

Investigating pooled sequences of *Apis mellifera*

Matthieu Guichard¹, Alain Vignal², Sonia Eynard², Benjamin Dainat¹, Markus Neuditschko¹

¹Agroscope, Swiss Bee Research Centre, Switzerland

²INRAE Toulouse, France

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Apis mellifera mellifera

- Native subspecies of the Western honeybee *Apis mellifera* in the Northern part of Switzerland
- Small population, two management strategies:
 - Selection by beekeepers (breeding programme)
 - Protection in conservation areas
- Main threats: Hybridization, *Varroa destructor*





Research Questions:

- 1/ Can genotypes associated with a resistance against Varroa destructor (and other traits) be identified?
- 2/ Can we assess the population structure of the sampled colonies using pooled sequences?

In Progress



Material and Methods

- Colony sampling (N=210):

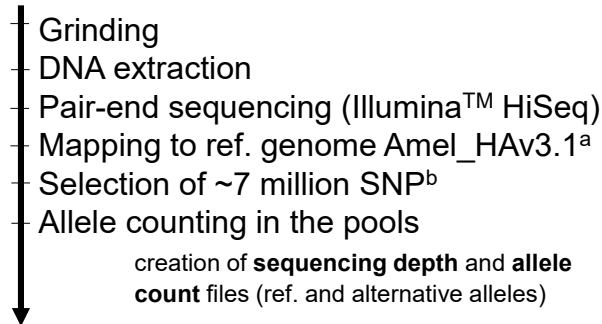
Population	Country of origin	Purpose	Code	N	Additional information
<i>A. m. mellifera</i>	Switzerland	Selection	MEL_SEL	112	+Pedigree information
<i>A. m. mellifera</i>	Switzerland	Conservation	MEL_CON	45	
<i>A. m. mellifera</i>	France, Savoie	Conservation	MEL_FRA	25	Evaluated in France
<i>A. m. carnica</i> -like	Switzerland	Selection	CAR_CHE	22	<i>V. destructor</i> Resistant?
<i>A. m. carnica</i> -like	Sweden	Selection	CAR_SWE	3	Surviving <i>V. destructor</i>
<i>A. m. carnica</i> -like	Norway	Selection	CAR_NOR	3	Surviving <i>V. destructor</i>

- Phenotypes: production, beekeeping abilities, resistance traits
- Genotypes



Material and Methods

- Colony: heterogenous assembly of workers (different patrilines) contributing to the phenotype.
- Sampling: 1 pool of 500 workers/colony



^a Wallberg, et al. A hybrid de novo genome assembly of the honeybee, *Apis mellifera*, with chromosome-length scaffolds. *BMC Genomics*. 2019;20(1):275
^b SeqApiPop project, France



Material and Methods

- Depth, reference allele count and alternative allele count files:
 - Chromosome, Position of SNP, Reference allele, alternative allele
 - + : - sequencing depth:
 - Or – frequency of reference allele
 - Or – frequency of alternative allele
- } For each pool (=colony)

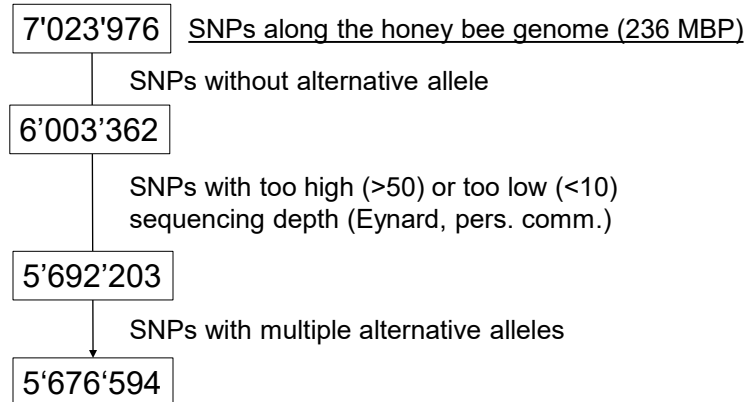
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NC_037638.1 7034 A G [23] [34] [34] [38] [16] [46] [36] [33] [15] [27] [41] [46] [15] [44] [37] [27] [30] [30] [28] [15] [13] [44] [25] [31] [29] [27] [9]
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[44] [32] [57] [49] [34] [46] [26] [37] [20] [23] [27] [29] [31] [32] [38] [36] [20] [78] [34] [18] [29] [36] [36] [43] [44] [25] [35] [22] [31] [28] [27]
[43] [32] [28] [40] [41] [30] [42] [40] [31] [25] [22] [33] [30] [33] [19] [32] [38] [33] [28] [63] [34] [40] [22] [36] [26] [17] [26] [22] [22] [38] [29]
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[22] [26] [21] [29] [21] [36] [26] [28] [21] [30] [33] [18] [28] [18] [11] [10] [13] [3] [10] [13] [14] [11] [18] [2] [16] [3] [3] [9] [30] [13] [22] [32]
[34]
  
