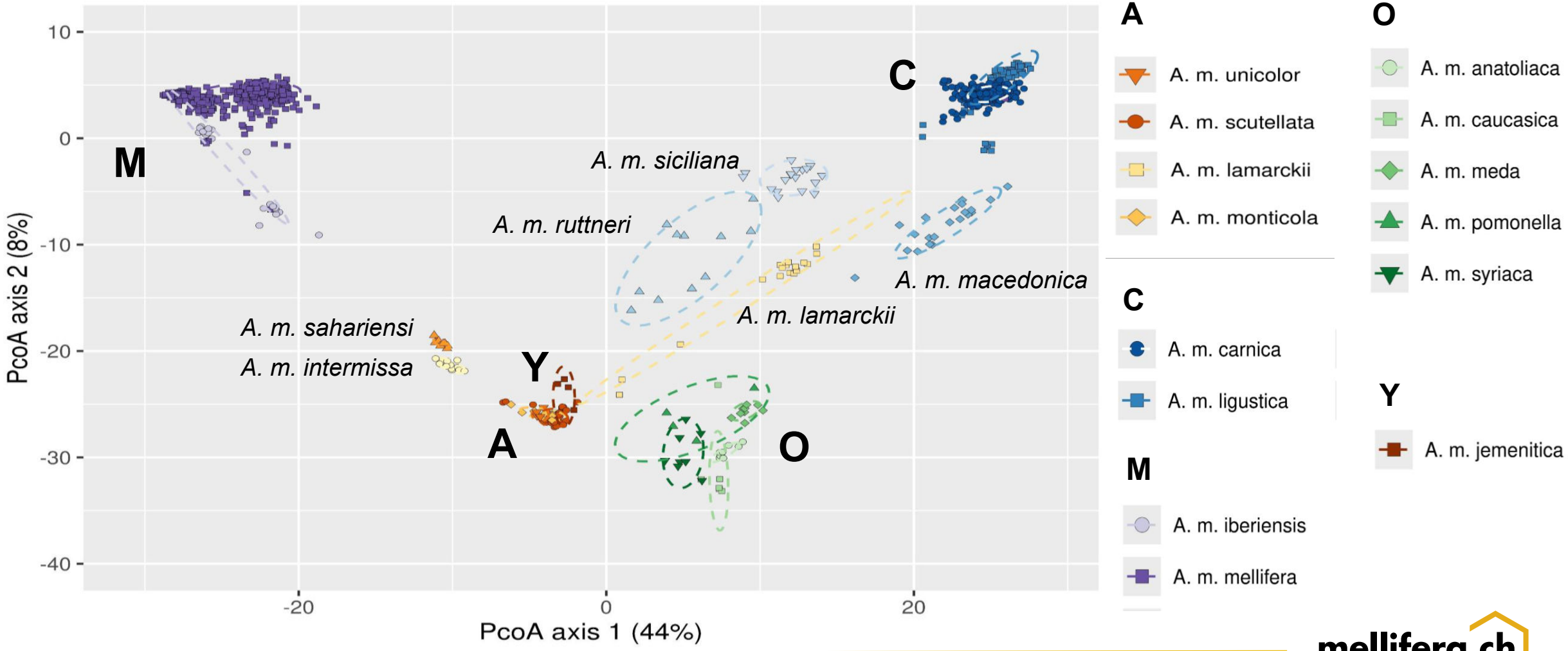
Y-lineage

M-lineage

C-lineage

First hybrid test including all major lineages of *Apis mellifera*

Six Reference Clusters: *A. m. carnica, A. m. ligustica,* lineage A, M, O, Y



Huml *et al.* (in Review)

