

OP-067 [Bee Biology]

From genomes to holobionts: can the reanalysis of large-scale bee sequencing projects open new perspectives?

Thibault Leroy

GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan 31326, France

Up to now, the BeeGEES lab, based in Toulouse, France, has resequenced more than 2,700 complete honey bee genomes, including 1,200 haploid drones and 1,500 queen genomes reconstructed from sequencing pools of workers using a pool-seq strategy. With this extensive dataset, our lab has generated fundamental insights into the population genomics of honey bees, shedding light on their population structure, long-term evolutionary history, and recent demographic trajectories in the context of modern beekeeping. Additionally, we have explored the genomic architecture of key traits associated with varroa resistance, contributing to a better understanding of their genetic basis. Beyond presenting these recent advances in honey bee genomics, this talk aims to introduce a new perspective: how reanalysis of large sequencing projects can help us better describe the bee holobiont, composed by the bee and its associated microbiota.

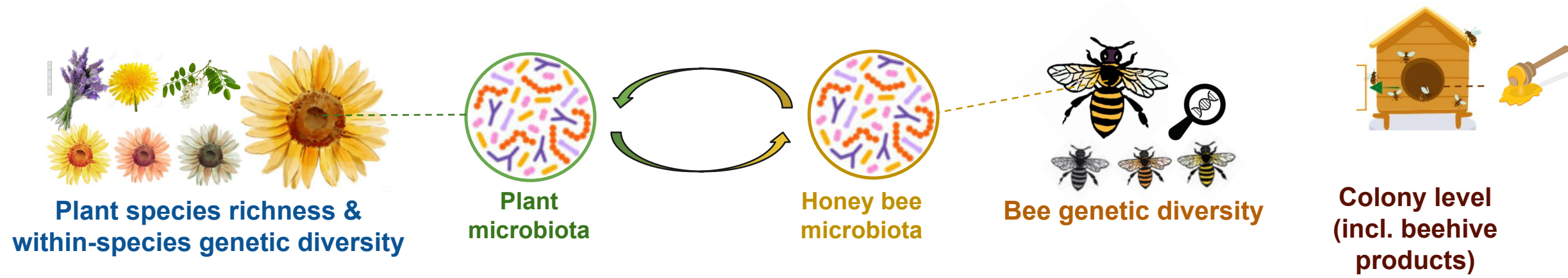
In practice, population genomics studies rely on mapping sequencing reads to a reference genome or a pangenome. However, unmapped reads - those that do not align with the reference - are assumed to be non-bee DNA and are discarded at the very first stages of analysis. Here, we propose that these unmapped reads contain valuable information and could provide new insights into the biological communities associated with bees. The presentation will focus on an ongoing study in which these unmapped reads are meta-assembled to reconstruct genomic information about the associated communities. In the long term, this approach could pave the way for in-depth metagenomic characterization of both bees and their associated organisms, including pathogens, using already available data. This would contribute to a more integrative understanding of bee biology and health.

➤ From genomes to holobionts: can the reanalysis of large-scale bee sequencing projects open new perspectives?

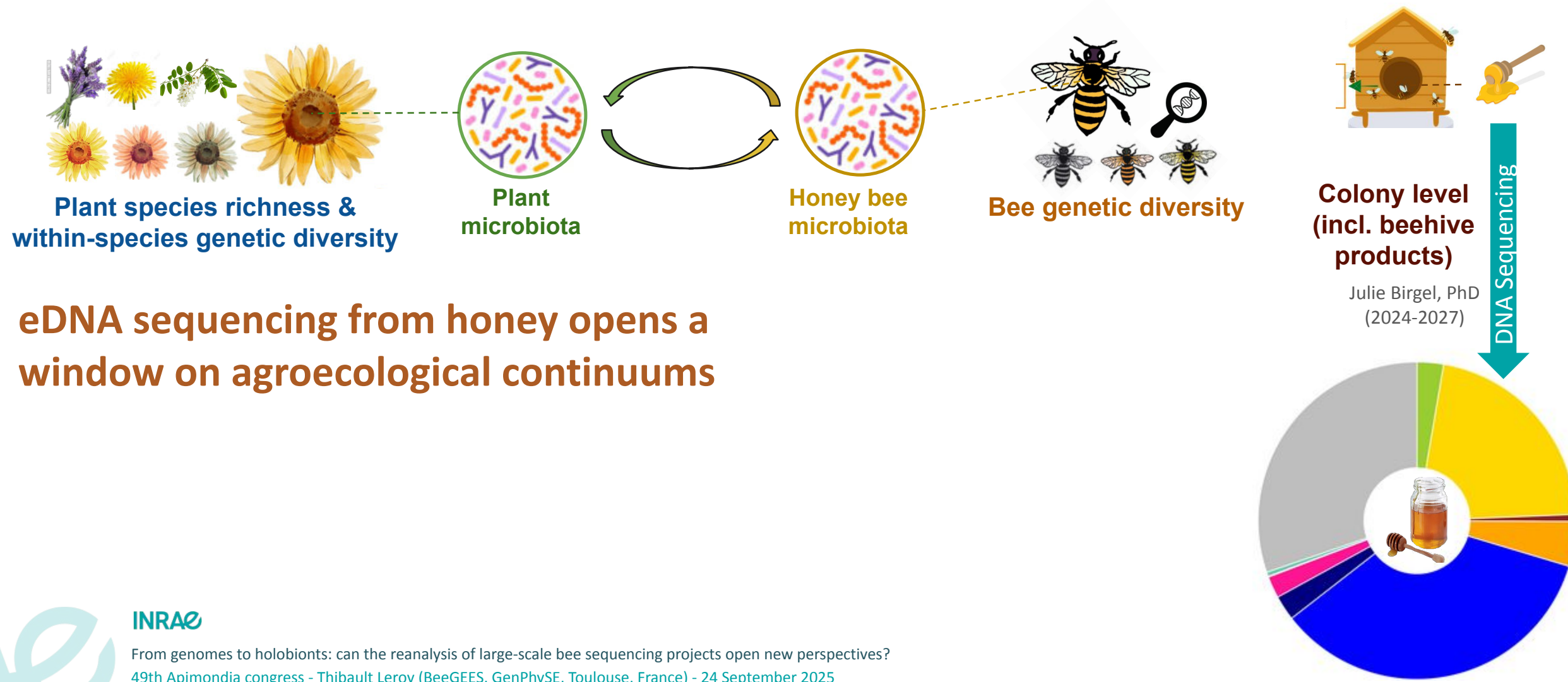
Thibault Leroy

BeeGEES lab, GenPhySE, INRAE Toulouse, France

Plant-bee pollinators as agroecological continuums



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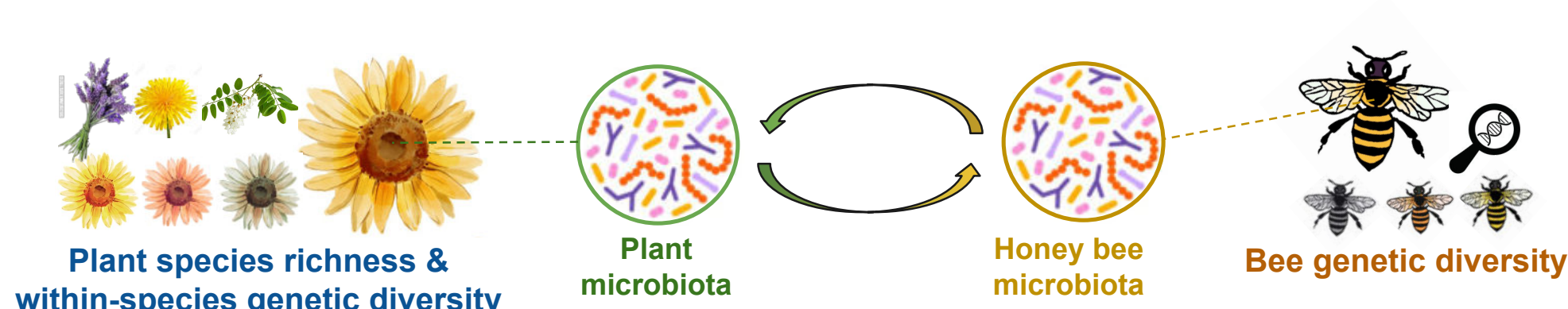
eDNA sequencing from honey opens a window on agroecological continuums



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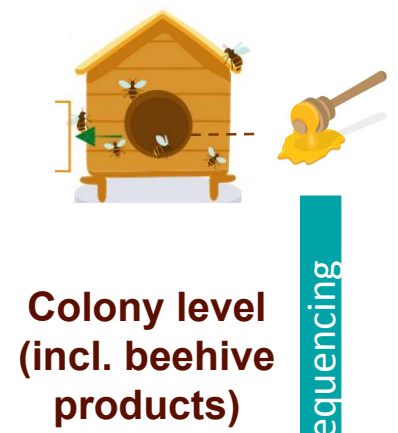
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To know more, visit the poster **#217** by Julie Birgel & collaborators (Wednesday & Thursday)

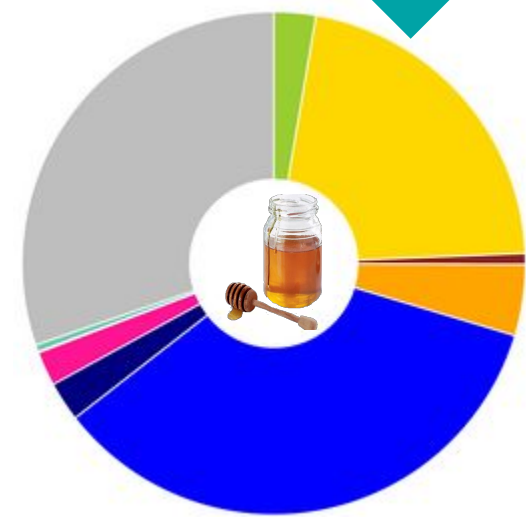
From hive to table: the promise of shotgun metagenomic to trace the honeys' journey.



