

## IMPROVEMENTS TO THE MITOCHONDRIAL RESTRICTION MAPS FOR ITALIAN AND AFRICANIZED HONEY BEES

M.C. Arias<sup>1,2</sup>, A.E.E. Soares<sup>2</sup> and F.G. Nóbrega<sup>1</sup>

### ABSTRACT

Mitochondrial DNA from New World descendents of the honey bee subspecies *Apis mellifera scutellata* (africanized) and *Apis mellifera ligustica* (italian) was extracted and characterized by digestion with the restriction endonucleases Acc I, BamH I, Bgl II, Cla I, EcoR I, EcoR V, Hind III, Pst I and Xho I. The results obtained allowed the construction of a circular map about 16,600 base pairs long for these subspecies. The two genomes could be distinguished by digestion with the enzymes Acc I, Bgl II and EcoR I. The mitochondrial DNA of the African subspecies presented no sites for the endonucleases BstE II, Kpn I, Sac I and Sty I.

### INTRODUCTION

The mitochondrial DNA (mtDNA) of eukaryotes is an abundant and easily extracted organelle genome of low complexity and high evolution rates (Wilson *et al.*, 1985). Consequently, a number of interesting populational and evolutionary studies in man (Brown, 1980; Cann *et al.*, 1987), *Drosophila* (Wolstenholme and Clary, 1985; DeSalle *et al.*, 1986) and *Xenopus* (Roe *et al.*, 1985; Carr *et al.*, 1987) have relied upon characterization of polymorphisms in mtDNA.

In the honey bee, comparative studies between species, subspecies and populations have employed enzymatic polymorphism as detected by electrophoretic procedures. Recently, study of honey bee mtDNA has resulted in subspecies charac-

---

<sup>1</sup> Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Caixa Postal 20780, 01498 São Paulo, SP, Brasil. Send correspondence to F.G.N.

<sup>2</sup> Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14049 Ribeirão Preto, SP, Brasil.

terization and detection of populational and subspecies differences (Moritz *et al.*, 1986; Smith and Brown, 1988 and Smith, 1989). As an initial step towards studying population structure and dispersion of the Africanized honey bee in Brazil, we used restriction endonuclease mapping to characterize the mtDNA genomes of Italian and Africanized honey bees. Our study extends the results of previously published work (Smith and Brown, 1988 and Smith, 1989) by using a number of additional enzymes in the mapping procedures. Consequently, a new map containing a total of 43 defined sites now describes the mtDNA of the honey bee.

## MATERIALS AND METHODS

Samples of approximately 300 white-eyed pupae, about 11 days old (roughly 35 grams) were collected from two africanized colonies (natural swarms captured in São Paulo and Ribeirão Preto and maintained respectively in the apiary of the Laboratório de Abelhas, Depto. de Ecologia Geral, IBUSP/SP and in the apiary of the Depto. de Genética, FMRP/USP). Italian honey bees were collected from two colonies of pure lineage maintained by instrumental insemination at the Depto. de Genética, FMRP/SP. The pupae were homogenized to obtain a purified mitochondrial fraction. The mtDNA was obtained by the procedure described by Moritz *et al.* (1986).

The mtDNA was digested with the following restriction endonucleases (New England Biolabs, Inc.) according to the manufacturers recommendations: Acc I, BamH I, Bgl II, Bst E II, Cla I, EcoR I, EcoR V, Hind III, Kpn I, PstI, Sac I, Sty I and Xho I. The digests were analyzed by electrophoresis in 0.9% agarose gels, stained with ethidium bromide and photographed under UV illumination. Molecular sizes were estimated by comparison with bacteriophage lambda DNA digested with Hind III. Double digestions were used to determine the relative position of restriction sites along the circular mtDNA molecule for the two subspecies.

## RESULTS

The eight restriction enzymes that cut honey bee mtDNA defined 16 sites in the Africanized mtDNA and 18 sites in Italian (*Apis mellifera ligustica*) mitochondrial genome. Polymorphism was detected in three digests (Figure 1): Acc I exhibits a smaller second fragment in the Italian honey bees, EcoR I with three sites (africanized) and four sites (italian) and Bgl II with one site (africanized) and two sites (italian). The number of sites detected with the different enzymes and the size of the mtDNA fragments generated is shown in Table I. The results from multiple enzyme digestions (not shown) were used to construct the circular restriction maps of both subspecies (Figure 2). The size of mtDNA was determined to be somewhere between 16,000 and 17,000 base pairs (Table I).

