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Sequencing haploid drones from royal jelly and honey bee populations for detection of differentiation and selective sweeps

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In France, until the middle of last century the endemic populations of honey bees were represented by a single subspecies, the black bee, *Apis mellifera mellifera*. More recently, apiculturists have demonstrated an interest in using other subspecies and hybrids between *A. m. ligustica*, *A. m. caucasica*, and *A. m. mellifera*, which have been found to be more efficient producers of honey and royal jelly.

Most population genetic studies to date in *A. mellifera* have been performed using microsatellite markers, and more recently with medium density SNP microarrays. However, recent advances in high-throughput sequencing and declining costs now make population studies at the genome level feasible and two recent publications[1,2] have proven its utility for worldwide surveys of bee populations. The SeqApiPop project is designed to study the structure of French bee populations by whole genome sequencing (Illumina™) of one drone per colony for 1000 colonies. The sequencing of haploid males rather than diploid worker individuals enables high confidence variant calling, reducing the need for greater depth of coverage and will also allow for easy determination of haplotypes.

METHODS

As a pilot study, one drone was sequenced per colony from a total of 60 colonies representing two distinct populations; one used in the **production of honey** (**HN**), and one for the **production of royal jelly** (**RJ**). Paired-reads were mapped to the reference (Amel4.5) using *BWA-MEM*[3]. Variants were called per individual using *GATK*[4], *Pileup*[5] and *Platypus*[6], and combined using *BAYSIC*[7]. VCF files were merged, and then filtered on call rate using Plink[8]. Missing genotypes were imputed with *BEAGLE*[9].

Diploids were constructed by combining haploids, population structure assessed using *ADMIXTURE*[10] with respect to a reference panel of diploid workers[1] from each of the main evolutionary lineages (A, M, C, O). The same data was used to construct a phylogentic tree, and the branches coloured according to the optimal *K* following *ADMIXTURE*.

Signatures of selection were detected by estimating F_{ST} and pooled heterozygosity (H_P) using a sliding window approach. Windows overlapped by 80% and were analysed at varying sizes (1-5, 10, 20, 50 kb). HP values were standardised to zero mean and unit variance. Significance thresholds were marked at the 99th and 99.9th percentiles for F_{ST}, and $|Z(H_P)| \geq 3.719$ and 4 for the H_P analysis. All plots were generated using R.

