

MOLECULAR DIVERSITY OF HONEYBEE *APIS MELLIFERA*
IBERICA L. (HYMENOPTERA: APIDAE)
FROM WESTERN ANDALUSIA

DIVERSIDAD MOLECULAR DE LA ABEJA *APIS MELLIFERA* *IBERICA* L.
(HYMENOPTERA: APIDAE) DE ANDALUCÍA OCCIDENTAL

De la Rúa, P.^{1*}, R. Hernández-García¹, B.V. Pedersen², J. Galián¹ and J. Serrano¹

¹Departamento de Zoología y Antropología Física. Facultad de Veterinaria. Apdo. 4021. Universidad de Murcia. 30071 Murcia. España. Tlf: 968 364908. Fax: 968 364906.

E-mail: mariap.delarua@carm.es; raquelhg@um.es; jgalian@um.es; jserrano@um.es

²Department of Evolutionary Biology. Zoological Institute. University of Copenhagen. Universitetsparken 15. 2100 Copenhagen Ø. Denmark. E-mail: bvpedersen@zi.ku.dk

*Corresponding author: IMIDA. C/ Mayor s/n. 30150 La Alberca. Murcia. España.

ADDITIONAL KEYWORDS

Mitochondrial DNA. RFLP-PCR. Microsatellites. Biodiversity. Population genetics. Spain. Europe.

PALABRAS CLAVE ADICIONALES

ADN mitocondrial. RFLP-PCR. Microsatélites. Biodiversidad. Genética de poblaciones. España. Europa.

SUMMARY

The molecular diversity of the honey bee *Apis mellifera iberica* in Western Andalusia has been analyzed through the study of mitochondrial and nuclear DNA. The mitochondrial haplotype corresponding to the intergenic region tRNA^{leu}-COII, and six microsatellite *loci* has been determined in hives distributed in 24 localities of the provinces of Malaga, Seville, Cadiz and Huelva. Six different haplotypes have been found, five of the African and one of the West European evolutionary lineage. These results corroborate the hybrid nature of the subspecies *Apis mellifera iberica*, which has a predominant influence of the African lineage in the South, that is gradually or steeply replaced northwards by the West European lineage. The variability of the microsatellite *loci* is similar to that found in African populations in relation to the detected number of alleles and the values of genetic diversity. These observations

show the genetic relationship between Andalusian honey bee populations and those ones from North Africa. Microsatellite data vary notably between the studied provinces. In the province of Cadiz the mitochondrial homogeneity contrasts with the microsatellite variability, what suggests a recent introgression event from African-like populations of unknown geographic origin.

RESUMEN

Hemos estudiado la diversidad molecular de 48 colmenas de la abeja doméstica, *Apis mellifera iberica*, situadas en 24 localidades de las cuatro provincias occidentales andaluzas. Para ello hemos determinado el haplotipo mitocondrial que corresponde a la región intergénica tRNA^{leu}-COII, así como la variabilidad que presentan seis *loci*

Arch. Zootec. 53: 195-203. 2004.

de microsatélites. Hemos encontrado seis haplotipos diferentes, de los que cinco corresponden al linaje evolutivo africano y uno al europeo occidental. Estos resultados corroboran la naturaleza híbrida de la subespecie *Apis mellifera iberica*, que tiene un componente norteafricano predominante en el sur peninsular, el cual es reemplazado gradual o abruptamente hacia el norte por el linaje europeo occidental. La variabilidad genética de los microsatélites es similar a la de las poblaciones africanas en cuanto al número de alelos detectado y los valores de diversidad génica. Estas observaciones indican la afinidad genética que hay entre las poblaciones andaluzas y las del norte de África. Los resultados de los microsatélites varían notoriamente entre provincias. En Cádiz la homogeneidad de haplotipos contrasta con la variabilidad de microsatélites, lo que sugiere la ocurrencia de fenómenos recientes de introgresión a partir de poblaciones con haplotipos africanos, cuyo origen está por determinar.

