Introgression of Lineage C Honey Bees into Black Honey Bees: a Genome-Wide estimation using SNPs

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Introduction

The black honey bee, *Apis mellifera mellifera L.*, is probably the subspecies more threatened by introgression from foreign subspecies, specially the C-lineage *A. m. carnica* and *A. m. ligustica*. In fact, in some areas of its distributional range, intensive beekeeping with foreign subspecies has driven *A. m. mellifera* populations to be nearly replaced (De la Rúa et al., 2009). While massive and repeated introductions may lead to loss of native genetic patrimony, a low level of gene flow can also be detrimental because it may compromise honey bee survival and local adaptation by disrupting co-evolved gene complexes. Assessing levels of introgression is an important activity in breeding programs, especially when conservation of native subspecies is a major concern. Previous surveys of *A. m. mellifera* populations estimated the introgression of C-lineage honey bees by using mtDNA and microsatellites markers (Jensen et al., 2005). Herein, we used both mtDNA (sequence data of the tRNA^{leu}-cox2 intergenic region) and 924 SNPs to ascertain introgression levels of *A. m. carnica* and *A. m. ligustica* in *A. m. mellifera* populations sampled in France, Switzerland, Denmark, the Netherlands, Norway, England, and Scotland.

Methods

Sampling

A total of 77 *A. m. mellifera* individuals from France (18), Denmark (10), the Netherlands (15), Switzerland (6), Scotland (10), Norway (10) and England (8) were collected. Samples of *A. m. carnica* (19) and *A. m. ligustica* (17) were included as reference populations of C-lineage (Figure 1).

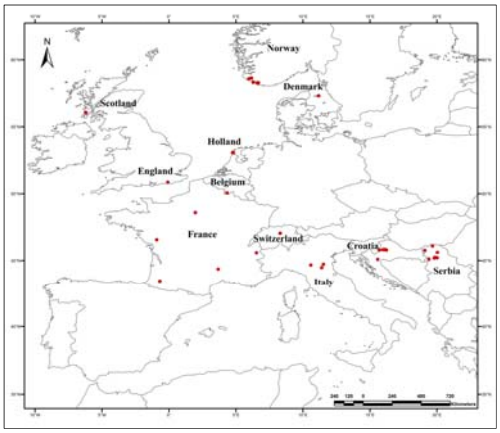

Figure 1

Genotyping

Individuals were genotyped for a panel of 1536 SNP's with Illumina Bead Station 500G using a custom Oligo Pool Assay. Individuals were scored using Illumina's Genome Studio software (Figure 2).

The monomorphic loci (cutoff 2%) and non-calls were removed from the dataset.

The SNP position in the bee genome was identified by using NCBI database (http://www.ncbi.nlm.nih.gov/genbank/).

The tRNA^{leu}-COX2 intergenic mtDNA region was amplified using the primers E2 and H2 and the PCR conditions recommended by Garnery *et al.* (1993). In order to have a high resolution this region was sequenced.

The sequences were manually checked and aligned with published data.

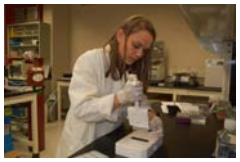

Figure 2

Analysis

To infer the introgression level of C-lineage in *A. m. mellifera* populations, we used the Bayesian clustering method implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000).

In the manual of STRUCTURE it is recommended that linked loci are removed from the dataset to prevent a bias. Considering that, in average, the honey bee recombines at every 50 000 bp, SNP loci that were below this physical distance were excluded.

Following exclusion of non-calls, monomorphic, and linked SNPs, the final number of loci was 924.

The STRUCTURE was run using the following settings: linkage model, correlated allele frequency, 125 000 burnin steps, 375 000 MCMC iterations, number of clusters K=1 to 5.

STRUCTURE provides probabilistic estimations of admixture coefficient (Q). Herein, the individuals exhibiting a value of Q ≥ 0.95 were considered as being pure *A. m. mellifera*.

Results and Discussion

For K=2 the STRUCTURE analysis separates *A. m. mellifera* populations from C-lineage (*A. m. ligustica* and *A. m. carnica*) populations (Figure 3). However, some *A. m. mellifera* individuals share an important component of their genome with C-lineage. For K=3 there is a substantial component (green) carried by four individuals from France. This component is also exhibited by individuals of *A. m. carnica* but in a small proportion (6.5%). For K=4 there is an additional component that appears mostly in three individuals from England.