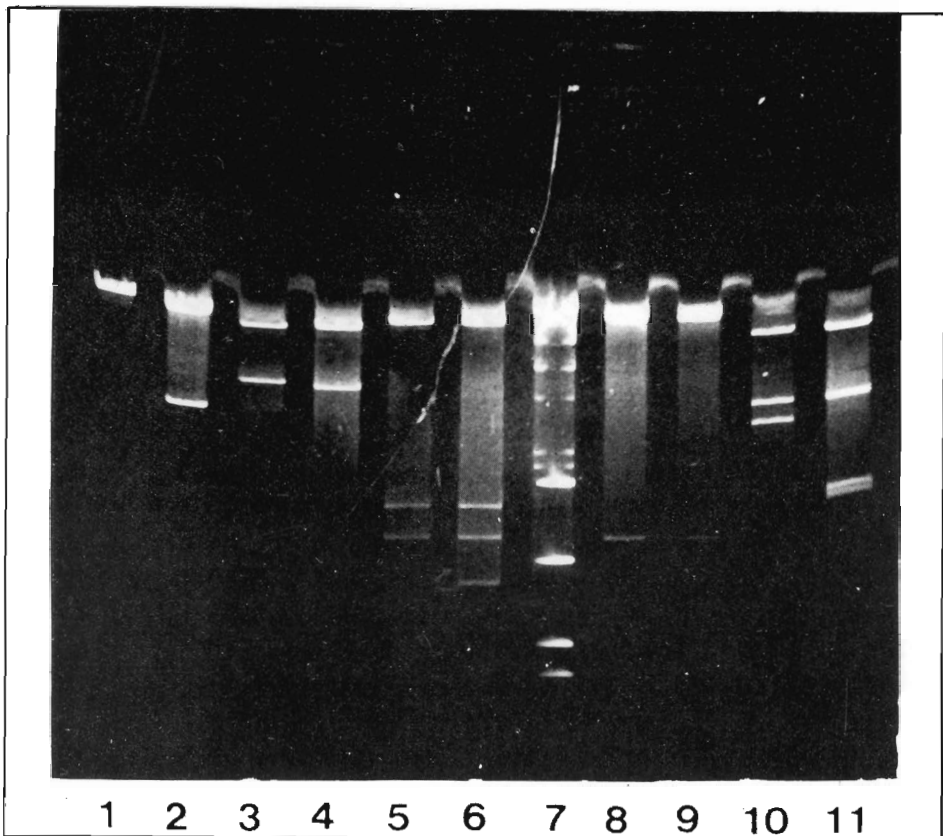


Figure 1 - Mitochondrial DNA fragments of honey bee subspecies *Apis mellifera scutellata* (lanes 1, 3, 5, 8 and 10) and *Apis mellifera ligustica* (lanes 2, 4, 6, 9 and 11) digested with the following restriction endonucleases: Bgl II (lanes 1 and 2), Acc I (lanes 3 and 4), Cla I (lanes 5 and 6), Hind III (lanes 8 and 9) and Eco RI (lanes 10 and 11). Lane 7, size standards: 23.1; 9.4; 6.6; 4.4; 2.3; 2.0 kilobases (Hind III digested lambda DNA) and 1.8; 1.0; 0.5 and 0.4 kilobases (pCL778/44 plasmid DNA digested with Hinf I).

## DISCUSSION

The reduced polymorphism detected between the samples analyzed from these two subspecies parallels the results of Moritz *et al.* (1986) who found differences only in Bgl II digests among seven other restriction endonucleases tested in the mtDNA of three subspecies (*Apis mellifera carnica*, *Apis mellifera caucasica* and *Apis mellifera ligustica*). One subspecies (*Apis mellifera caucasica*) exhibited two sites for Bgl II while the other two had single sites for this enzyme. The authors proposed that the reduced variability was caused by the use of domesticated populations. In our case