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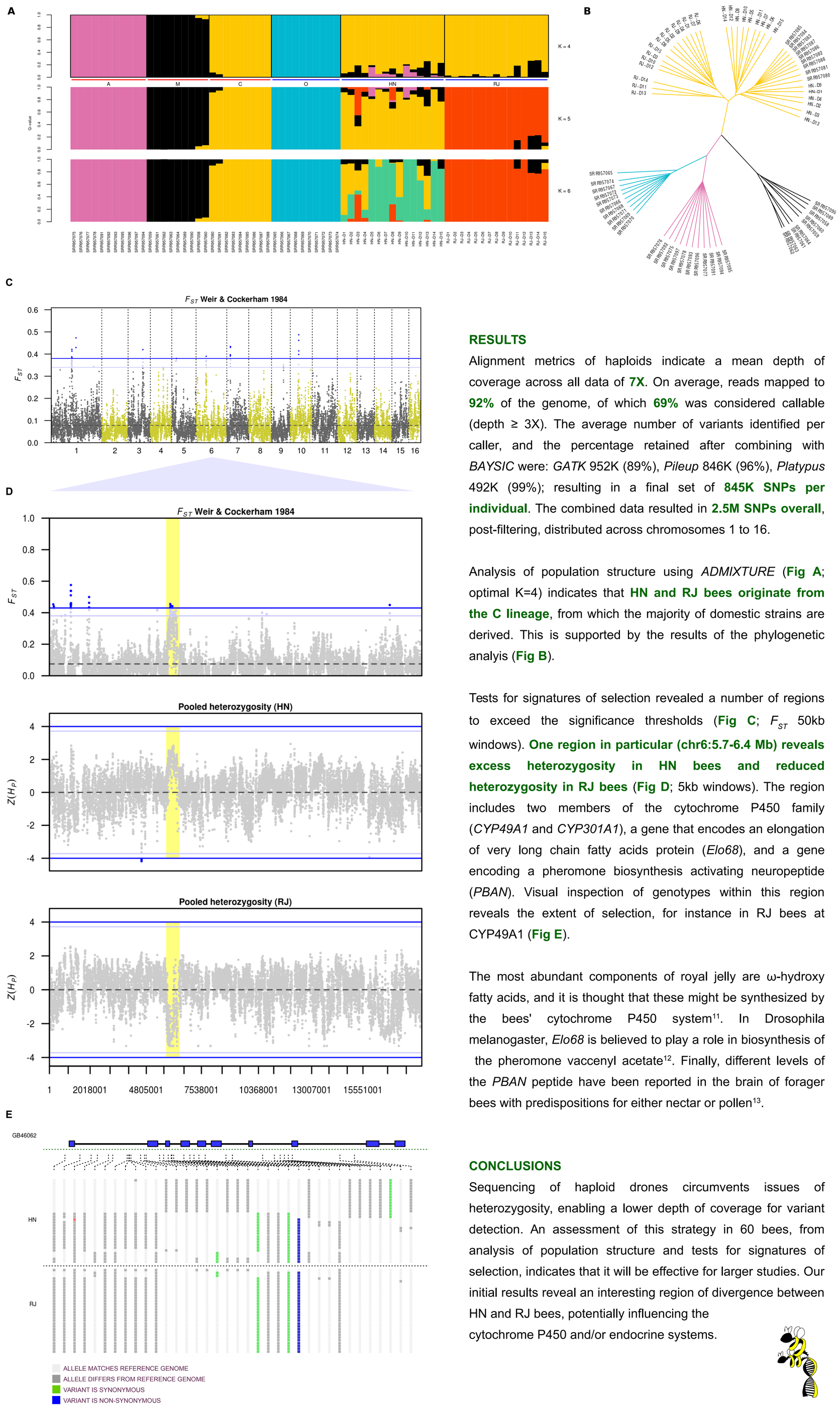

RESULTS

Alignment metrics of haploids indicate a mean depth of coverage across all data of **7X**. On average, reads mapped to **92%** of the genome, of which **69%** was considered callable (depth ≥ 3X). The average number of variants identified per caller, and the percentage retained after combining with *BAYSIC* were: *GATK* 952K (89%), *Pileup* 846K (96%), *Platypus* 492K (99%); resulting in a final set of **845K SNPs per individual**. The combined data resulted in **2.5M SNPs overall**, post-filtering, distributed across chromosomes 1 to 16.

Analysis of population structure using *ADMIXTURE* (**Fig A**; optimal K=4) indicates that **HN and RJ bees originate from the C lineage**, from which the majority of domestic strains are derived. This is supported by the results of the phylogenetic analyis (**Fig B**).

Tests for signatures of selection revealed a number of regions to exceed the significance thresholds (**Fig C**; F_{ST} 50kb windows). **One region in particular (chr6:5.7-6.4 Mb) reveals excess heterozygosity in HN bees and reduced heterozygosity in RJ bees** (**Fig D**; 5kb windows). The region includes two members of the cytochrome P450 family (*CYP49A1* and *CYP301A1*), a gene that encodes an elongation of very long chain fatty acids protein (*Elo68*), and a gene encoding a pheromone biosynthesis activating neuropeptide (*PBAN*). Visual inspection of genotypes within this region reveals the extent of selection, for instance in RJ bees at CYP49A1 (**Fig E**).

The most abundant components of royal jelly are ω-hydroxy fatty acids, and it is thought that these might be synthesized by the bees' cytochrome P450 system[11]. In Drosophila melanogaster, *Elo68* is believed to play a role in biosynthesis of the pheromone vaccenyl acetate[12]. Finally, different levels of the *PBAN* peptide have been reported in the brain of forager bees with predispositions for either nectar or pollen[13].

CONCLUSIONS

Sequencing of haploid drones circumvents issues of heterozygosity, enabling a lower depth of coverage for variant detection. An assessment of this strategy in 60 bees, from analysis of population structure and tests for signatures of selection, indicates that it will be effective for larger studies. Our initial results reveal an interesting region of divergence between HN and RJ bees, potentially influencing the cytochrome P450 and/or endocrine systems.

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