INTRODUCTION

The Iberian honeybee, *Apis mellifera iberica* (Goetze, 1964; Ruttner, 1988) has been the subject of numerous studies about its molecular diversity (Smith *et al.*, 1991; Garnery *et al.*, 1995, 1998a, b; Sheppard *et al.*, 1996; Franck *et al.*, 1998; De la Rúa *et al.*, 1999, 2002; Cánovas *et al.*, 2002; Hernández-García *et al.*, 2002) and morphometrical variation (Izquierdo *et al.*, 1985; Cornuet and Fresnaye 1989; Orantes-Bermejo and García-Fernández, 1995; Padilla *et al.*, 1998, 2001). Earlier analyses showed that two out of the five evolutionary lineages of *A. mellifera* coexist on the Iberian Peninsula (Smith *et al.*, 1991; Garnery *et al.*, 1995). In the North, the Iberian honeybee populations are more

similar to the West European lineage as they bear particular sequences in the intergenic tRNA^{leu}-COII region (so called mitochondrial haplotypes), whereas in the South they show predominantly African haplotypes. These results led Smith *et al.* (1991) to postulate that *A. mellifera iberica* is the result of the hybridization between the West European *A. mellifera mellifera* and the North African *A. mellifera intermissa*.

In spite of the occurrence of this generalized pattern of mitochondrial haplotypes across the Iberian Peninsula, the studies realized at the regional level have demonstrated that every region holds a peculiar composition of molecular markers. Thus, the honeybees from Murcia show a homogeneous composition of mitochondrial haplotypes (De la Rúa *et al.*, 1999), and neither traces of recent introgression events from African subspecies (De la Rúa *et al.*, 2002), nor the effects of transhumance movements (Hernández-García *et al.*, 2002). In Galicia (NW Spain) the gradient of lineage distribution shows a rather steep transition, as the Southern provinces of Pontevedra and Orense have predominantly (>90 percent) African haplotypes, whereas La Coruña and Lugo in the North show almost only (>90 percent) West European haplotypes (Cánovas *et al.*, 2002). In contrast, in East Spain the percentage of African haplotypes smoothly decreases northwards along the Mediterranean provinces of Valencia (De la Rúa *et al.*, submitted).

The purpose of this work is to characterize the populations of *A. m. iberica* from Western Andalusia, according to the variability shown by

MOLECULAR CHARACTERIZATION OF WEST-ANDALUSIAN HONEYBEES

mitochondrial haplotypes and microsatellite *loci*. It is expected to find a high proportion of African haplotypes, as predicted by the hypothesis stated above about the major influence in South Iberia of the subspecies *A. m. intermissa*, which is found in North Africa. It is also expected to find a moderate degree of differentiation in microsatellite *loci*, denoting the separate evolution of Iberian populations due to the isolating effect of the Straight of Gibraltar and the Pyrenees (De la Rúa *et al.*, 2002).

MATERIAL AND METHODS

A total of 48 colonies were sampled during 2001 and 2002 from 24 localities in the four Western Andalusian provinces (**table I**). Bees were immediately killed by immersion in absolute ethanol and kept at -20 °C until they were processed in the laboratory. The same individual honeybees were used for both mitochondrial and microsatellite analyses.

DNA isolation was performed following the *Chelex* method (Walsh *et al.*, 1991) with slight modifications. One or two worker legs (one worker bee per colony) were dried for 30 min at 37 °C and then homogenized with 100 µL of 5 percent *Chelex* solution and proteinase K (10 mg/ml). This mix was incubated for 1 h at 55 °C, 15 min at 99 °C, 1 min at 37 °C, and finally 15 min at 99 °C in a thermocycler. Two µL of this solution were used for the PCR amplification.

MITOCHONDRIAL ANALYSIS

The intergenic tRNA^{leu}-COII region was amplified and digested following Garnery *et al.* (1993), with the primers E2 located at the 5'-end of the tRNA^{leu} gene, and H2 located at the 5'-end of the COII gene. The size of the amplified products was determined after electrophoretic separation on 1.5 percent (w/v) agarose gels. Ten µL aliquots of the PCR products were digested with five units of the *DraI* enzyme at 37 °C for 4-12 h. The resulting fragments were visualized in 5 percent agarose (Nusieve)

Table I. Haplotype frequency distribution among the samples, gene diversity (*D*) and standard error (*SD*) in the honeybee populations from western Andalusia. (*N* = Number of localities, *n* = number of colonies). (Distribución de la frecuencia de haplotipos, diversidad génica (*D*) y error estándar (*SD*) en las poblaciones de abejas de Andalucía occidental (*N* = número de localidades, *n* = número de colmenas).