Colony level (incl. beehive products)

Julie Birgel, PhD
(2024-2027)

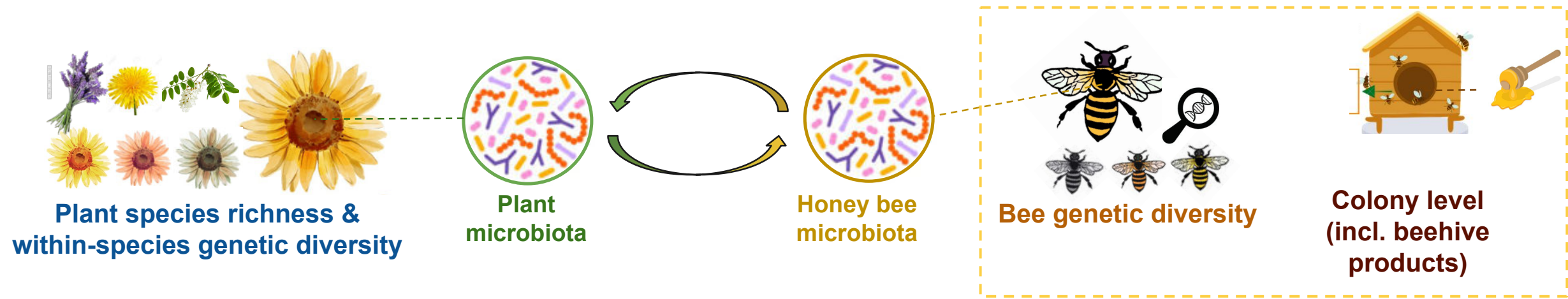
DNA Sequencing



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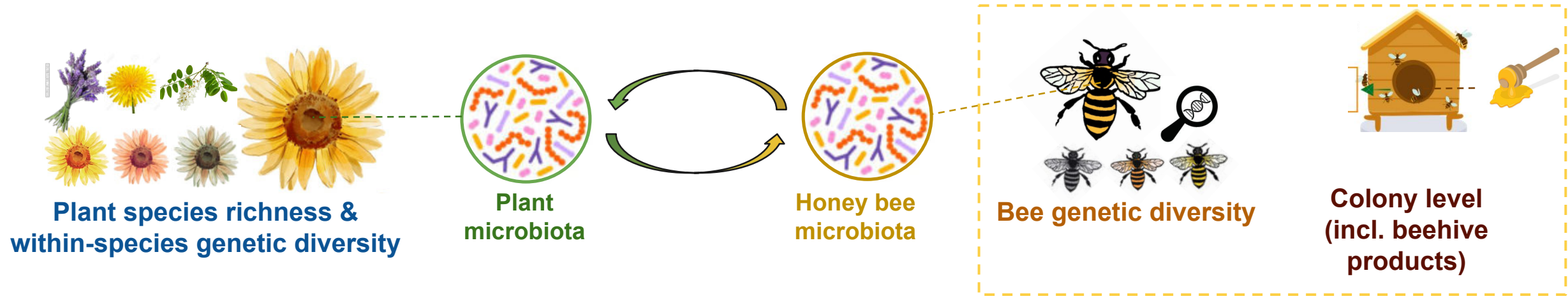
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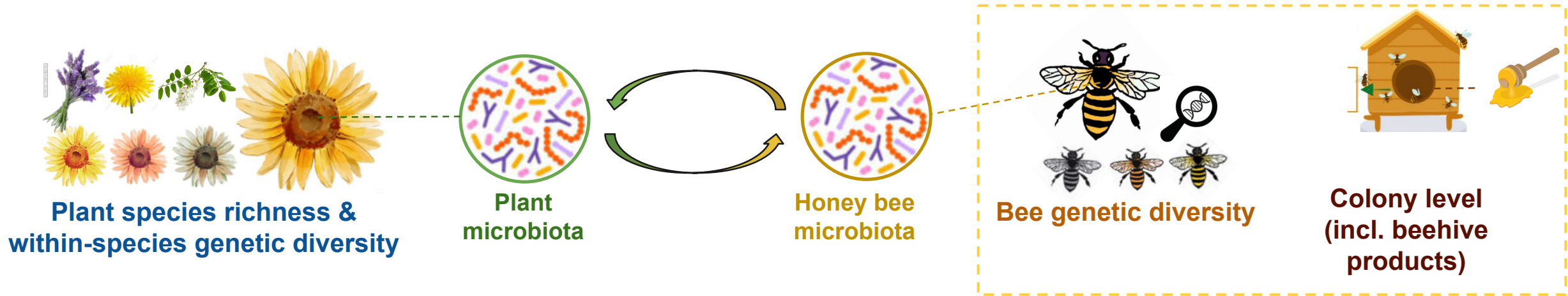


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- the population structure and evolutionary history of honey bees
- their genetic diversity
- their adaptation to different stressors (Varroa, climate, ...)



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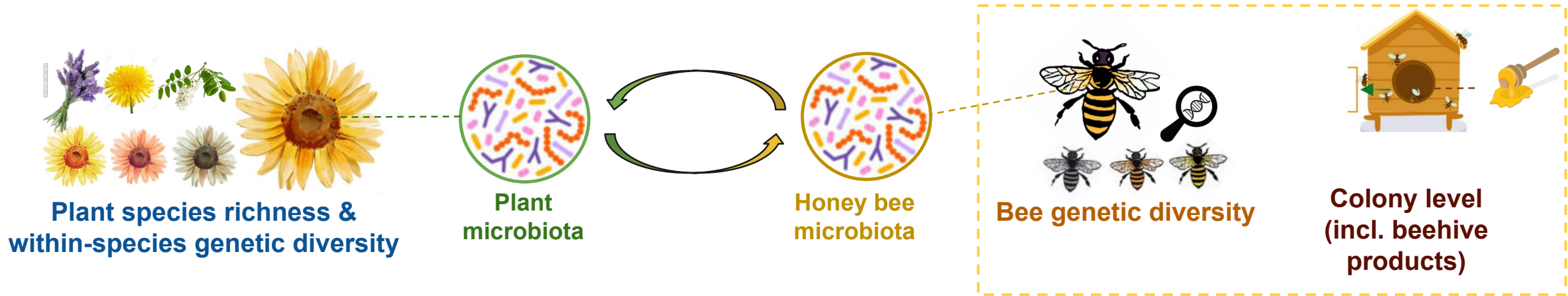
1500+ pool of workers (PoolSeq, colony level)



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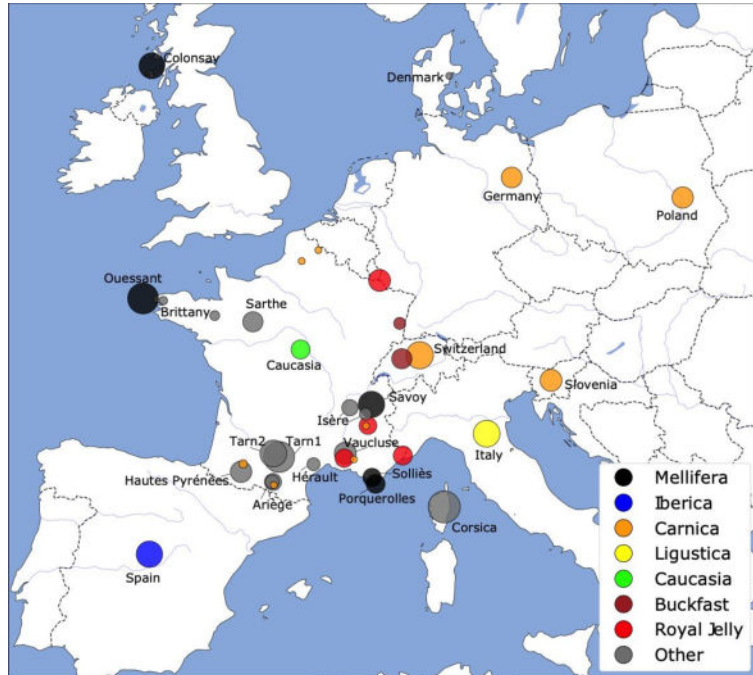
Population structure in Western Europe

RESOURCE ARTICLE

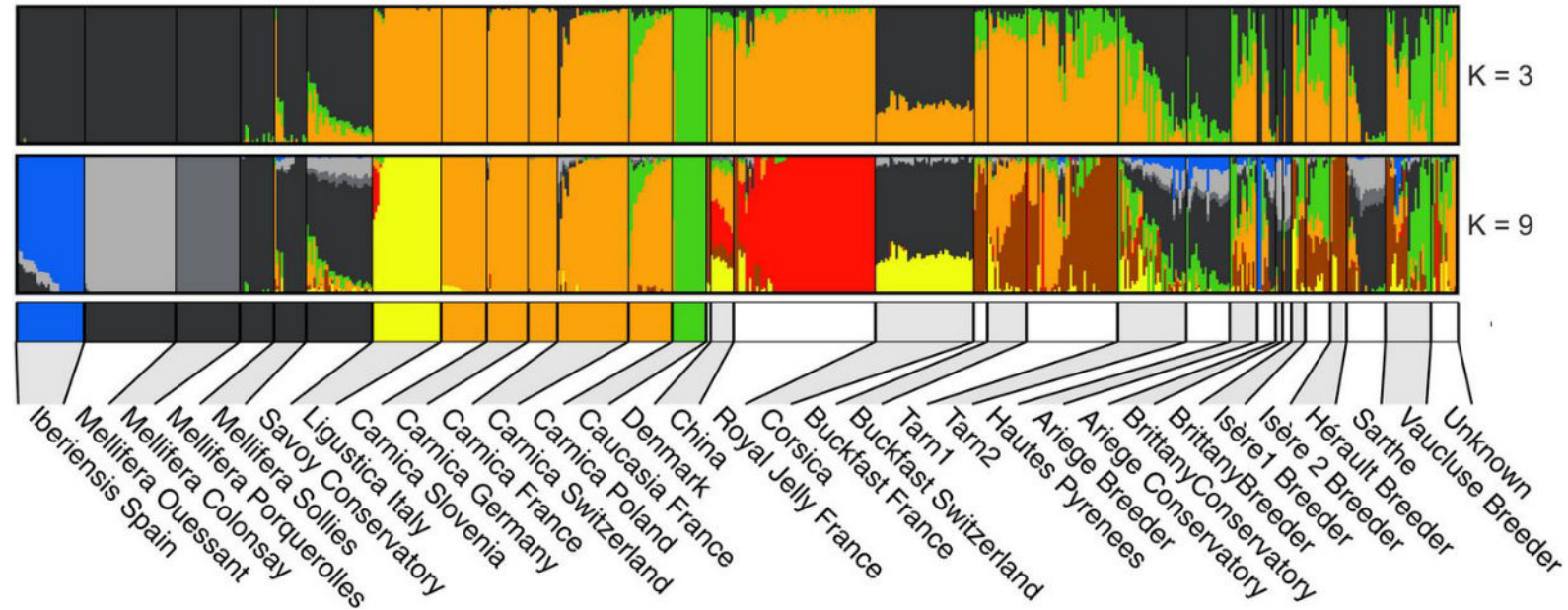
MOLECULAR ECOLOGY
RESOURCES WILEY

Complex population structure and haplotype patterns in the Western European honey bee from sequencing a large panel of haploid drones

David Wragg¹ | Sonia E. Eynard¹ | Benjamin Basso² | Kamila Canale-Tabet¹ | Emmanuelle Labarthe¹ | Olivier Bouchez³ | Kaspar Bienefeld⁴ | Małgorzata Bienkowska⁵ | Cecilia Costa⁶ | Aleš Gregorc⁷ | Per Kryger⁸ | Melanie Parejo⁹ | M. Alice Pinto¹⁰ | Jean-Pierre Bidanel¹¹ | Bertrand Servin¹ | Yves Le Conte¹² | Alain Vignal¹



Sub-
species
Beek.



