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P129 Contribution to the characterization of the genetic diversity of the honeybee *Apis mellifera*: case of the sex determination locus csd

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Sex determination in the honeybee is under the control of the csd gene composed of 9 exons, the seventh of which being hypervariable. A bee will be a female worker or a queen only if it is heterozygote at this specific locus and individuals having a single allele for the exon 7 of csd will develop as males, which is typically the case of the normal haploid males. Diploid individuals, homozygotes for the locus will also develop as male larvae, that will be killed by the workers. Within the framework of the INRA-ITSAP SeqApiPop project, we focused here on the diversity at csd by Sanger sequencing PCR products of the hypervariable exon. By amplifying DNA from haploid males, the direct sequencing of PCR products was possible without a sub-cloning step. We analyzed a total of 183 individuals from the 3 subspecies *A. m. mellifera* (n=86), *A. m. ligustica* (n=29) and *A. m. carnica* (n=37), in addition to bees from French beekeepers (n=51), which are a mixed type.
A total of 77 DNA and 73 amino acid haplotypes were observed, 25 of which are new. Polymorphisms include a variation of length of a short tandem repeat-like sequence, causing the length of csd exon 7 to varie between 73 and 101 amino acids, small indels and SNPs. Most mutations observed at the DNA level translate into amino acid changes. Across all the studied population, the mean number of individuals in which one specific amino acid haplotype is detected is 2.5 ± 2.1. When excluding the 51 mixed type bees, amongst 33 amino acid haplotypes observed in at least two individuals, 8 (24 %) could be detected in both M-type and C-type samples. Such a high level of allele sharing between otherwise genetically distant sub-species is surprising. This suggests either pressure for high allele diversity acting against loss by drift which could be due to the sex determination mechanism, or the convergent appearance of alleles in different populations due to a high mutation and fixation rate.

P130 Applying molecular tools for conservation of wild and managed black bees in Ireland

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Apis mellifera mellifera (Amm) is threatened over much of its natural range. However, in Ireland microsatellite and mitochondrial data have shown that a significant population of this subspecies exists in pure form and is spread over a large geographical region on the Island. Black bees have been managed and protected by beekeepers on the island, some of whom formed the Native Irish Honeybee Society (NIHBS)in 2012 and a breeding programme was initiated for Amm in 2014/2015.
The application of a SNP panel that detects hybridization between M and C lineages clearly supports other data showing that the majority of beekeepers included in the breeding programme indeed have bees with very low to no introgression from the C lineage.
Furthermore, SNP data has also been applied to the first feral bee colonies located in Ireland subsequent to the introduction of Varroa. Here we will present on the use of molecular data as an aid to manage and conserve honeybees in Ireland, and to elucidate patterns in colour variation and honeybee subspecies purity in wild and managed bees with a view towards improving conservation approaches in the face of a potential hybridization threat.