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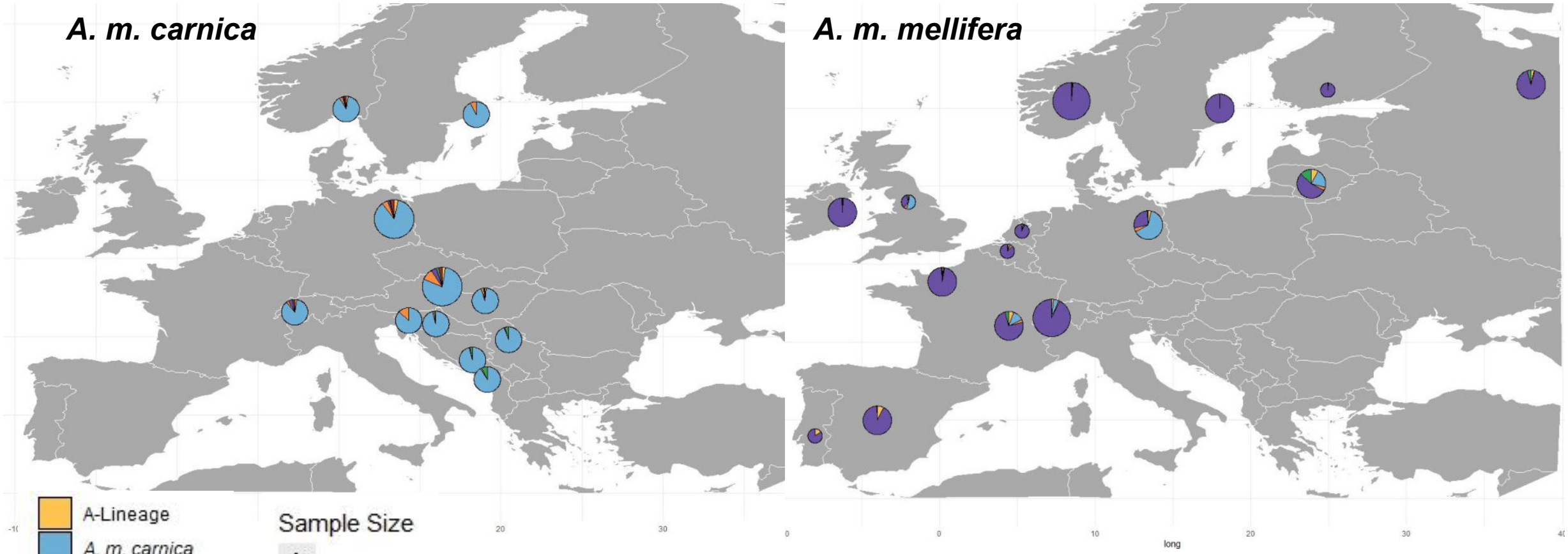


Huml *et al.* (in Review)

