

INTRODUCTION

The ectoparasite *Varroa destructor* Anderson and Trueman is the most dangerous pest for the worldwide beekeeping. Some honeybee strains bred for tolerance against Varroa had showed a positive expression for mite control. Varroa Sensitive Hygiene bees (VSH, Harris, 2007) remove the infested pupae from capped brood cells. Another trait that allows the bees to control the mite population affects the non-reproductive success of Varroa (Ibrahim and Spivak, 2006), that is: i) it dies in the cell without reproducing, ii) when the mite is infertile, iii) when the offspring is only male, and iv) when the offspring lasts too much to reach the maturity before the post-capping period is finished.

The aim of this research is to investigate the effect of the brood genotype on the degree of Varroa infestation. Three types of pupae were genotyped using microsatellite markers: non-infested pupae (NP), infested pupae with mites without reproductive success (IM) and infested pupae with mites with reproductive success (FM). To this aim we have also considered the mitochondrial lineage (west European or African) of the investigated bees, and the mite genotype, either the Japanese or the Korean genotype.

MATERIAL AND METHODS

Varroa mites and honeybees for genetic analysis were collected from *A. m. iberiensis* colonies at the University of Cordoba (Spain) during Autumn 2006. Mites (46) were collected alive from capped worker brood cells. Three types of honeybee pupae were sampled from 23 colonies: pupae not parasitized (NP = 74), infested pupae with mites without reproductive success (IM = 32) and infested pupae with mites with reproductive success (FM = 76).

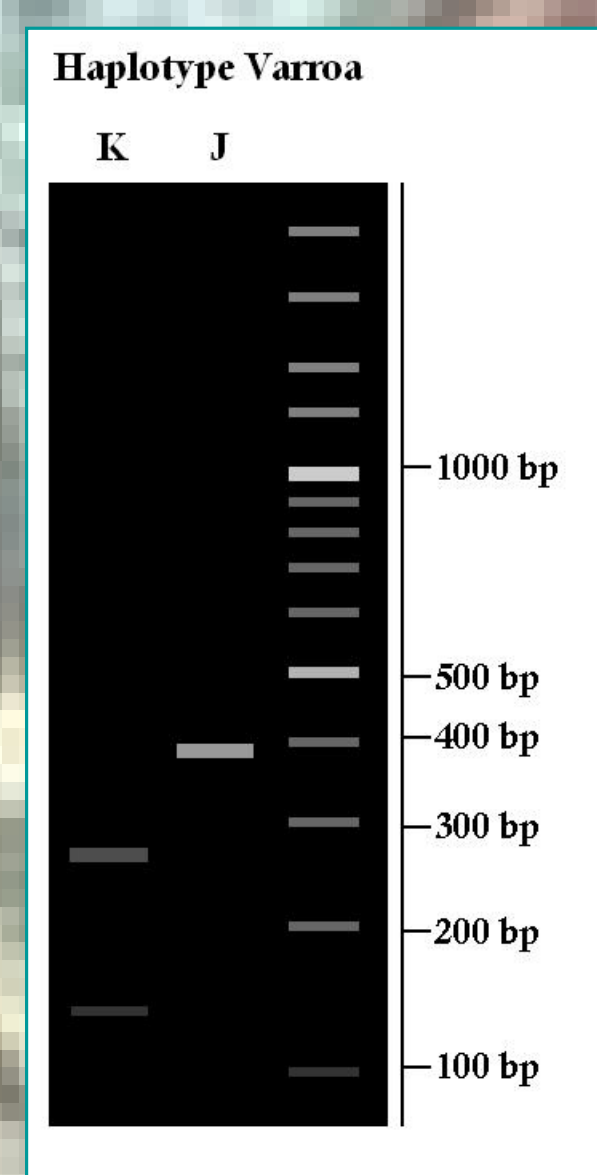


Five microsatellite loci analyzed in 182 pupae: A113, A7, Ap43, Ap55 and B124 (Solignac *et al.*, 2003).