3 main genetic backgrounds (K=3) in Western Europe

Black (M-lineage), yellow (C) and Caucasian (O) honey bees

9 genetic groups in this dataset (K=9):

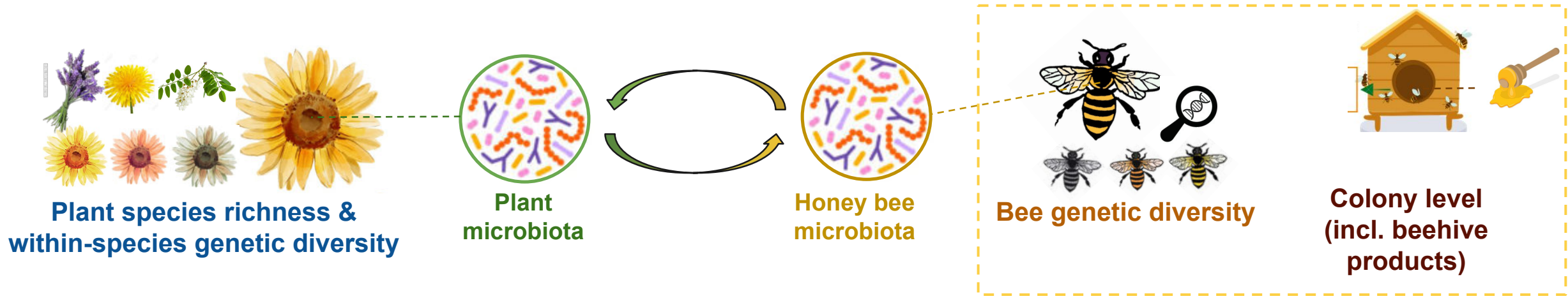
- Black honey bees (M) subdivided in 4 groups (within *A. m. mellifera* & *iberiensis* subsp)
- Yellow honey bees (C) subdivided in 3 groups (within *A. m. carnica* & *ligustica* subsp)
- A single Caucasian group (O) in our dataset (*A. m. caucasia*)
- A specific background found for the Buckfast lineage

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Same dataset: estimating diversity and the impact of modern beekeeping



Inferring Long-Term and Short-Term Determinants of Genetic Diversity in Honey Bees: Beekeeping Impact and Conservation Strategies

Thibault Leroy ^{1,*} Pierre Faux ¹ Benjamin Basso ² Sonia Eynard ¹ David Wragg ³
Alain Vignal ¹

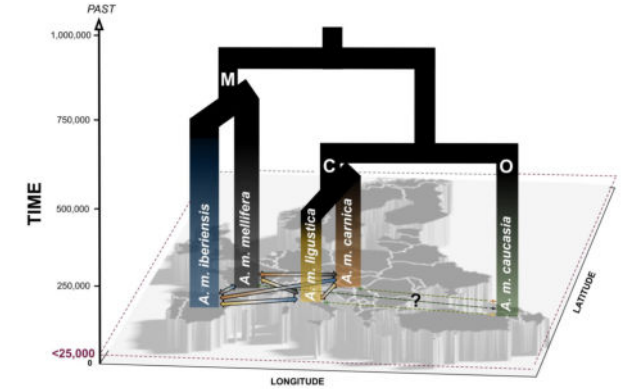
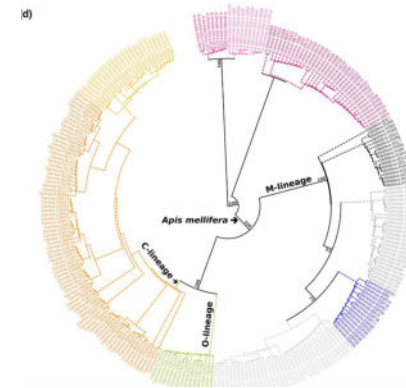
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*Corresponding author: E-mail: thibault.leroy@inrae.fr.

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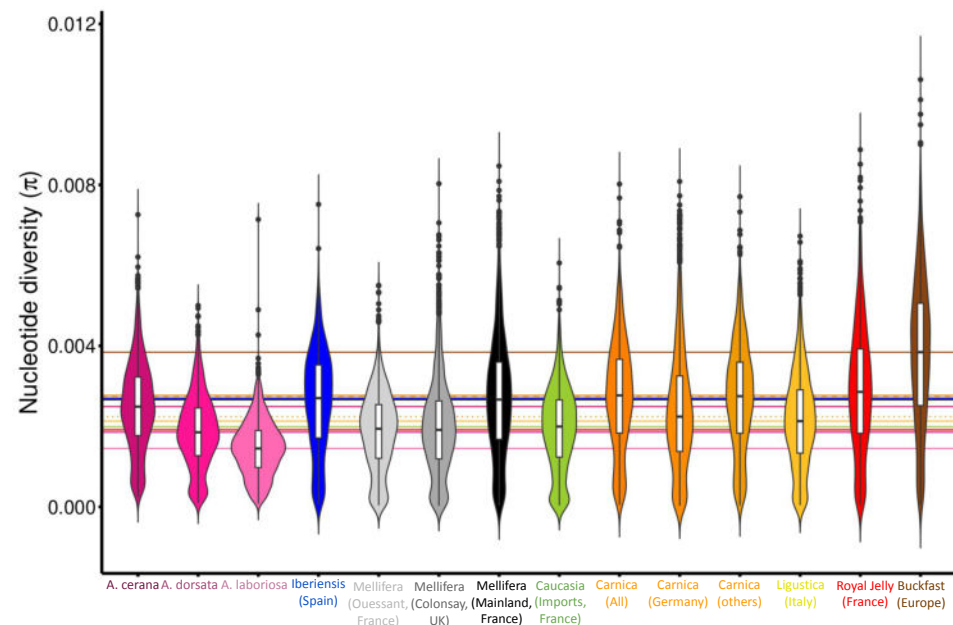
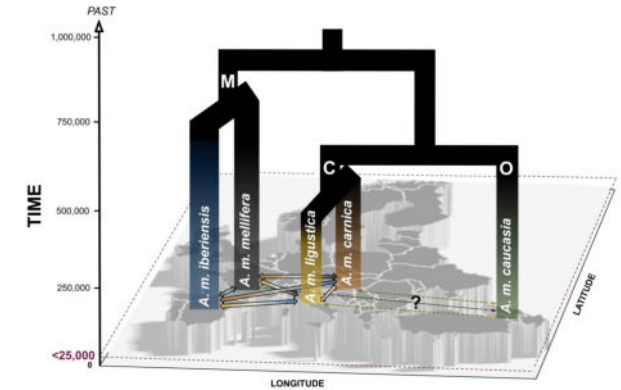
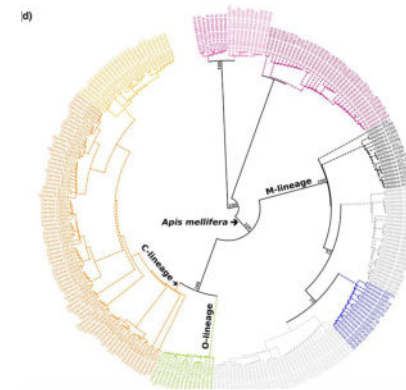
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Reusing these data:

- We precisely estimate the levels of diversity in the nine genetic groups
- Inferred recent changes due to beekeeping

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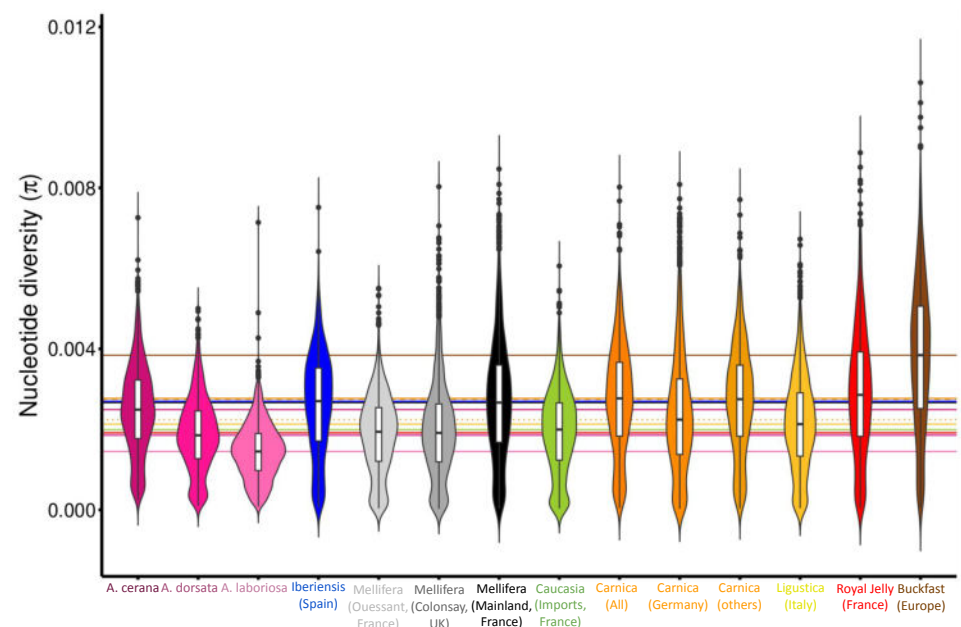
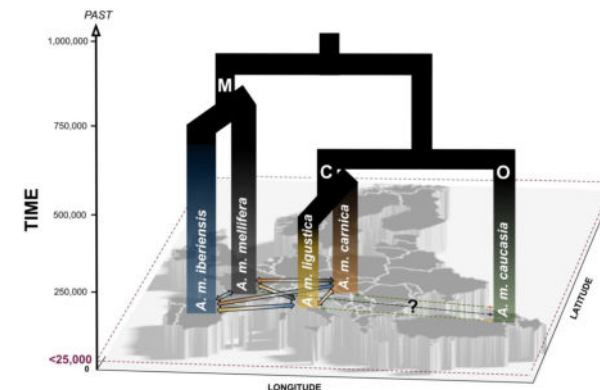
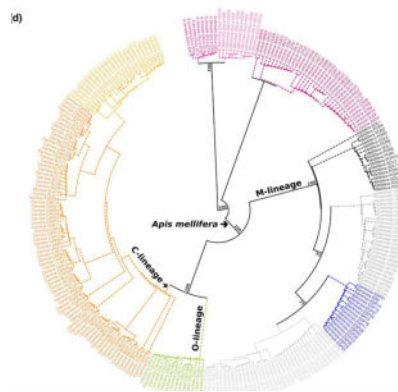
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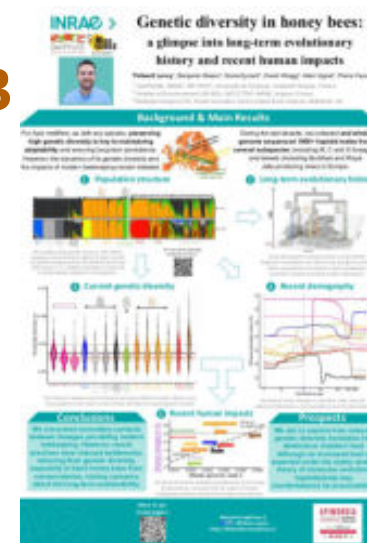


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To know more, visit my poster #453
(Friday and Saturday)

Genetic diversity in honey bees: a glimpse into long-term evolutionary history and recent human impacts