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Material and Methods

- SNP pre-filtering: R



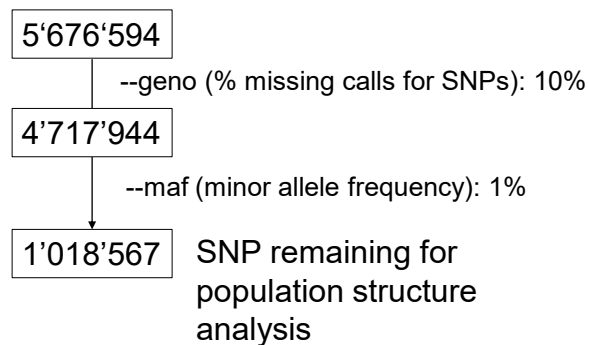
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matthieu.guichard@agroscope.admin.ch

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Material and Methods

- Preparing dosage file: Plink 2
- hard-call-threshold: Threshold to attribute an allele to frequencies
Default: 0.10 but only 100'000 SNP left: set to 0.40.



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Material and Methods

- 3-step population structure analysis
 - ROH identification: regions of the genome without variation

Hap1: CCAGGTATTC**AAAAAAAAAAAA**GGACT.....
 Hap2: TTGAACGCCT**AAAAAAAAAAAA**AAGTC.....
 - ROH can be used to calculate the inbreeding coefficient of individuals: $F_{ROH} = \sum \frac{L_{ROH}}{L_{AUTO}}$
 - Admixture calculation (ADMIXTURE): proportion of hybridization
- > NetView: Vizualisation of genetic relationship structure, hybridization, inbreeding

Results

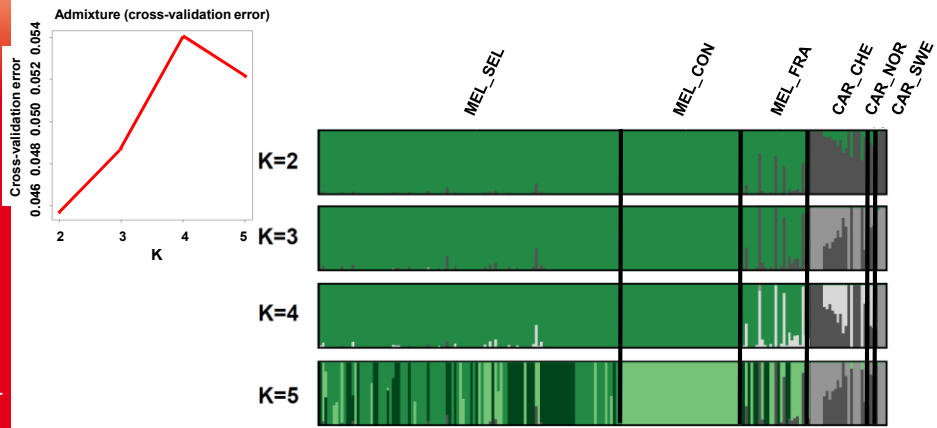
- F_{ROH} (%) as a calculation of inbreeding