Province	N	n	A1	A2	A8	A9	A14	M7	D ± SD
Malaga	4	8	–	7	–	–	1	–	0.25 ± 0.18
Cadiz	10	20	–	18	–	–	2	–	0.19 ± 0.11
Seville	7	14	1	11	1	1	–	–	0.39 ± 0.16
Huelva	3	6	1	3	–	–	–	2	0.73 ± 0.15
Total	24	48	2	39	1	1	3	2	

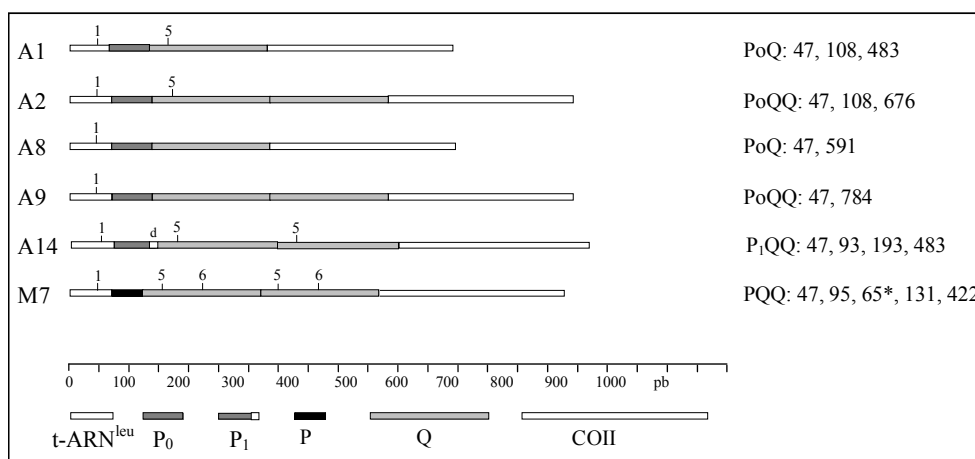


Figure 1. Composition and restriction size fragments of the tRNA^{leu}-COII intergenic region. Superscripts indicate the number of restriction fragments with the same size. (Composición y tamaño de los fragmentos de restricción de la región intergénica tARN^{leu}-COII. Los exponentes indican el número de fragmentos de restricción con el mismo tamaño).

stained with ethidium bromide.

Unbiased estimates and standard deviations of mtDNA gene diversity (Nei, 1973) were calculated using the Arlequin program (Schneider *et al.*, 1997).

MICROSATELLITE ANALYSIS

Six polymorphic microsatellite *loci* were analyzed: B124, A113, A7, A24, A28 and A8. Multiplex PCR reactions were performed when the annealing temperature and the MgCl₂ concentration coincided. The reactions were done with fluorescent labeled primers and separated on a DNA automated sequencer (ABI 377, Applied Biosystems).

Microsatellite allele sizes were scored by comparing the length of the PCR fragments to the standard 100 bp ROX (Applied Biosystems). The Excel Microsat Toolkit (<http://acer.gen.tcd.ie/>

~sdeparck/ms-toolkit) was used to obtain population parameters and the files for the statistical packages. The exact test for Hardy-Weinberg equilibrium, genotypic linkage disequilibrium and genetic structure (genotypic differentiation) were computed with GENEPOP web version 3.1c (<http://wbiomed.curtin.edu.au/genepop>).

RESULTS

MITOCHONDRIAL VARIABILITY

Six different haplotypes have been detected in the 48 analyzed colonies. Five of them correspond to the African evolutionary lineage as they bear the P₀ sequence in the intergenic region. They are differentiated by the number of Q sequences (one in A1 and A8 and two in A2 and A9) and the position of the restriction sites (**figure 1**). The

MOLECULAR CHARACTERIZATION OF WEST-ANDALUSIAN HONEYBEES

most common haplotype is A2, that is present at high frequencies in the four provinces (ranging from 0.90 in Cadiz to 0.50 in Huelva). Haplotypes A8 and A9 have been observed only in one colony each in Seville, A1 in two colonies in Seville and Huelva, and A14 in two colonies in Malaga and Cadiz. The haplotype M7 has the P sequence and two Q sequences showing

the typical restriction pattern of the West European lineage. This haplotype has been observed only in Huelva, with a frequency of 0.333. The gene diversity increases from East to West ranging from 0.19 ± 0.11 observed in Cadiz to 0.73 ± 0.15 detected in Huelva, but this result should be corroborated by increasing the number of sampled colonies in Huelva.

Table II. *Microsatellite allele frequencies for the Western Andalusian provinces by locus.* (Frecuencias de los alelos de microsatélites por *locus* en las cuatro provincias andaluzas occidentales).