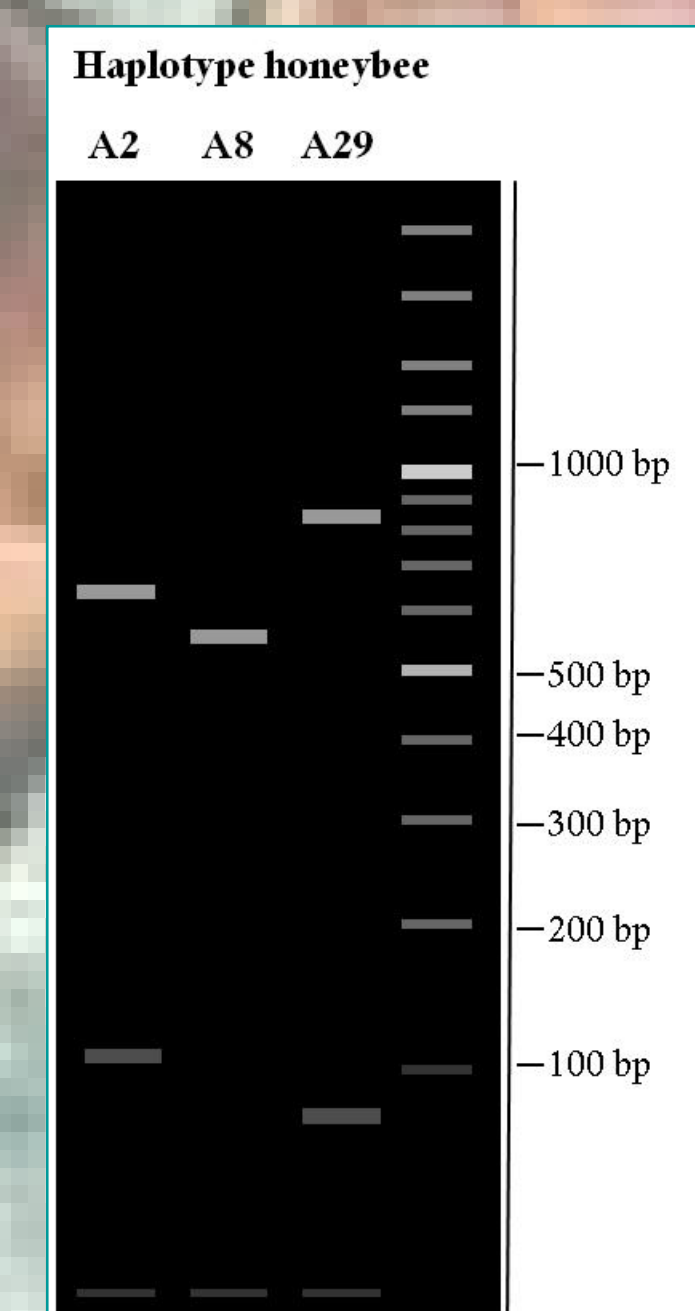
- ❖ population genetic parameters
- ❖ principal coordinate analysis based on the genetic distance (PCA) (GeneAlex, Peakall and Smouse, 2006)
- ❖ population structure and assignment of individuals to populations (Bayesian model-based clustering method, STRUCTURE v 2.2, Pritchard *et al.*, 2000)



PCR of the mitochondrial *cox1* gene and RFLP with *XhoI* (Anderson and Fuchs, 1998)



PCR of the *trnA^{leu}-cox2* intergenic region (Garnery *et al.*, 1993) and RFLP with *DraI*.



RESULTS

- ❖ All mites (with and without reproductive success) infesting *A. m. iberiensis* were the Korean genotype of *V. destructor*.
- ❖ Three mitochondrial haplotypes were found in the honeybee colonies: A2 (0.435), A8 (0.261) and A29 (0.304).
- ❖ Population genetic parameters of the three types of honeybee pupae are shown in Table I.

Table I. Parameters of the three types of honeybee pupae across all loci. N = mean sample size, Na = no. of different alleles, Ne = no. of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity.

Pupae	N ± SE	Na ± SE	Ne ± SE	Ho ± SE	He ± SE
NP	73.800 ± 0.200	10.800 ± 1.881	4.126 ± 0.446	0.813 ± 0.036	0.747 ± 0.024
IM	31.200 ± 0.583	9.800 ± 1.800	4.784 ± 0.590	0.808 ± 0.087	0.779 ± 0.025
FM	74.600 ± 0.510	9.400 ± 0.872	3.847 ± 0.663	0.673 ± 0.083	0.696 ± 0.069

- ❖ In the PCA, two main clusters of individuals were distinguished (Fig. 1).
- ❖ Assignment test: when using a model with two groups of pupae 95.9% of NP pupae and 68.8% of IM pupae belong to cluster-1, and 31.2% and 94.1% of IM and FM pupae respectively belong to cluster-2 (Fig. 2a).

