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A scientific note on the ITS-1 region of *Apis mellifera* subspecies*

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Apis mellifera / honeybee subspecies / ITS-1 region / DNA sequence / molecular markers

Several molecular markers have been widely used in insect molecular systematics in the last 20 years; among them nuclear DNA markers as the internal transcribed spacers (ITS1 and ITS2) of the nuclear rDNA (revision in Caterino et al., 2000). The sequence of these regions, which lie between functional 18S, 5.8S and 28S ribosomal genes, has proven suitable for phylogenetic studies at the species and intraspecific levels due to the fact that they are relatively free from selective constraints and correspondingly accumulate sequence differences rapidly. The sequence variation of this marker has been characterized in several genera within the order Hymenoptera as in *Ageniaspis* (Álvarez and Hoy, 2002) and in *Tetranychus* (Navajas et al., 1998) and also it has been applied to phylogenetic analysis in *Melipona* species (Fernandes-Salomão et al., 2005). Although the size of the ITS-1 region was determined by Sheppard and McPherson (1991) to 132 bp in a sample of *Apis mellifera* from Argentina, nothing is published regarding sequence characteristics. Therefore, we characterized the sequence of the internal transcribed region (ITS-1) and evaluated its potential usefulness for phylogenetic and population studies among subspecies of *A. mellifera*.

The ITS-1 region was PCR-amplified (Ji et al., 2003) in ten subspecies of *Apis mellifera* (GenBank accession numbers DQ195225–DQ195236) (see material online at <http://www.apidologie.org>). It had a size of 132 bp, one of the smallest ITS-1 regions known so far in bees. In other Apidae this region varies from 1387–1417 bp in *Melipona*

subspecies (Fernandes-Salomão et al., 2005) to 289 bp in *Bombus lapidarius* (Ji et al., 2003). The G+C content (21.6%) was similar to that found in *Drosophila melanogaster* (i.e. 27%, Tautz et al., 1988) but in contrast, it was different to that observed in *Melipona* in which it varies from 49 to 54%. This A-T richness of the ITS sequence can be explained by processes as biased occurrence or fixation of point mutations. The presence of microsatellite loci located within the ITS-1 region is well documented in many organisms (see Harris and Crandall, 2000 for rationale) and has also been observed in *Melipona* subspecies, in the form of repeated elements of one to four nucleotides (Fernandes-Salomão et al., 2005). Despite the variation found in these regions, the ITS-1 region had the same sequence in eight of the ten *mellifera* subspecies studied and differed only in two, *A. m. scutellata* and *A. m. intermissa*, with exhibit a southern and northern African distribution, respectively.

The homogeneity observed in the ITS-1 region of the honeybee presumably results from the process of concerted evolution leading to a homogenization of the rDNA copies within a species. Vogler and DeSalle (1994) hypothesized that the homogenization proceeds more efficiently within a single chromosome than among different chromosomes, which is in congruence with the observations of Beye and Moritz (1993) concerning the location of the rDNA copies in only two chromosomes of the chromosomal set of the honeybee ($n = 16$). In contrast, a considerable amount of sequence divergence in the mitochondrial DNA molecule exists within *Apis mellifera*, which has been widely used to establish the phylogenetic relationships

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among the *A. mellifera* subspecies (Garnery et al., 1992; Arias and Sheppard, 1996; De la Rúa et al., 2005 and references therein). These results suggest that suitable molecular markers may vary notably within the order Hymenoptera. Each potential marker has to be carefully evaluated to test its suitability for phylogenetic or population use within particular taxa. In the case of *Apis mellifera* we conclude that the ITS-1 region is unlikely to be useful at the subspecific level. Detailed analyses of the ITS-2 region will provide a more complete resolution of the sequence variation of the intergenic transcribed spacers of honeybee rDNA.

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Note scientifique sur la région ITS-1 chez les sous-espèces d'*Apis mellifera*.

Zusammenfassung – Eine wissenschaftliche Notiz über die ITS-1 Region der Unterarten von *Apis mellifera*.

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Figure 1. Sequence alignment of the partial 18S, complete ITS-1 and partial 5.8S sequence of the ten *A. mellifera* subspecies. Gaps are indicated by hyphens and identical nucleotides by dots. An asterisk indicates the location of the repeated unit.