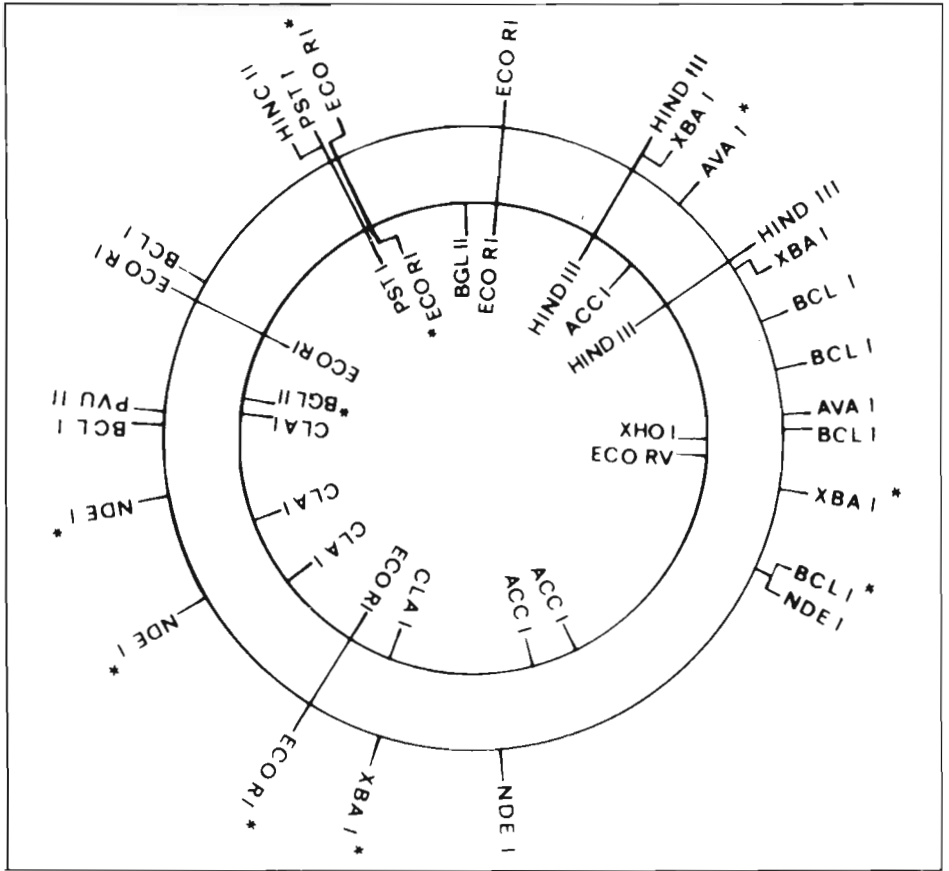


Figure 2 - Composite physical map of the honey bee *Apis mellifera* mitochondrial DNA. Restriction sites located in the outer circle (9 enzymes) are adapted from the data of Smith and Brown (1988) and Smith (1989). Inner circle data (8 enzymes) derives from the present work. This representation exhibits a total of 43 mapped restriction endonuclease sites. The asterisks indicate the 10 polymorphic sites found in different subspecies or populations studied.

we analyzed the mtDNA from honey bees derived from only two colonies for each subspecies, Smith and Brown (1988) studied mtDNA polymorphism in 3 colonies of european descent and five brazilian africanized colonies and the patterns analyzed clearly showed that the differences between samples within the same lineage weren't smaller than the differences between subspecies. Recently Smith (1989) reported the study of additional subspecies (*Apis mellifera capensis*, *Apis mellifera caucasica* and *Apis mellifera ligustica*) along with two africanized honey bee colonies from Mexico, and a few more polymorphic sites were found. In the present work, three of the en-

Table 1 - Number and approximate sizes (in base pairs) of mt DNA restriction fragments from *Apis mellifera scutellata* (AF) and *Apis mellifera ligustica* (IT). The size standard used was bacteriophage lambda DNA digested with HIND III<sup>a</sup>. The number in parenthesis under the subspecies designation indicates the number of fragments or restriction sites observed after digestion with the endonuclease indicated above the corresponding column. The asterisk shows enzyme sites found to be polymorphic.

Enzymes	ACCI*	BGL II*		CLA I	ECO RI*	ECO RV	HIND III	PST I	XHO I
Lineages	AF (3)	IT (3)	AF (1)	IT (2)	AF/IT (4)	AF (3)	IT (4)	AF/IT (1)	AF/IT (1)
Restriction fragments	11.700 4.530 510	11.700 4.900 510	17.900 17.900	14.000 3.900	14.000 1.480 1.200	9.400 3.800 2.800	9.400 3.800 1.500	17.000 1.200	15.400 17.500
Total	16.740	17.110	17.900	17.900	17.480	16.000	16.150	17.000	16.600
								17.500	17.500

<sup>a</sup> Szybalski and Szybalski (1979).

zymes used by us were also utilized by Smith and Brown (1988) and Smith (1989) for mapping purposes. This allowed for the necessary comparisons between our study and theirs. In addition, we used five new enzymes and our combined data can now define a total of 43 sites in honey bee mtDNA (Figure 2).