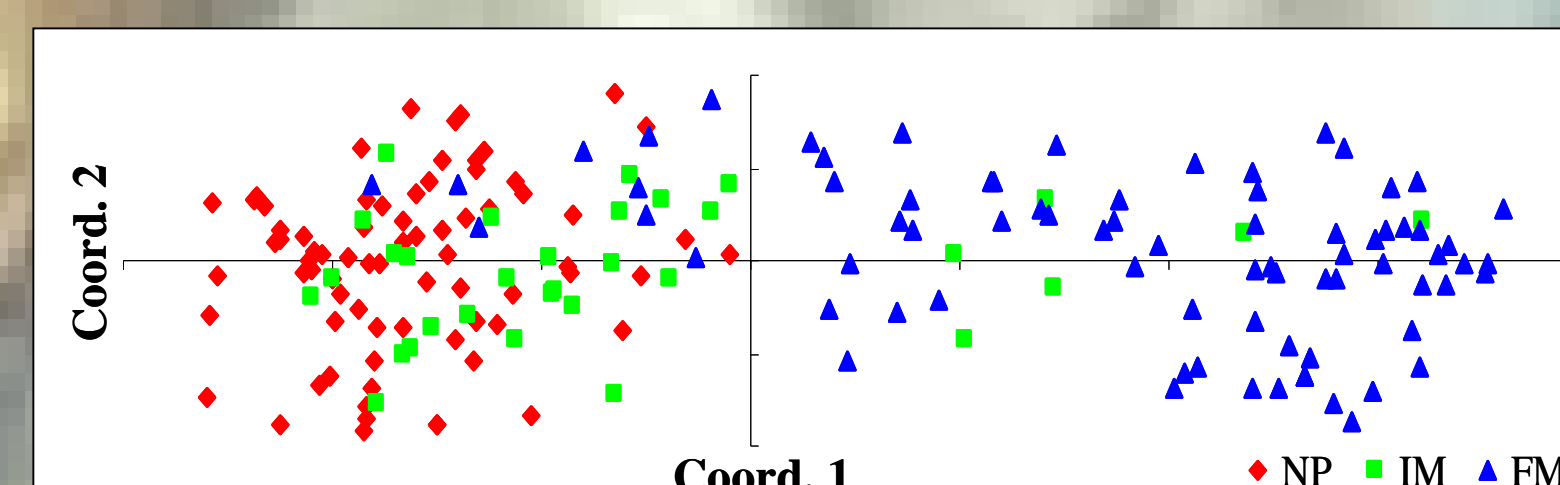


Figure 1. Principal coordinate analysis (PCA) of the three types of pupae.

- ❖ When using a model with three groups NP and IM pupae clustered together and differed from FM pupae (Fig. 2b).

