

SPATIAL PATTERNS OF GENETIC VARIATION IN THE IBERIAN HONEY BEE HYBRID ZONE - A COMPARISON BETWEEN MITOCHONDRIAL DNA AND NUCLEAR DNA -

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INTRODUCTION

>The Iberian honey bee (*Apis mellifera iberiensis*) has been intensely surveyed for genetic variation with nuclear (morphology, allozymes, and microsatellites) and mitochondrial DNA (mtDNA) markers. Morphology and allozymes revealed the existence of a smooth gradient extending from northern Africa to northern Europe, with Iberian honey bees showing intermediate phenotypes. This clinal pattern of variation raised the hypothesis of an African origin and a process of differentiation by local adaptation (primary contact hypothesis) for the Iberian honey bee (Ruttner 1988, Smith and Glenn 1995).

>Patterns of mtDNA variation and phylogenetic data led to suggestion of an alternative evolutionary hypothesis. According to Garnery et al. (1992) the coexistence of highly divergent African and European mtDNA types (mitotypes) forming a sharp southwestern-northeastern cline in Iberia is more compatible with a secondary contact hypothesis. Adding to the complexity, microsatellites were unable to capture any structure and no signs of African genes in Iberian honey bee populations (Franck et al. 1998). Based on these findings, these authors speculate a recent human-mediated secondary contact during Moorish invasions of the Iberian Peninsula and that natural selection has shaped and maintained the clinal mtDNA and allozymic patterns of variation (Franck et al. 1998).

>While complex patterns are typical of glacial refuges, such as the Iberian Peninsula, the use of more powerful molecular and analytical tools and the fine-scale sampling promises to help better dissecting the complexities of Iberian honey bees. In this study, we used high throughput molecular technology to score single nucleotide polymorphisms (SNPs) across the genome of numerous Iberian honey bee individuals. This is the first fine-scale genomic and geographical sampling of the Iberian honey bee and therefore the best representation, so far, of the patterns of genetic variation which will ultimately help disentangling underlying evolutionary processes.

SAMPLING

>A total of 711 Iberian honey bee individuals (each representing a single colony) was collected in 2010 across three North-South transects in the Iberian Peninsula. A reference sample (N=31) of the northern African subspecies *A. m. intermissa* was also analyzed (Figure 1).

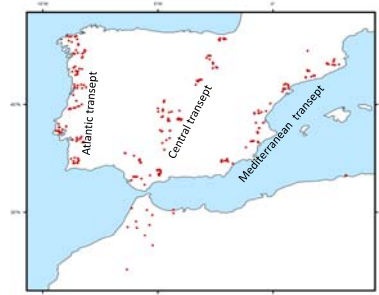


Figure 1 – Sampling transects across the Iberian Peninsula. Each dot represents an apiary where 3 individuals (corresponding to 3 colonies) were sampled. Location of the reference *A. m. intermissa* in North Africa.

MOLECULAR ANALYSIS

>MtDNA: the tRNA^{leu}-cox2 intergenic region was amplified, using the primers and PCR conditions recommended by Garnery et al. (1993), and then sequenced. The sequence data was used to identify the mitotypes as being of African (lineage A and further identification into sub-lineages AI, AII, and AIII) or western European (lineage M) ancestry.

>Nuclear DNA: over 1536 SNPs were genotyped using Illumina's Technology (GoldenGate Assay and Genome Studio software). A final dataset of 383 SNPs was obtained after excluding monomorphic loci (2% cutoff) and non-calls.

DATA ANALYSIS

>Population structure was inferred using the Bayesian clustering softwares *Structure* (Pritchard et al. 2000) and *Tess* (François and Durand 2010). *Tess* uses tessellations and Markov models for the spatial structure analysis. *Structure* model: admixture, correlated allele frequencies. *Tess* model: BYM with linear trend degree. Run parameters: 250000 burnin steps, 750000 MCMC iterations, minimum 3 reps per K.

>Loci under selection were detected using F_{ST} -based approaches.