The overall size of the mitochondrial genome was estimated to be between 16,000 to 17,000 bases pairs. The uncertainty in the evaluation of molecular size is considerable and we fear that the estimates of Smith (1989) proposing 100 to 270 base pair differences in genomes about 17,000 bases long are quite risky. Nevertheless we have some evidence that goes in the same direction: the Acc I digestion (Figure 1) suggests that the africanized honey bees we studied exhibit a restriction fragment about 370 base pairs larger than the corresponding fragment in the digest of mtDNA from bees of Italian descent. The simplest explanation for this difference, seen only in the Acc I digest, is the presence of one or more extra sites for this endonuclease about 300-400 base pairs away from one of the three mapped sites; the resulting small fragment(s) could be easily overlooked.

Because our sampling was limited we think that the present data does not allow any solid conclusions about the degree of polymorphism between the two subspecies studied.

Further molecular studies encompassing the 24 subspecies of *Apis mellifera* and also utilizing larger sample size will certainly help towards a better understanding of the population dynamics, phylogeny and evolutionary rates within honey bees.

### ACKNOWLEDGMENTS

We thank the help of colleagues from the Laboratório de Abelhas, Depto. de Ecologia Geral, IBUSP and the Laboratório de Genética de Hymenopteros, Depto. de Genética, USP/RP. The efficient technical help of Jose Lino and Cristina Keiko Takahashi is gratefully acknowledged as well as the counsel of Dr. Claudio A. Bonjardim and Dr. Daniel Delouya during the course of this work. Financial support was provided by FAPESP, CNPq and FINEP.

Publication supported by FAPESP.

### RESUMO

O DNA mitocondrial (mtDNA) de abelhas locais descendentes das subespécies *Apis mellifera scutellata* (africanizada) e *Apis mellifera ligustica* (italiana) foi extraído e caracterizado por digestão com as endonucleases de restrição Acc I, Bgl II, Cla I, EcoR I, EcoR V, Hind III, Pst I e Xho I de maneira a definir um mapa circular compreendendo cerca de 16,600 pares de bases para o genoma mitocondrial destas subespécies. Os dois genomas podem ser diferenciados por meio de digestão com as enzimas Acc I, Bgl II e EcoR I. O DNA mitocondrial da subespécie africanizada não apresenta sítios para as enzimas de restrição BstE II, Kpn I, Sac I e Sty I.

## REFERENCES

- Brown, W.M. (1980). Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analyses. *Proc. Nat. Acad. Sci.* 77: 3605-3609.
- Cann, R.L., Stoneking, M. and Wilson, A.C. (1987). Mitochondrial DNA and human evolution. *Nature* 325: 31-36.
- Carr, S.M., Brothers, A.J. and Wilson, A.C. (1987). Evolutionary inferences from restriction maps of mitochondrial DNA from nine taxa of *Xenopus* frog. *Evolution* 41: 176-190.
- DeSalle, R., Giddings, L.V. and Templeton, A.R. (1986). Mitochondrial DNA variability in natural populations of Hawaiian *Drosophila*. I. Methods and levels of variability in *D. silvestris* and *D. heteroneura* populations. *Heredity* 56: 75-85.
- Moritz, R.F.A., Hawkins, C.F., Crozier, R.H. and MacKinley, A.G. (1986). A mitochondrial DNA polymorphism in honeybees (*Apis mellifera* L.). *Experientia* 42: 322-324.
- Roe, B.A., Ma, D.P., Wilson, R.K. and Wong, J.F.H. (1985). The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *J. Biol. Chem.* 260: 9759-9774.
- Smith, D.R. and Brown, W.M. (1988). Polymorphisms in mitochondrial DNA of European and Africanized honey bees (*Apis mellifera*). *Experientia* 44: 257-260.
- Smith, D.R. (1989). Mitochondrial DNA polymorphisms in five Old World subspecies of honey bee and in New World hybrids. In: *Africanized honey bees and bee mites*. (Needham, G.R., Page, R.E., Jr., Delfinado-Baker M. and Bowman, C.L., eds.). John Wiley & Sons, New York, pp. 303-312.
- Szybalski, E.H. and Szybalski, W. (1979). A comprehensive molecular map of bacteriophage lambda. *Gene* 7: 217-270.
- Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higushi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D. and Stoneking, M. (1985). Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26: 375-400.
- Wolstenholme, D.R. and Clary, D.O. (1985). Sequence evolution of *Drosophila* mitochondrial DNA. *Genetics* 109: 725-744.

(Received July 24, 1989)