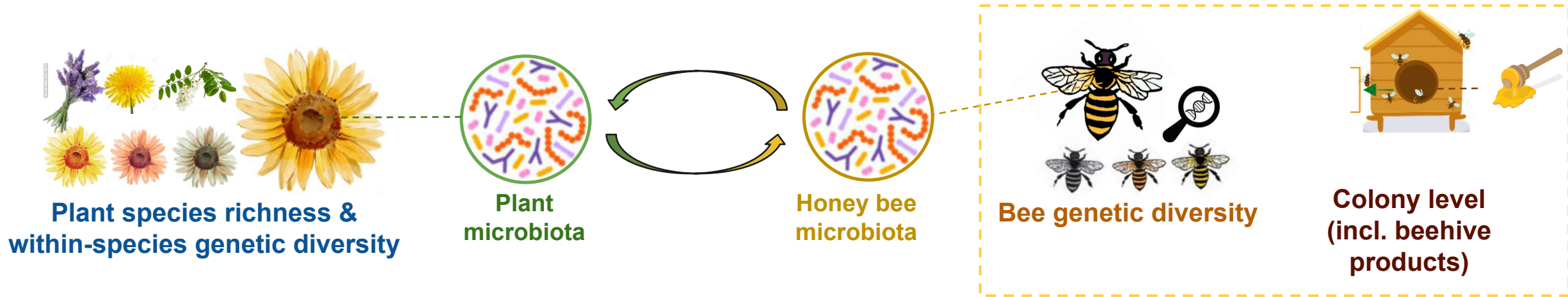


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Identifying genetic basis for resistance to varroa

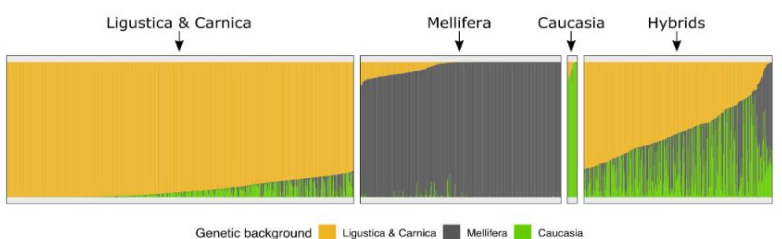


Sequence-Based Multi Ancestry Association Study Reveals the Polygenic Architecture of *Varroa destructor* Resistance in the Honeybee *Apis mellifera*

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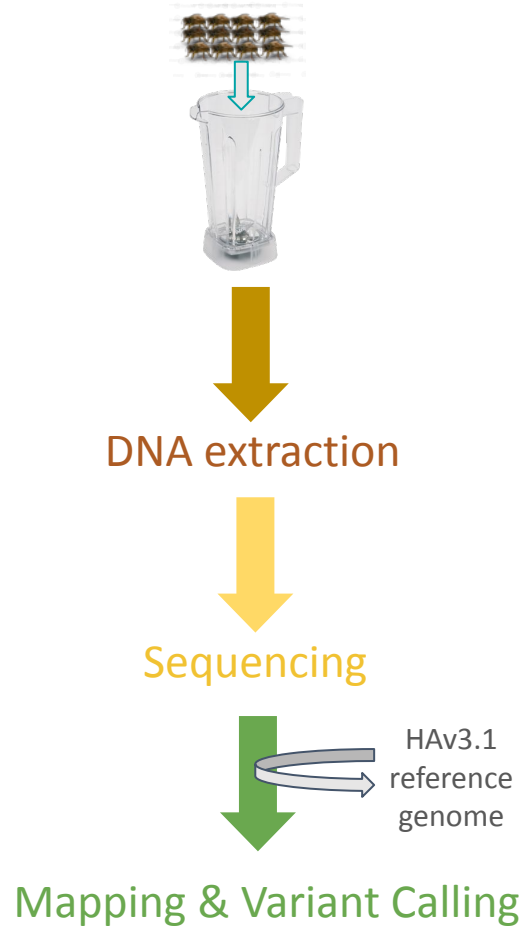
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1508 pools of workers (PoolSeq, colony level) mapped against HAv3.1



703 A. m. carnica or ligustica (yellow honey bees, C)
407 A. m. mellifera (black honey bees, M)
21 A. m. caucasia (Caucasian honey bees, O)
382 hybrids (Q-values < 0.8)

300-500 workers / colony



Identifying genetic basis for resistance to varroa

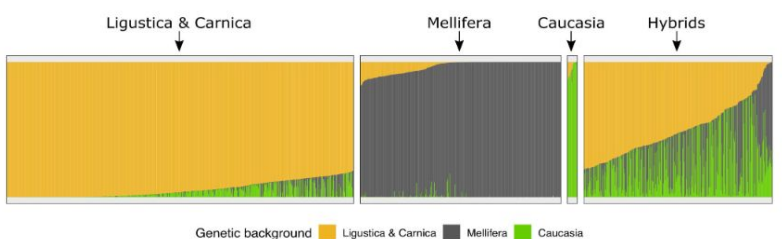


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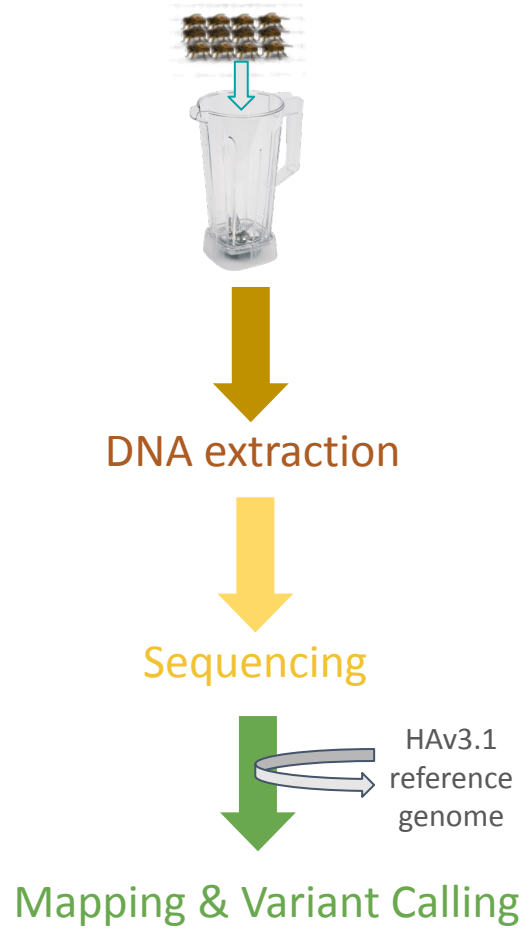
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Different phenotypes (varroa counts, recapping, MNR, ...)

Varroa resistance is substantially heritable (0.065-0.331)...

300-500 workers / colony



Identifying genetic basis for resistance to varroa

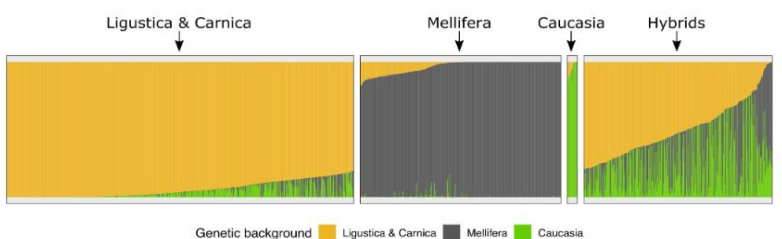


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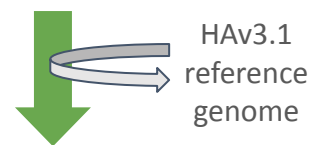
... but is highly polygenic (i.e. not simply driven by a few genes with large effects)

300-500 workers / colony

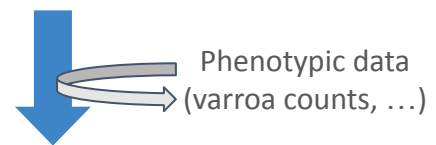


DNA extraction

Sequencing



Mapping & Variant Calling



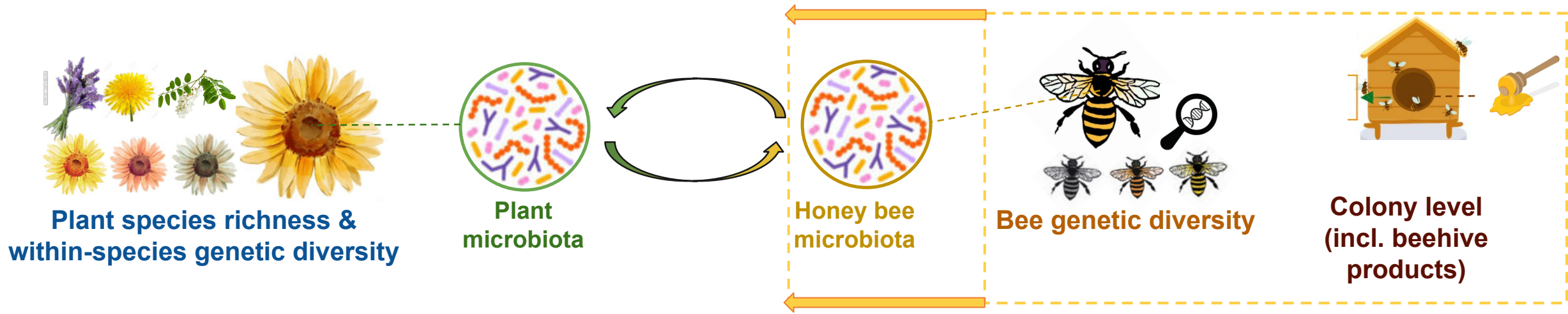
Associations with phenotypes (genetic basis for resistance) p. 17



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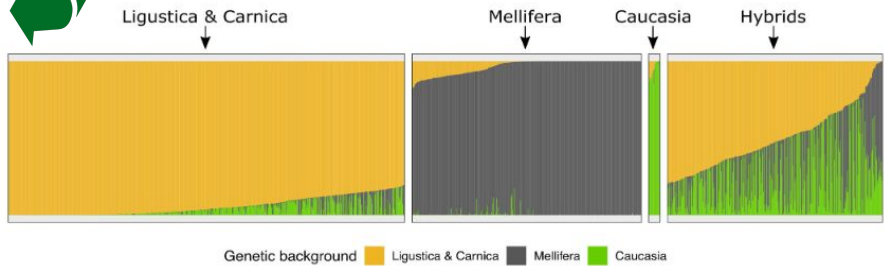
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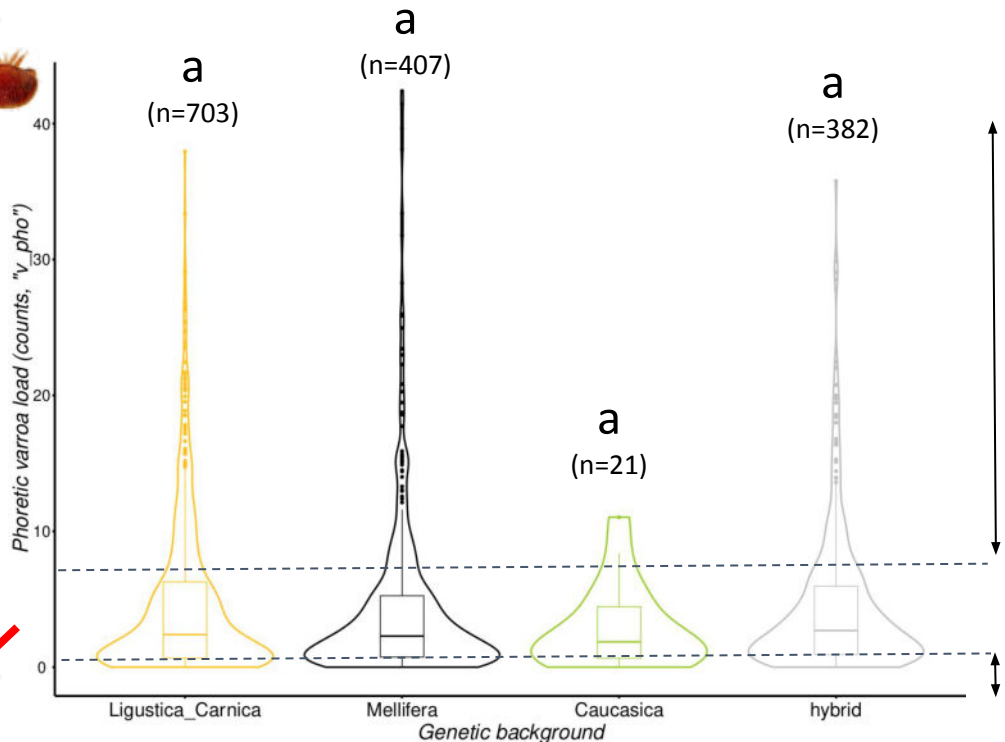
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Same dataset: describing sources of microbiota variation



No significant differences in the level of infestation depending on the genetic backgrounds (M, C or O)

Selecting colonies well assigned to the three genetic backgrounds (M, C or O) with very high or low infestation level:



Colonies with a high infestation

Colonies with a low infestation

	Ligustica/Carnica	Mellifera	Caucasia
High Inf.	n = 64	n = 28	n = 4
Low inf.	n = 34	n = 44	n = 3

Total: 177 colonies



More non-mapped reads for highly infested colonies ?