Population	Country	Origin	Code	N	F_{ROH} mean (%)	F_{ROH} min (%)	F_{ROH} max (%)	F_{PED} Mean (%)	F_{PED} min (%)	F_{PED} max (%)
<i>A. m. mellifera</i>	Switzerland	Selection	MEL_SEL	112	1.9	0.5	3.1	1.7	0.0	5.2
<i>A. m. mellifera</i>	Switzerland	Conservation	MEL_CON	45	2.3	1.1	3.1	-	-	-
<i>A. m. mellifera</i>	France, Savoie	Conservation	MEL_FRA	25	1.0	0.1	2.2	-	-	-
<i>A. m. carnica</i> -like	Switzerland	Selection	CAR_CHE	22	1.5	0.1	4.0	-	-	-
<i>A. m. carnica</i> -like	Sweden	Selection	CAR_SWE	3	2.5	2.5	2.6	-	-	-
<i>A. m. carnica</i> -like	Norway	Selection	CAR_NOR	3	0.4	0.2	0.7	-	-	-



Results

- Admixture : Cross-validation error graph and barplot



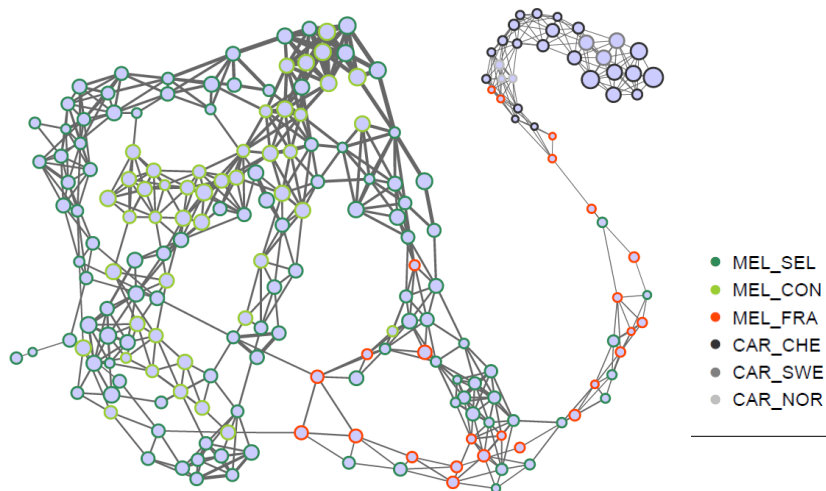
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Material and Methods

- Vizualisation of population network according to sample origin
 - Circle size: inbreeding, edge thickness: relatedness

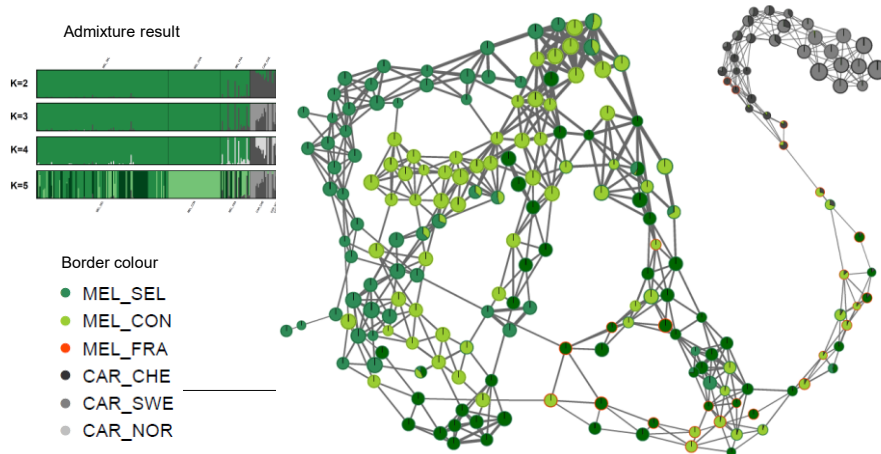


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Material and Methods

- Netview visualisation of population according to K=5 cluster result
 - Node: admixture result; border: sample origin



Conclusion

- Population structure at subspecies level can be ascertained
- Inbreeding levels are lower compared to other livestock (e.g. horses) : high recombination rate of *A. mellifera*
- Savoie population (France) is highly admixed
- Conservation+selection populations are closely related: exchanges between both?
- Substructure of the Swiss *A. m. mellifera* population might be explained by different drone pools at the mating stations?