Genetic monitoring of honeybees: Applications

Individual

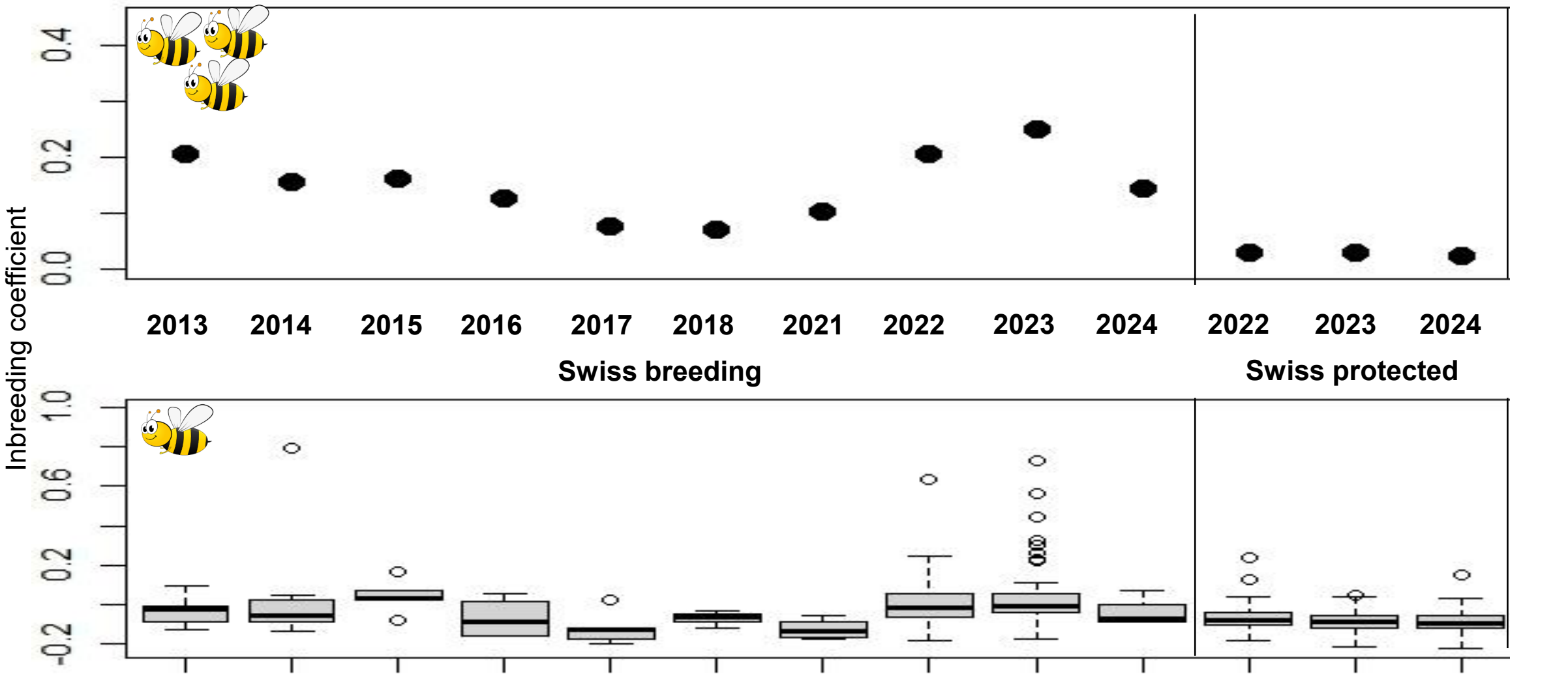
Illumina Genotype 70K SNPs

Population

- Hybridization & Introgression
- Genetic diversity & Inbreeding
- Paternity & Pairwise relationship
- Genomic breeding value

- Genetic diversity & Inbreeding
- Effective population size
- Differentiation & Migration

Genetic diversity & Inbreeding



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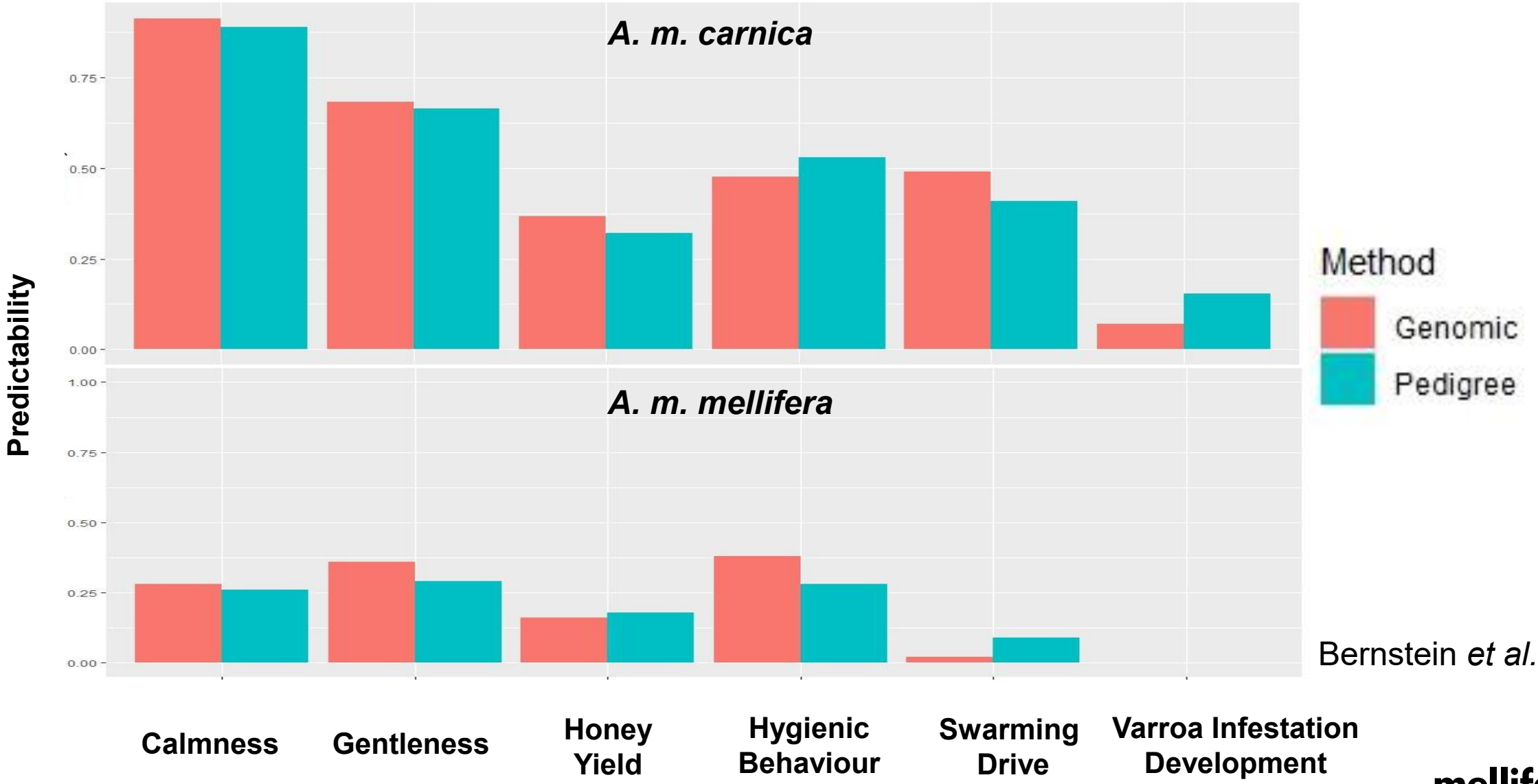
Genomic breeding value