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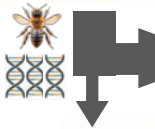
Total: 177

For each library:

Reads
(raw data)



3 references
Amel_HAv3.1+
Amelmel1.1+
Amel_DK2

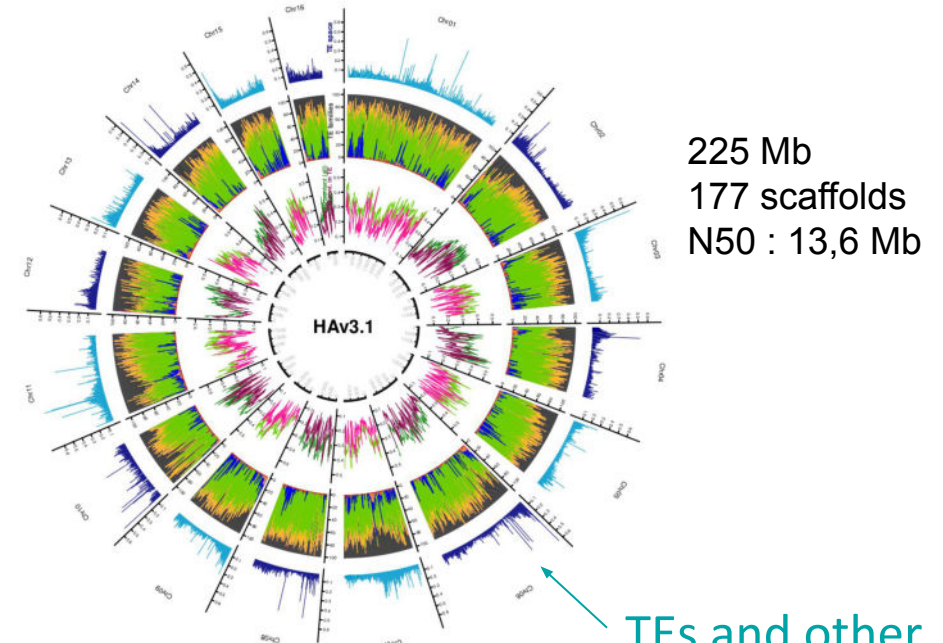


Mapped reads
(i.e. honey bee DNA)

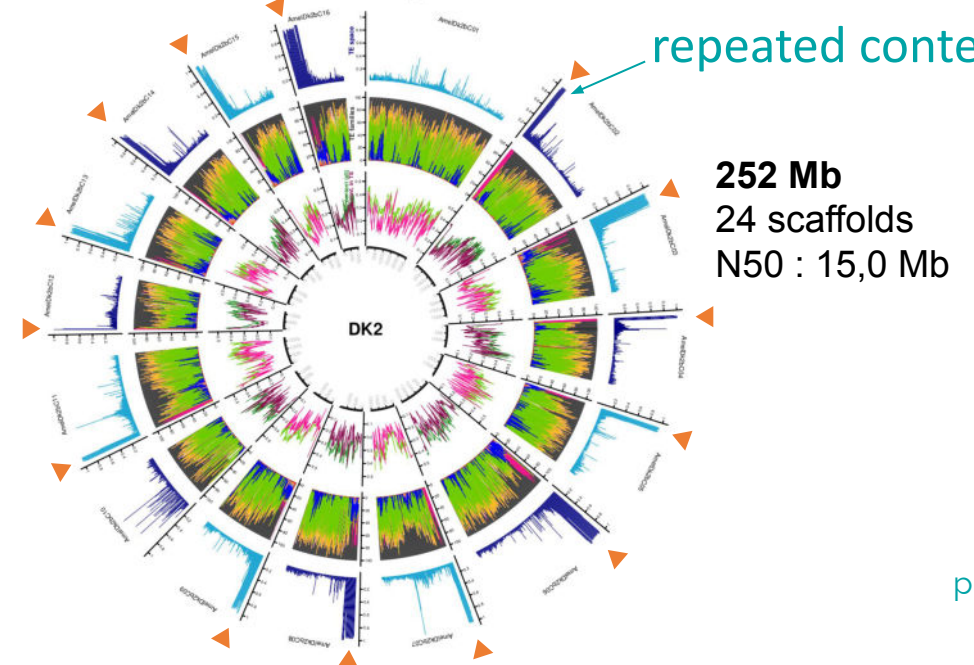


**% of unmapped reads
(i.e. non-honey bee DNA)**

Collab. LIPME:
 Baptiste Mayjonade
 Jérôme Gouzy



TEs and other
repeated content



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High Inf.	n = 64	n = 28	n = 4
Low inf.	n = 34	n = 44	n = 3

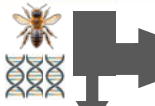
Total: 177

For each library:

Reads
(raw data)



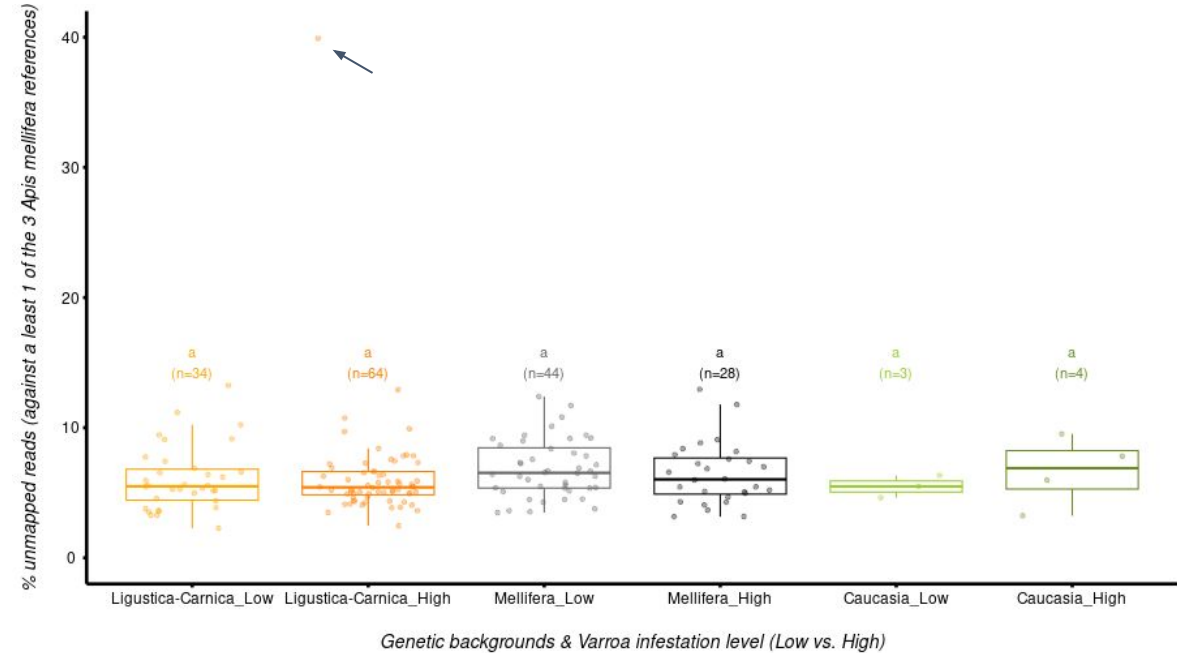
3 references
Amel_HAv3.1+
Amelmel1.1+
Amel_DK2



Mapped reads
(i.e. honey bee DNA)



**% of unmapped reads
(i.e. non-honey bee DNA)**



No significant difference in the proportion of non-bee reads associated with their genetic background and infestation level (high vs. low)

~ 6 % of the total number of reads
 (383 millions / 6.1 billion for the 177 colonies selected),
 i.e. > 3 billion reads expected for the 1508 colonies)

How does the microbiota vary among colonies?