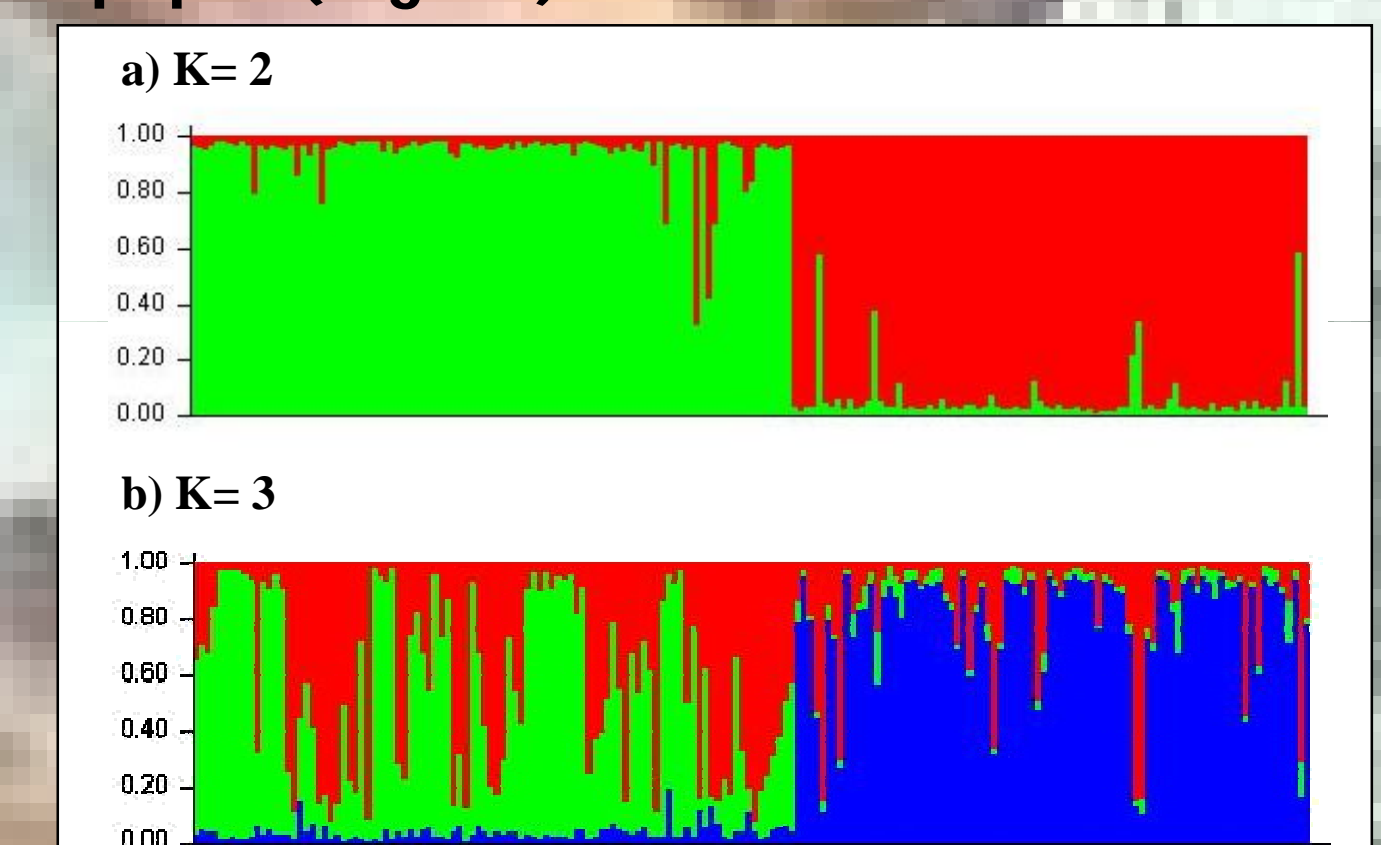


Figure 2. Results of STRUCTURE analysis. Division of individuals into colored segments represents the probability of assignment of that individual to each of K (arbitrary number of hypothetical groups). Colors for K= 3 (Fig. 2b) correspond to those used in the PCA (Fig. 1).

CONCLUSIONS

- ❖ The Korean genotype was observed in both types of mites, suggesting that the mite genotype does not determine the reproductive success or fertility of the mite.
- ❖ Honeybee colonies selected for Varroa tolerance corresponded to the African-derived *A. m. iberiensis* populations adapted to the mild southern Iberian climates.
- ❖ The results indicate that honeybee pupae genotype is related to the hygienic response of the worker bees in charge of detecting the mite inside the capped cell. This results in two different behaviours depending whether pupae are not parasitized or have mites without reproductive success, or the pupae are parasitized with mites with reproductive success. These two groups of pupae could differentially express genes associated to the production of chemical substances (= odors) that promote the cleaning behaviour.
- ❖ Microsatellite analysis reveals that both groups of pupae can be also distinguished by a different allelic composition. This preliminary conclusion must be further assessed by studying a large number of colonies with different geographic and racial origins.
- ❖ Preliminary studies of the genetic variation of larvae of the honeybee colonies submitted to a Varroa tolerance selection process are of interest to infer the drone genotypes, and to select for artificial insemination of daughter queens those showing the same genotype than the NP or IM pupae.

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