Bernstein *et al.* (2023)

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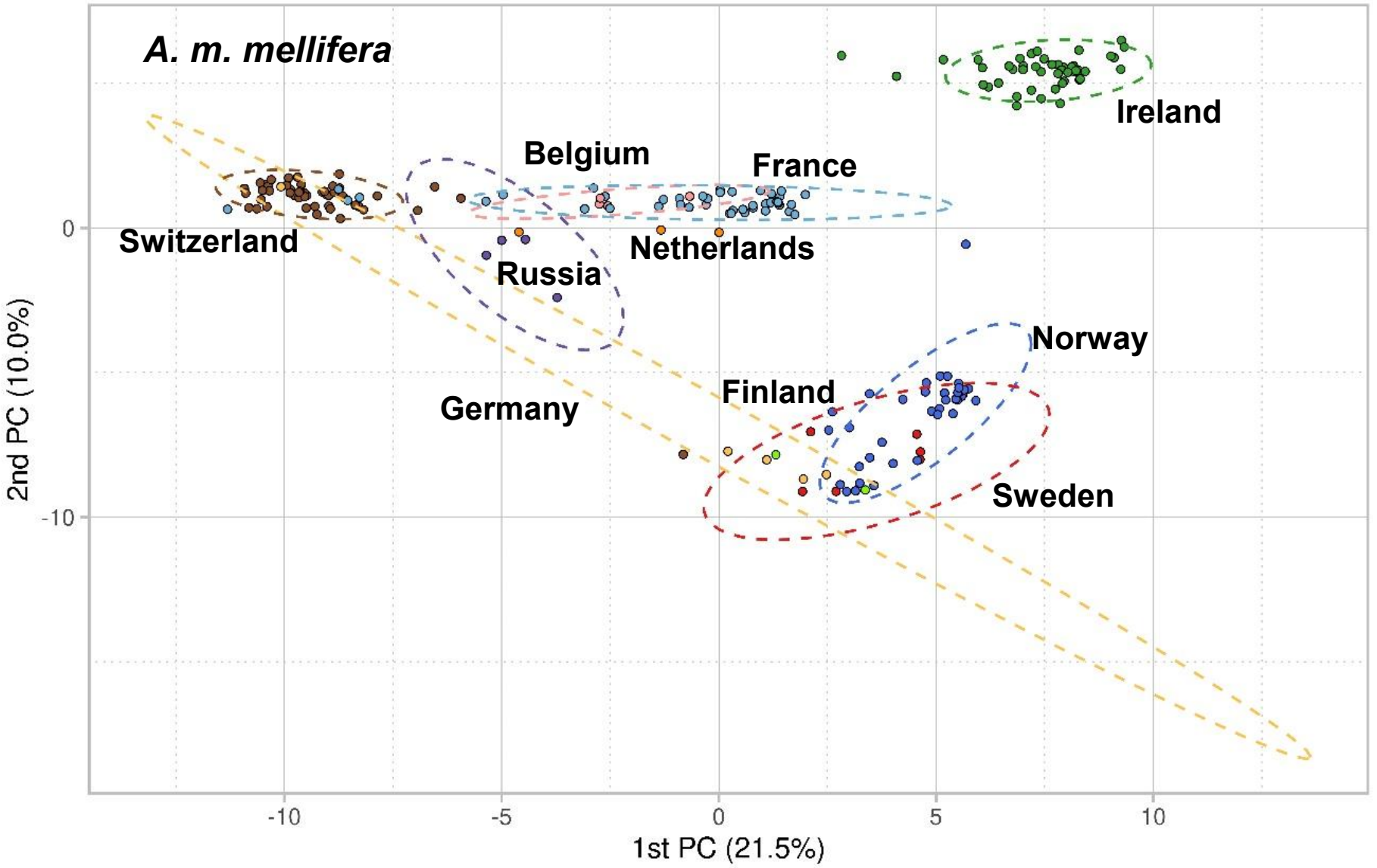
Differentiation & Migration



Huml *et al.* (in Review)

Implementation of tools for routine applications

- **Hybrid test**
  - ➽ Accurate quantification of introgression
- **Individual Inbreeding estimation**
  - ➽ Keep your stock healthy and diverse
- **Kinship analysis**
  - ➽ Improve your line management and pooled matings
- **Genetic Monitoring**
  - ➽ Keep your population genetically monitored to keep it diverse
- **Individualised Conservation Concepts**
  - ➽ Get help to establish your individual conservation concept
- **Application writing**
  - ➽ Get help finding the right words for your donors

Apigenix
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www.apigenix.com

info@apigenix.com

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Thank you for your attention!

Questions?

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