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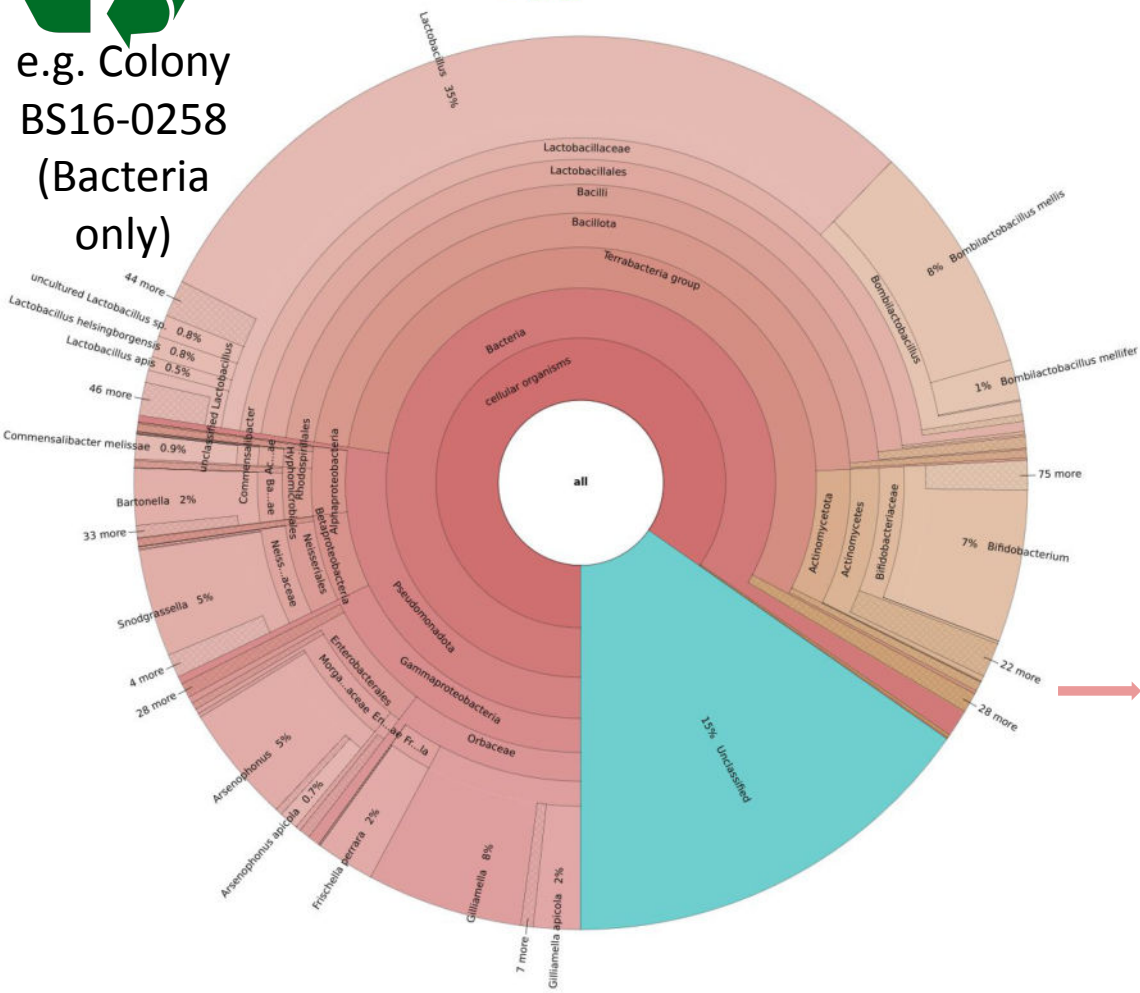
From genomes to holobionts: can the reanalysis of large-scale bee sequencing projects open new perspectives?

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Taxonomically assigning non-bee reads

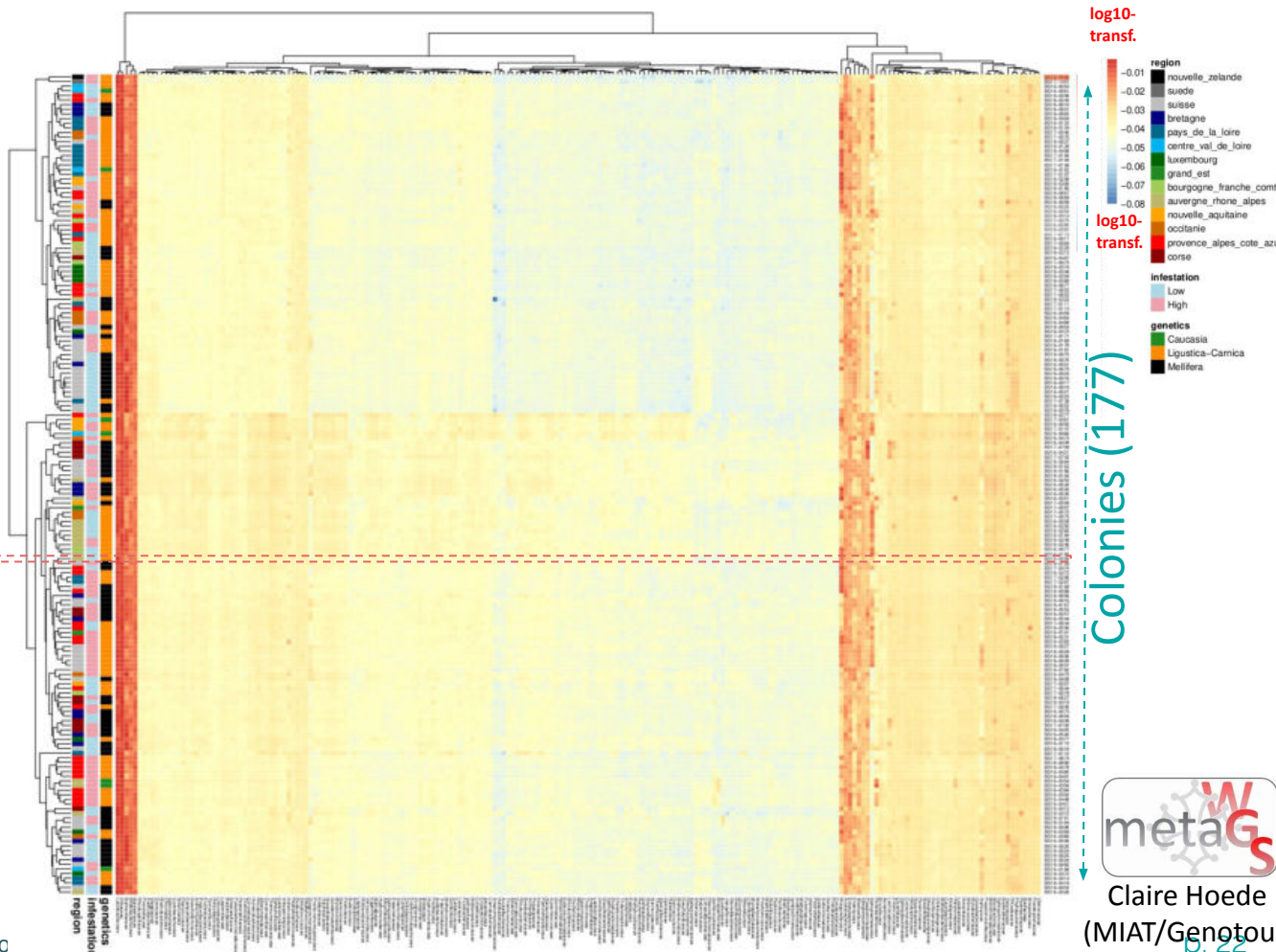


e.g. Colony
BS16-0258
(Bacteria
only)



211 bacterial families:
5 main families: ~ 2/3 of all sequencing reads

- Orbaceae (24.8%)
- Lactobacillaceae (24.0%)
- Neisseriaceae (6.3%)
- Bartonellaceae (6.1%)
- Bifidobacteriaceae (5.4%)



log10-transf.
region
nouvelle_zelande
suede
suisse
bretagne
pays_de_la_loire
centre_val_de_loire
luxembourg
grand_est
bourgogne_franche_comte
auvergne_rhone_alpes
nouvelle_aquitaine
ocotanie
provenance_alpes_cote_azur
corse
infestation
Low
High
genetics
Caucasia
Ligustica-Carnica
Mediterranea

Colonies (177)

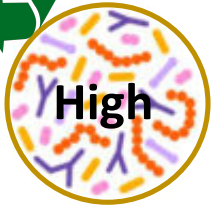
Bacterial families (211)



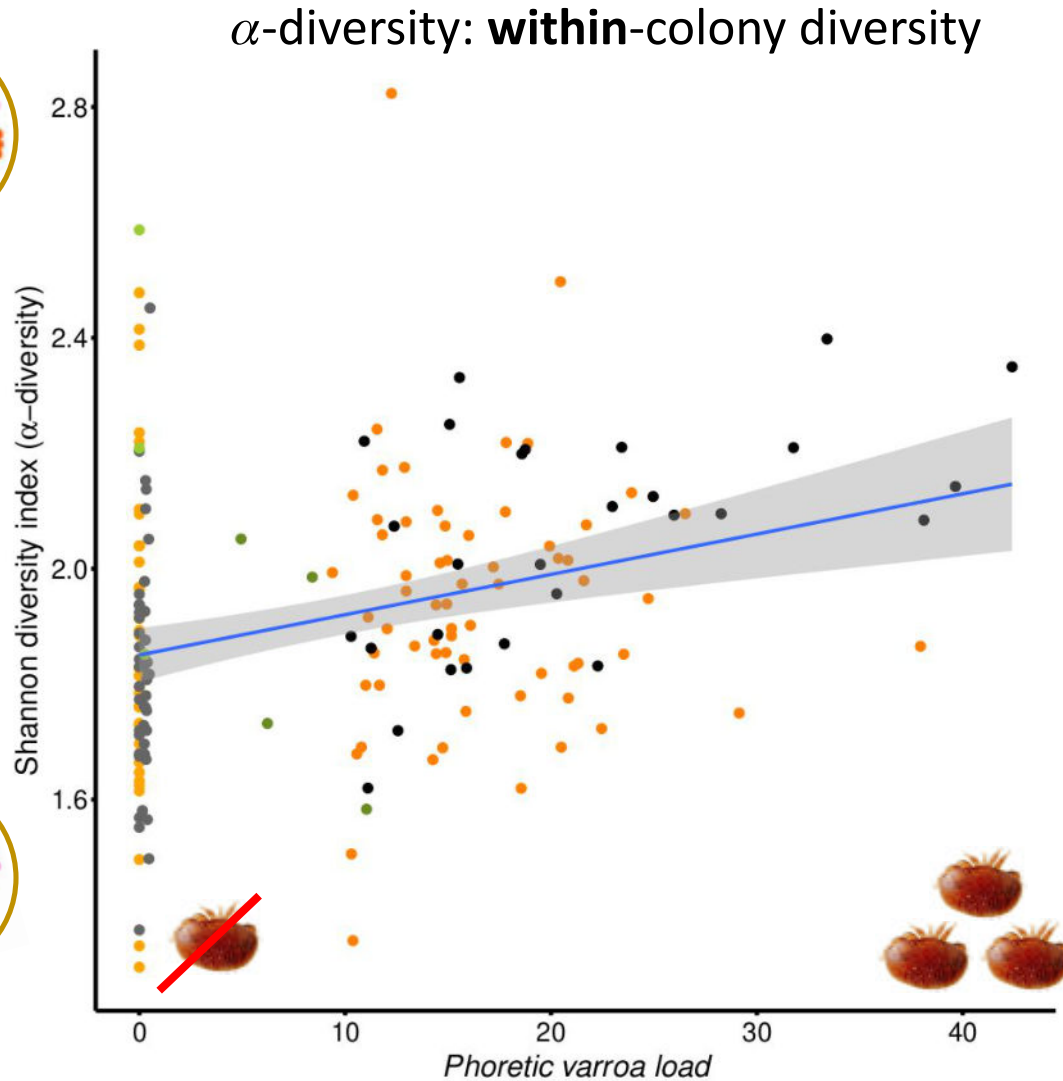
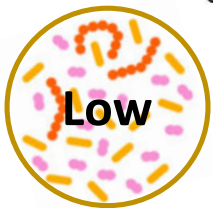
From genomes to holobionts: can the reanalysis of large-scale bee sequencing projects c
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metaG
Claire Hoede
(MIAT/Genotoul)

What shapes the diversity of the bee microbiota ?



