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Scientific note

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

Pilar De La RÚA[a], Stefan FUCHS[b], José SERRANO[a]

[a] Área de Biología Animal, Facultad de Veterinaria, Apdo. 4021, Universidad de Murcia, 30100 Murcia, Spain
[b] Institut fuer Bienenkunde, J.W. Goethe-Universitaet Frankfurt-am-Main, Karl von Frisch-Weg 2, 61440 Oberursel, Germany

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

Several molecular markers have being 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 McPheron (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 being widely used to establish the phylogenetic relationships

Corresponding author: P. De La Rúa,
pdelarua@um.es
* Manuscript editor: Walter S. Sheppard

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.

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                    [18S⟶
A. m. mellifera1  AGTGAGGTCT TCGGACTGGT GCGCGGCCAA TGTCTCGGCA 40
A. m. mellifera2  .......... .......... .......... ..........
A. m. iberiensis1 .......... .......... .......... ..........
A. m. iberiensis2 .......... .......... .......... ..........
A. m. caucasica   .......... .......... .......... ..........
A. m. carnica     .......... .......... .......... ..........
A. m. ligustica   .......... .......... .......... ..........
A. m. scutellata  .......... .......... .......... ..........
A. m. sahariensis .......... .......... .......... ..........
A. m. intermissa  .......... .......... .......... ..........
A. m. jemenitica  .......... .......... .......... ..........
A. m. meda        .......... .......... .......... ..........

A. m. mellifera1  TTGCCGATGT TACCGGGAAG ATGACCAAAC TTGATCATTT 80
A. m. mellifera2  .......... .......... .......... ..........
A. m. iberiensis1 .......... .......... .......... ..........
A. m. iberiensis2 .......... .......... .......... ..........
A. m. caucasica   .......... .......... .......... ..........
A. m. carnica     .......... .......... .......... ..........
A. m. ligustica   .......... .......... .......... ..........
A. m. scutellata  .......... .......... .......... ..........
A. m. sahariensis .......... .......... .......... ..........
A. m. intermissa  .......... .......... .......... ..........
A. m. jemenitica  .......... .......... .......... ..........
A. m. meda        .......... .......... .......... ..........

A. m. mellifera1  AGAGGAAGTA AAAGTCGTAA CAAGGTTTCC GTAGGTGAAC 120
A. m. mellifera2  .......... .......... .......... ..........
A. m. iberiensis1 .......... .......... .......... ..........
A. m. iberiensis2 .......... .......... .......... ..........
A. m. caucasica   .......... .......... .......... ..........
A. m. carnica     .......... .......... .......... ..........
A. m. ligustica   .......... .......... .......... ..........
A. m. scutellata  .......... .......... .......... ..........
A. m. sahariensis .......... .......... .......... ..........
A. m. intermissa  .......... .......... .......... ..........
A. m. jemenitica  .......... .......... .......... ..........
A. m. meda        .......... .......... .......... ..........

                                              [ITS-1⟶
A. m. mellifera1  CTGCGGAAGG ATCATTAACG AATGAAACAT ACAAAAAGAG 160
A. m. mellifera2  .......... .......... .......... ..........
A. m. iberiensis1 .......... .......... .......... ..........
A. m. iberiensis2 .......... .......... .......... ..........
A. m. caucasica   .......... .......... .......... ..........
A. m. carnica     .......... .......... .......... ..........
A. m. ligustica   .......... .......... .......... ..........
A. m. scutellata  .......... .......... .......... ..........
A. m. sahariensis .......... .......... .......... ..........
A. m. intermissa  .......... .......... .......... ..........
A. m. jemenitica  .......... .......... .......... ..........
A. m. meda        .......... .......... .......... ..........
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Figure 1. Continued.

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A. m. mellifera1  AAAATGCGTA ACAAAACAAA CAGAGAATTG AAAGAAGGGG 200
A. m. mellifera2  .......... .......... .......... ..........
A. m. iberiensis1 .......... .......... .......... ..........
A. m. iberiensis2 .......... .......... .......... ..........
A. m. caucasica   .......... .......... .......... ..........
A. m. carnica     .......... .......... .......... ..........
A. m. ligustica   .......... .......... .......... ..........
A. m. scutellata  .......... .......... .......... ...A..-A..
A. m. sahariensis .......... .......... .......... ..........
A. m. intermissa  .......... .......... .......... ...R..R...
A. m. jemenitica  .......... .......... .......... ..........
A. m. meda        .......... .......... .......... ..........
                                                      *
A. m. mellifera1  ATGATAAATA TATATATTTT ATATATATAT ATATTTATAA 240
A. m. mellifera2  .......... .......... .......... ----......
A. m. iberiensis1 .......... .......... .......... ..........
A. m. iberiensis2 .......... .......... .......... ----......
A. m. caucasica   .......... .......... .......... ----......
A. m. carnica     .......... .......... .......... ----......
A. m. ligustica   .......... .......... .......... ----......
A. m. scutellata  .......... .......... .......... ----......
A. m. sahariensis .......... .......... .......... ----......
A. m. intermissa  .......... .......... .......... ----......
A. m. jemenitica  .......... .......... .......... ----......
A. m. meda        .......... .......... .......... ----......
                                                        [5.8S⟶
A. m. mellifera1  AATGGATTTT TTGAATCCAT TATCAAATAC CAAAACTTTG 280
A. m. mellifera2  .......... .......... .......... ..........
A. m. iberiensis1 .......... .......... .......... ..........
A. m. iberiensis2 .......... .......... .......... ..........
A. m. caucasica   .......... .......... .......... ...-------
A. m. carnica     .......... .......... .......... ..........
A. m. ligustica   .......... .......... .......... ..........
A. m. scutellata  .......... .......... .......... ..........
A. m. sahariensis .......... .......... .......... ..........
A. m. intermissa  .......... .......... .......... ..........
A. m. jemenitica  .......... .......... .......... ..........
A. m. meda        .......... .......... .......... ...-------

A. m. mellifera1  AACATCGACA TTTCGAACCG CACATA 306
A. m. mellifera2  .......... .......... ...... 302
A. m. iberiensis1 .......... .......... ...... 306
A. m. iberiensis2 .......... .......... ...... 302
A. m. caucasica   ---------- ---------- ------ 269
A. m. carnica     .......... .......... ...... 302
A. m. ligustica   .......... .......... ...... 302
A. m. scutellata  .......... .......... ...... 301
A. m. sahariensis .......... .....---- ------ 292
A. m. intermissa  .......... .......... ...... 300
A. m. jemenitica  .......... .......... ...... 302
A. m. meda        ---------- ---------- ------ 269
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