At the population level, introgression values provided by mtDNA and nuclear DNA are roughly concordant (Figure 4). The populations with the highest values of nuclear introgression are those from France and England (Figures 3, 4). Individuals of C-lineage maternal ancestry were detected only in these two populations (France=28% ; England=50%; Figure 4).

At the individual level, nuclear and mtDNA data are not always concordant. For example, the most introgressed (58%) individual of the Netherlands population carries an M haplotype (Figure 3). On the other hand, for the population of England the introgression values are similar among individuals of C and M maternal ancestry. These results emphasize the importance of screening both nuclear and mtDNA for certification of "pure" *A. m. mellifera* individuals.

The populations exhibiting the lowest introgression levels and with the greatest proportion of "pure" individuals (Q ≥ 0.95) are those from Scotland and Norway (Figures 3, 4). These levels of introgression are roughly concordant with those reported by Jensen et al. (2005) using microsatellites.

Populations without "pure" individuals are those from England and Switzerland, although the average introgression level of the Switzerland population is below 12% (Figure 4). These results contrast with those reported by Soland-Reckeweg et al. (2009) who identified (with microsatellites) a greater proportion of pure individuals in Switzerland. However, these authors scored a greater number of individuals (n=100).

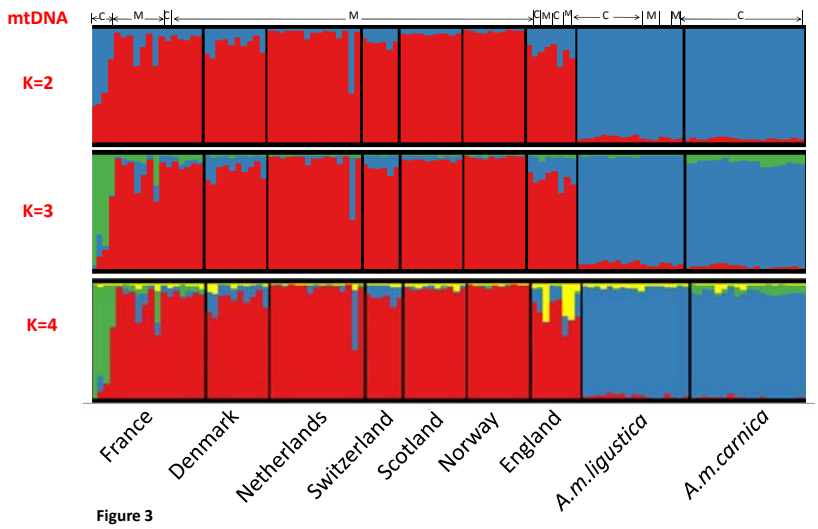

Figure 3

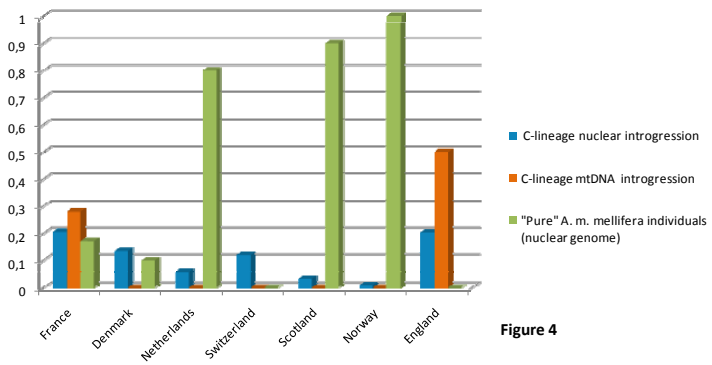

Figure 4

Literature

1. De la Rúa P., Jaffé R., Dall'Olio R., Muñoz I., Serrano J. 2009. Biodiversity, conservation and current threats to European honeybees. Apidologie 40: 263-284.
2. Garnery L., Solignac M., Celebrano G., Cornuet J. M. 1993. A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure of *Apis mellifera* L. Experientia 49: 1016-1021
3. Jensen, A.B., Palmer., K.A., Boomsma, J.J., Pedersen, B.V. 2005. Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honeybee, *Apis mellifera mellifera*, in northwest Europe. Molecular Ecology 14: 93-106.
4. Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959.
5. Soland-Reckeweg, G., Heckel, G., Neumann, P., Fluri, P., Excoffier, L. 2009. Gene flow in admixed populations and implications for the conservation of the Western honeybee, *Apis mellifera*. Journal of Insect Conservation 13: 317-328.

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