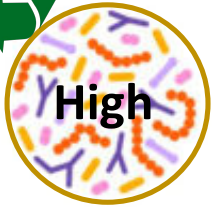
“Species richness” ↑



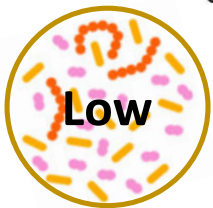
Microbial diversity tends to be greater in colonies with high Varroa loads

Shannon: $p\text{-value} < 1.10^{-4}$, $R^2=0.082$; Simpson: $p < 1.10^{-4}$, $R^2=0.093$

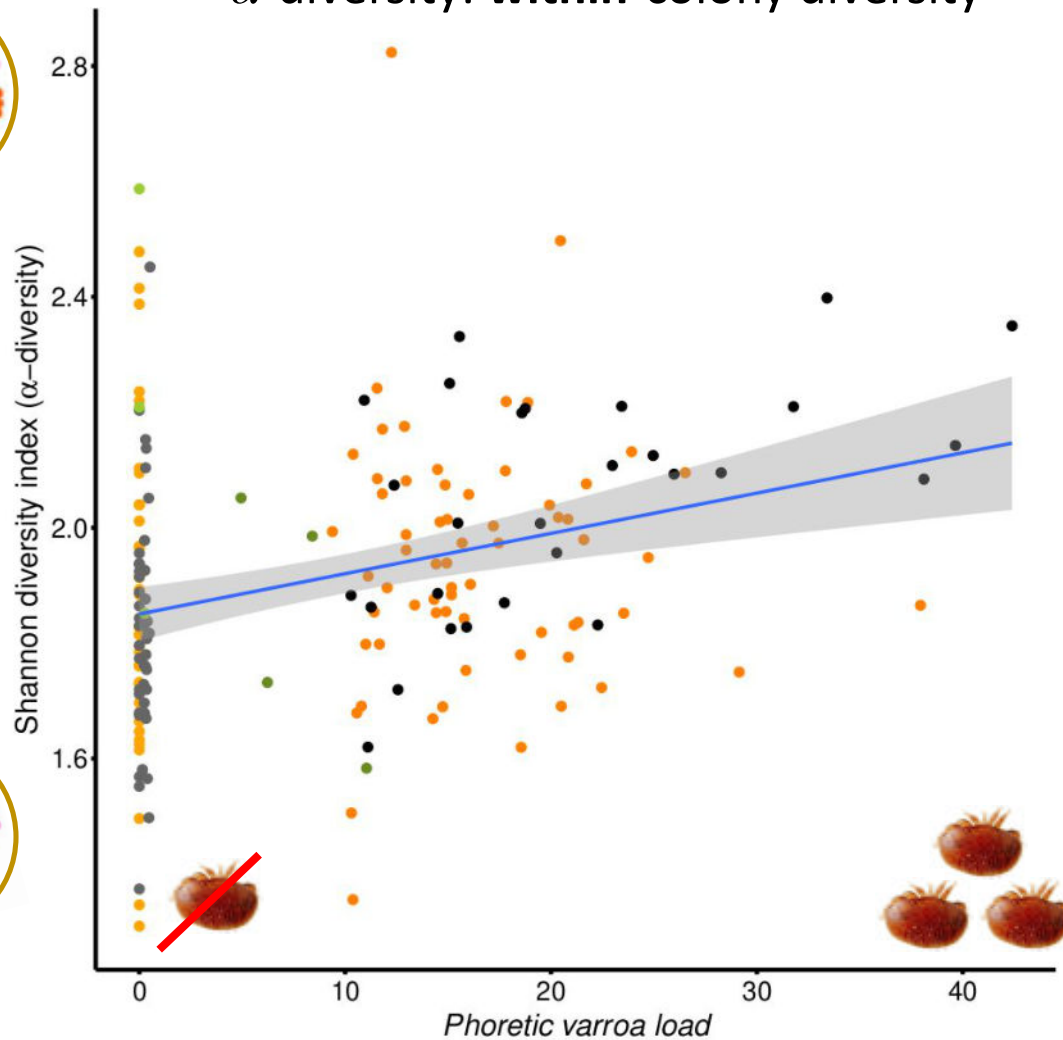
What shapes the diversity of the bee microbiota ?



“Species richness” ↑



α -diversity: **within-colony** diversity

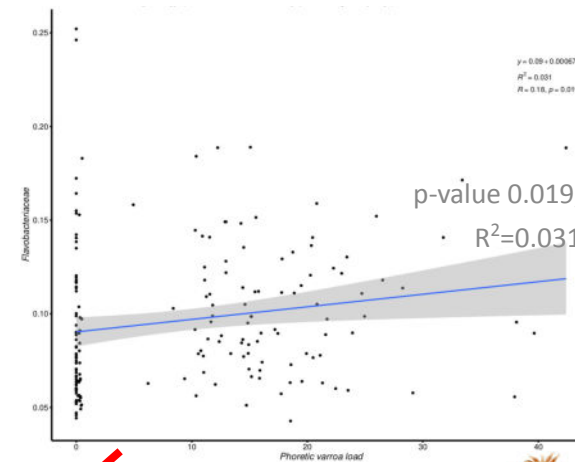


Microbial diversity tends to be greater in colonies with high Varroa loads

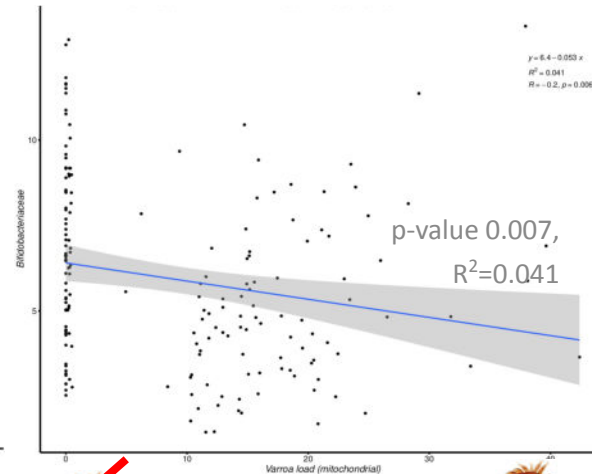
Shannon: $p\text{-value} < 1.10^{-4}$, $R^2=0.082$; Simpson: $p < 1.10^{-4}$, $R^2=0.093$

Identification of bacterial families whose abundances covary with Varroa loads

Positive relationships:
e.g. Flavobacteriaceae



Negative relationships:
e.g. Bifidobacteriaceae



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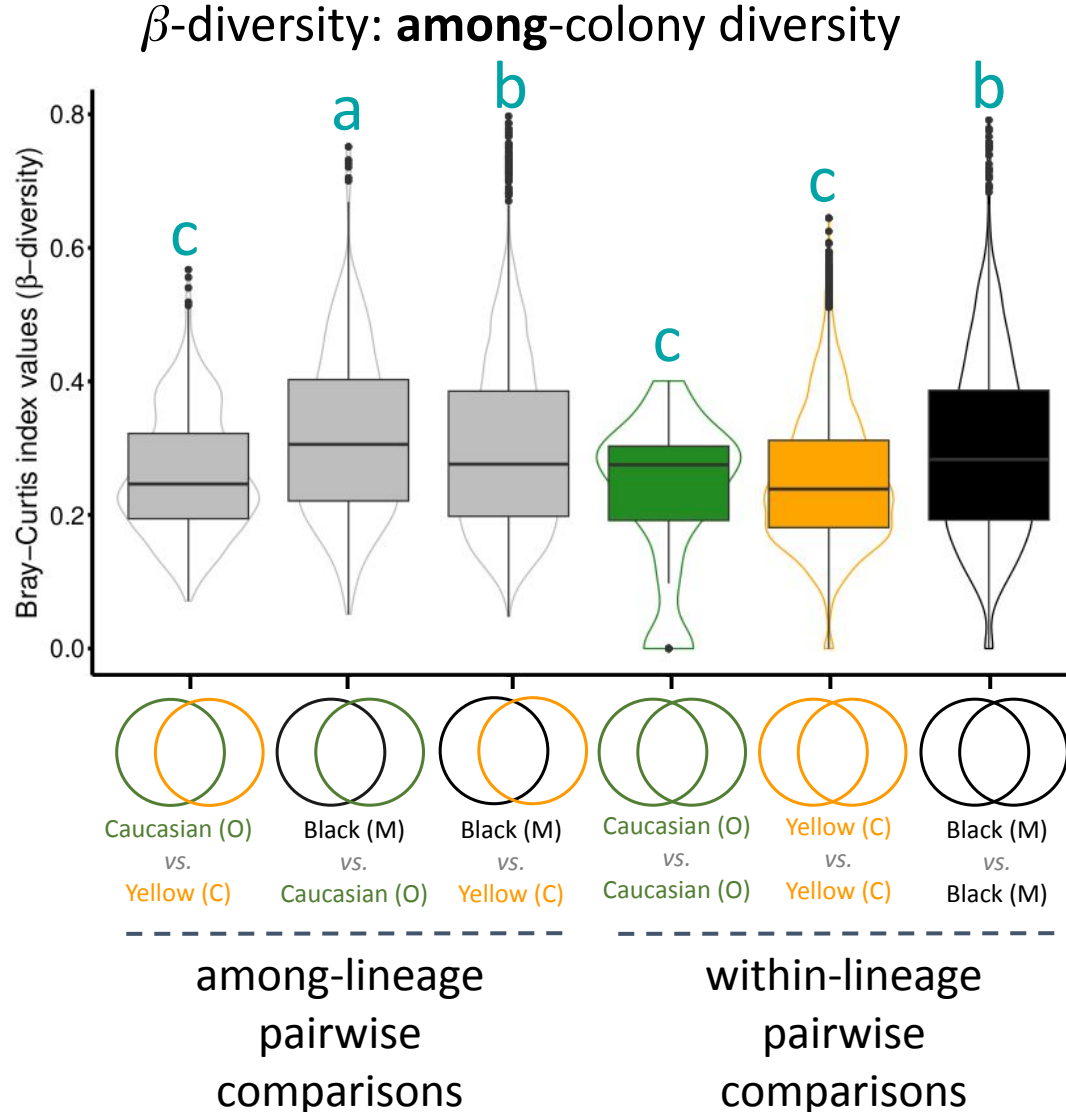
What shapes the diversity of the bee microbiota ?