> F_{ST} values, obtained with *Arlequin* (Excoffier and Schneider 2005), were summarized in 2 major dimensions using multidimensional scaling (MDS) analysis, implemented by *Statistica*. The values obtained in the first MDS dimension were interpolated through ordinary kriging, based on an omnidirectional spheric variogram model, to generate a synthetic map.

RESULTS AND DISCUSSION

>The spatial representation of mtDNA reveals a sharp transition of European-derived mitotypes in the northeastern part of Iberia to African-derived mitotypes in the southwestern part of Iberia (Figure 2A,B).

>The maternal composition of the southwestern African component is complex and dramatically different from that of northern African honey bees (Figure 2B). This pattern (and further data not shown here) does not support the hypothesis proposed by Franck et al. (1998) of a recent human-mediated colonization of the Iberian Peninsula.

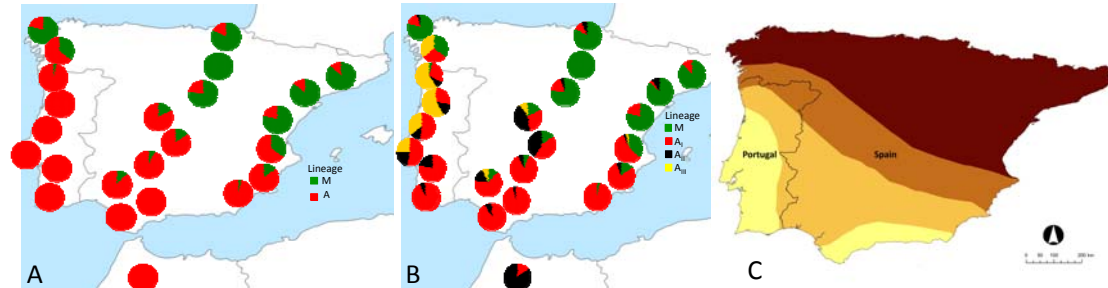
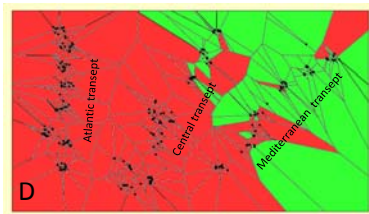
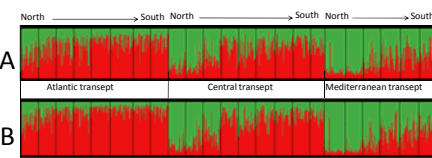


Figure 2 – Mitochondrial DNA patterns of variation. (A) Proportions of honey bees of western European (M) and African (A) lineage ancestry. (B) Proportions of honey bees of western European lineage (M) and African sub-lineage (AI, AII, and AIII) ancestry. (C) Synthetic map generated by interpolating the first dimension of MDS using F_{ST} values obtained with lineage frequency data displayed in (A).



>The spatial representation of mitotypes and SNPs reveals a concordant northeastern-southwestern clinal pattern of variation (Figures 2, 3). The SNP pattern is also roughly concordant with morphology (Ruttner 1988) and allozymes (Smith and Glenn 1995), which contrasts with the absence of structure revealed by microsatellite markers (Franck et al. 1998).

>The structure analysis using the total SNP dataset (neutral SNPs plus SNPs under selection, Figure 3B) reveals a sharper northeastern-southwestern pattern of variation than that of using only neutral SNPs (Figure 3A). This pattern suggests that other evolutionary processes, besides selection, are shaping Iberian honey bee genetic variation.



Figure 3 – Nuclear DNA patterns of variation obtained with SNPs. (A) Proportions of ancestry inferred by *Structure* software for each individual (represented by vertical lines), when the number of groups is two ($k=2$), using the neutral SNP dataset (SNPs that are candidates for selection were removed). Division of individuals into colored segments represents the probability of assignment of that individual to each of the two groups (represented by red and green). (B) *Structure* analysis using all the 383 polymorphic SNPs. (C) The individual probabilities displayed in (B) were averaged across each sampling location and represented spatially. (D) The genetic clustering of individuals in the presence of a spatial geographic network inferred with *Tess*. (E) Synthetic map obtained from MDS analysis. The map was generated by interpolating the first dimension of MDS analysis using F_{ST} values obtained with SNP data.

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