	[18S→				
<i>A. m. mellifera</i> 1	AGTGAGGTCT	TCGGACTGGT	GCGCGGCCAA	TGTCTCGGCA	40
<i>A. m. mellifera</i> 2					
<i>A. m. iberiensis</i> 1					
<i>A. m. iberiensis</i> 2					
<i>A. m. caucasica</i>					
<i>A. m. carnica</i>					
<i>A. m. ligustica</i>					
<i>A. m. scutellata</i>					
<i>A. m. sahariensis</i>					
<i>A. m. intermissa</i>					
<i>A. m. jemenitica</i>					
<i>A. m. meda</i>					
<i>A. m. mellifera</i> 1	TTGCCGATGT	TACCGGGAAG	ATGACCAAAC	TTGATCATTT	80
<i>A. m. mellifera</i> 2					
<i>A. m. iberiensis</i> 1					
<i>A. m. iberiensis</i> 2					
<i>A. m. caucasica</i>					
<i>A. m. carnica</i>					
<i>A. m. ligustica</i>					
<i>A. m. scutellata</i>					
<i>A. m. sahariensis</i>					
<i>A. m. intermissa</i>					
<i>A. m. jemenitica</i>					
<i>A. m. meda</i>					
<i>A. m. mellifera</i> 1	AGAGGAAGTA	AAAGTCGTAA	CAAGGTTTCC	GTAGGTGAAC	120
<i>A. m. mellifera</i> 2					
<i>A. m. iberiensis</i> 1					
<i>A. m. iberiensis</i> 2					
<i>A. m. caucasica</i>					
<i>A. m. carnica</i>					
<i>A. m. ligustica</i>					
<i>A. m. scutellata</i>					
<i>A. m. sahariensis</i>					
<i>A. m. intermissa</i>					
<i>A. m. jemenitica</i>					
<i>A. m. meda</i>					
	[ITS-1→				
<i>A. m. mellifera</i> 1	CTGCGGAAGG	ATCATTAACG	AATGAAACAT	ACAAAAAGAG	160
<i>A. m. mellifera</i> 2					
<i>A. m. iberiensis</i> 1					
<i>A. m. iberiensis</i> 2					
<i>A. m. caucasica</i>					
<i>A. m. carnica</i>					
<i>A. m. ligustica</i>					
<i>A. m. scutellata</i>					
<i>A. m. sahariensis</i>					
<i>A. m. intermissa</i>					
<i>A. m. jemenitica</i>					
<i>A. m. meda</i>					

Figure 1. Continued.

<i>A. m. mellifera</i> 1	AAAATGCGTA	ACAAAACAAA	CAGAGAATTG	AAAGAAGGGG	200
<i>A. m. mellifera</i> 2					
<i>A. m. iberiensis</i> 1					
<i>A. m. iberiensis</i> 2					
<i>A. m. caucasica</i>					
<i>A. m. carnica</i>					
<i>A. m. ligustica</i>					
<i>A. m. scutellata</i>				...A..-A..	
<i>A. m. sahariensis</i>					
<i>A. m. intermissa</i>				...R..R...	
<i>A. m. jemenitica</i>					
<i>A. m. meda</i>					
				★	
<i>A. m. mellifera</i> 1	ATGATAAATA	TATATATTTT	ATATATATAT	ATATTTATAA	240
<i>A. m. mellifera</i> 2				-----	
<i>A. m. iberiensis</i> 1				-----	
<i>A. m. iberiensis</i> 2				-----	
<i>A. m. caucasica</i>				-----	
<i>A. m. carnica</i>				-----	
<i>A. m. ligustica</i>				-----	
<i>A. m. scutellata</i>				-----	
<i>A. m. sahariensis</i>				-----	
<i>A. m. intermissa</i>				-----	
<i>A. m. jemenitica</i>				-----	
<i>A. m. meda</i>				-----	
				[5.8S→	
<i>A. m. mellifera</i> 1	AATGGATTTT	TTGAATCCAT	TATCAAATAC	CAAAACTTTG	280
<i>A. m. mellifera</i> 2					
<i>A. m. iberiensis</i> 1					
<i>A. m. iberiensis</i> 2					
<i>A. m. caucasica</i>					
<i>A. m. carnica</i>					
<i>A. m. ligustica</i>					
<i>A. m. scutellata</i>					
<i>A. m. sahariensis</i>					
<i>A. m. intermissa</i>					
<i>A. m. jemenitica</i>					
<i>A. m. meda</i>					
<i>A. m. mellifera</i> 1	AACATCGACA	TTTCGAACCG	CACATA		306
<i>A. m. mellifera</i> 2					302
<i>A. m. iberiensis</i> 1					306
<i>A. m. iberiensis</i> 2					302
<i>A. m. caucasica</i>	-----	-----	-----		269
<i>A. m. carnica</i>					302
<i>A. m. ligustica</i>					302
<i>A. m. scutellata</i>					301
<i>A. m. sahariensis</i>		-----	-----		292
<i>A. m. intermissa</i>					300
<i>A. m. jemenitica</i>					302
<i>A. m. meda</i>	-----	-----	-----		269