High value
= different
communities

Differences in
communities
pairwise comparisons

Low value =
same
communities



Differences in microbial
composition:

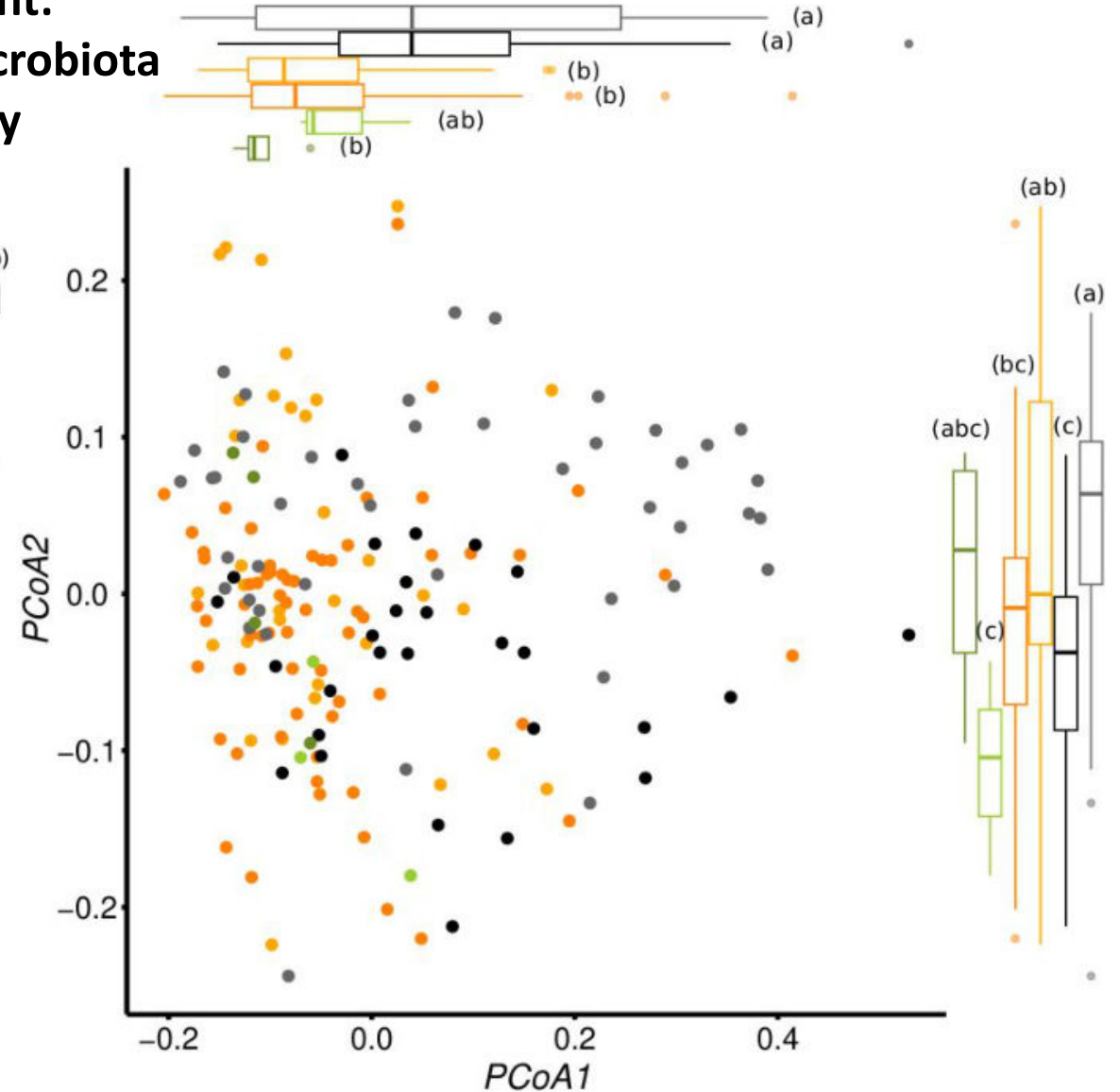
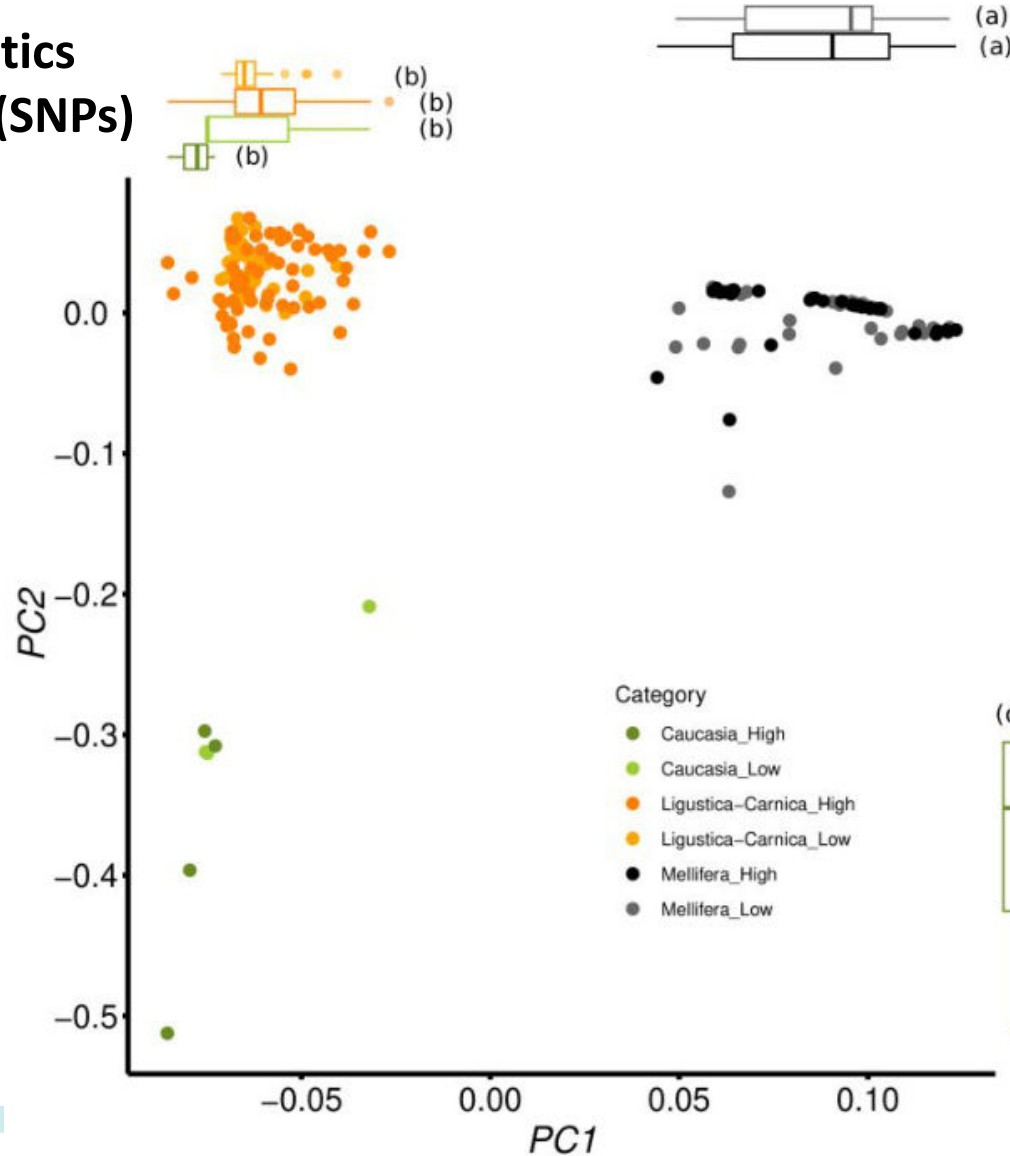
Yellow (C) and Caucasian (O) honey
bee colonies seem to share relatively
similar microbiota...

... while more differences in
microbial composition are observed
comparing black honey bees (M) and
the two other lineages (C & O)

What shapes the diversity of the bee microbiota ?

Left:
Genetics
only (SNPs)

Right:
Microbiota
only



Conclusions

Sharing and reusing sequencing data allows to:

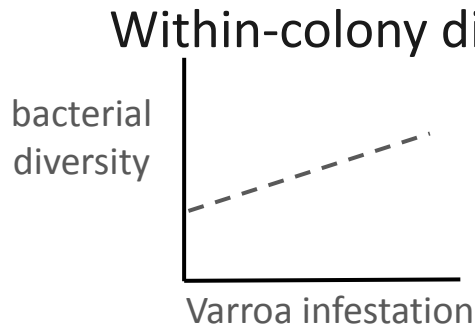


Generate new knowledge in areas the original authors may not have anticipated

‘Win-win strategy’: opportunities for collaboration with the original authors (incl. publications)

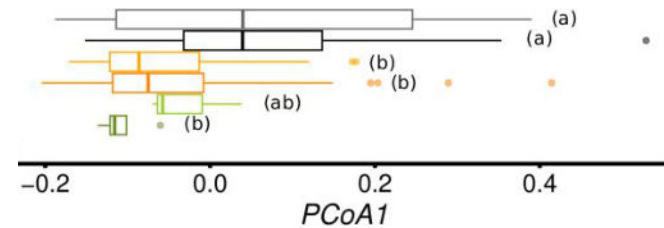
Ensures that valuable research continues... even in the face of grant proposal rejections!!

From genomes to holobionts: what shapes the diversity of the bee microbiota ?



Varroa bringing its own diversity?

Among-colony differences:



honey bee genetics shape variation in microbiota composition (along many other factors!)

What's next ?



Soon applying this approach to the full dataset (177 → 1,508 colonies), also incl. eukaryotic microbiota
Expanding the catalog of genomes of honey bee-associated organisms (*i.e.* MAG reconstruction)

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Collaborators:



Julie Birgel
Kamila Canale-Tabet
Sonia Eynard
Alain Vignal



Benjamin Basso
Fanny Mondet

Computing cluster:



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All authors of the previous studies:



Complex population structure and haplotype patterns in the Western European honey bee from sequencing a large panel of haploid drones

David Wragg¹ | Sonia E. Eynard¹ | Benjamin Basso² | Kamila Canale-Tabet¹ | Emmanuelle Labarthe¹ | Olivier Bouchez³ | Kaspar Bienefeld⁴ | Małgorzata Bieńkowska⁵ | Cecilia Costa⁶ | Aleš Gregorc⁷ | Per Kryger⁸ | Melanie Parejo⁹ | M. Alice Pinto¹⁰ | Jean-Pierre Bidanel¹¹ | Bertrand Servin¹ | Yves Le Conte¹² | Alain Vignal¹

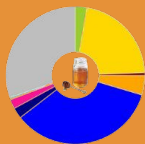
Sequence-Based Multi Ancestry Association Study Reveals the Polygenic Architecture of *Varroa destructor* Resistance in the Honeybee *Apis mellifera*

Sonia E. Eynard^{1,2,3} | Fanny Mondet^{3,4} | Benjamin Basso^{3,4,5} | Olivier Bouchez⁶ | Yves Le Conte^{3,4} | Benjamin Dainat⁷ | Axel Decourtie^{3,5} | Lucie Genestout² | Matthieu Guichard^{2,8} | François Guillaume⁹ | Emmanuelle Labarthe^{1,3} | Barbara Locke¹⁰ | Rachid Mahla² | Joachim de Miranda¹⁰ | Markus Neuditschko⁸ | Florence Phocas^{3,11} | Kamila Canale-Tabet^{1,3} | Alain Vignal^{1,3} | Bertrand Servin^{1,3}

¹GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France | ²LABOGENA DNA, Palaiseau, France | ³UMT PrADE, Avignon, France | ⁴INRAE, UR 406 Abeilles et Environnement, Avignon, France | ⁵ITSAP, Avignon, France | ⁶GeT-PlaGe, Genotoul INRAE, Castanet-Tolosan, France | ⁷Agroscope Swiss Bee Research Centre, Bern, Switzerland | ⁸Animal GenoPhenomics, Agroscope, Posieux, Switzerland | ⁹SYNETICS, Noyal-sur-Vilaine, France | ¹⁰Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden | ¹¹INRAE, AgroParisTech, GABI, Université Paris-Saclay, Jouy-en-Josas, France

See you later this week at our posters!

Julie Birgel
(#217)



From hive to table: the promise of shotgun metagenomic to trace the honeys' journey

Wednesday and Thursday



Thibault Leroy
(#453)

Genetic diversity in honey bees: a glimpse into long-term evolutionary history and recent human impacts

Friday and Saturday