<i>Locus</i> /Allele	Malaga	Cadiz	Seville	Huelva	<i>Locus</i> /Allele	Malaga	Cadiz	Seville	Huelva
<i>B124</i>					128	0.06	–	–	–
212	–	0.03	–	–	129	0.13	–	–	0.33
218	0.06	0.03	0.08	0.08	130	0.19	0.10	0.46	0.17
220	–	0.08	0.17	0.33	132	0.06	0.08	–	–
222	0.44	0.31	0.21	–	133	0.38	0.45	0.35	0.42
224	0.06	0.14	0.04	0.17	143	0.13	0.23	0.12	0.08
226	–	0.14	0.17	0.17	<i>A24</i>				
228	0.25	0.17	0.21	–	93	–	0.11	–	–
230	0.06	0.11	0.04	0.17	94	0.79	0.56	0.86	1.00
232	0.06	–	0.04	–	102	0.21	–	0.09	–
234	0.06	–	–	–	104	–	–	0.05	–
238	–	–	–	0.08	105	–	0.06	–	–
240	–	–	0.04	–	120	–	0.28	–	–
<i>A113</i>					<i>A8</i>				
202	0.06	–	–	–	123	–	0.08	–	–
204	–	0.05	–	–	128	0.10	–	–	–
210	–	–	0.14	0.08	129	0.20	0.12	0.05	0.50
214	0.06	–	0.07	0.08	130	0.40	0.58	0.95	0.33
218	–	–	0.04	–	132	–	–	–	0.08
220	0.63	0.66	0.43	0.42	133	0.30	0.15	–	0.08
222	0.19	0.05	0.07	–	143	–	0.08	–	–
224	–	0.13	0.14	0.25	<i>A28</i>				
226	–	–	–	0.08	129	0.25	0.10	–	0.33
228	0.06	0.05	–	–	130	0.69	0.83	0.75	0.50
230	–	0.05	0.11	–	131	–	0.03	–	–
234	–	–	–	0.08	133	0.06	0.03	–	–
<i>A7</i>					139	–	0.03	–	–
123	0.06	0.15	0.08	–	140	–	–	0.25	–0.17

MICROSATELLITE VARIABILITY

One bee of each of the 48 colonies was analyzed with six microsatellite *loci*. The allele frequencies of the colonies grouped in provinces and by *locus* are shown in **table II** (detailed data are available upon request). Twelve alleles have been observed at the *loci* B124 and A113, seven at the *loci* A7 and A8 and six at the *loci* A24 and A28. The overall population parameters per province (medium number of alleles detected and the observed and expected heterozygosities, H_o and H_e) are shown in **table III**. The gene diversity varied between 0.51 ± 0.13 (Seville) and 0.64 ± 0.07 (Malaga).

Six significant departures from the Hardy-Weinberg equilibrium were detected among 24 (6 x 4) *loci* per population combinations, when only one was expected by chance at the 5 percent level. Three significant tests were observed in Cadiz, at *loci* A7, A8 and A28 and one in Malaga, Seville and Huelva at the *locus* A7. Exact test for linkage disequilibrium resulted in no significant value out of 44 pairwise

comparisons. Fisher's method was used to perform the genic and genotypic differentiation tests, to check whether the honeybees sampled in each province could be considered as one single population. The genic differentiation tests (concerning the distribution of alleles in the provinces) were highly significant ($p < 0.002$) at every *locus*, except for B124. The genotypic differentiation (taking into account the distribution of genotypes among the four provinces) was significant ($p < 0.03$) at *loci* A113, A24 and A28.

DISCUSSION

The high percentage of African haplotypes (95.8 percent) in the four occidental provinces of Andalusia fits the expectations of the hypothesis about the predominance of African haplotypes in South Iberia and of haplotypes of the Western European lineage in the Northern Peninsula (Smith *et al.*, 1991; Garnery *et al.*, 1995). The cumulative evidence that we are obtaining in the whole Iberian

Table III. Multilocus microsatellite variation in the Western Andalusian honeybee populations. *N*, mean sample size; *n*, mean and standard error of the observed number of alleles; H_o and H_e are the observed and the expected heterozygosity, respectively. (Variación de microsatélites multilocus en las poblaciones de abejas de Andalucía occidental. *N* es el tamaño muestral medio; *n* es la media y el error estándar del número observado de alelos; H_o y H_e son respectivamente la heterocigosidad observada y la esperada).

Province	N	n	H_o	H_e
Malaga	8	4.67 ± 2.07	0.47 ± 0.07	0.64 ± 0.07
Cadiz	20	5.50 ± 1.38	0.43 ± 0.05	0.62 ± 0.07
Seville	14	4.50 ± 2.88	0.44 ± 0.06	0.51 ± 0.13
Huelva	6	4.00 ± 1.90	0.47 ± 0.08	0.62 ± 0.13

Peninsula (De la Rúa *et al.*, 1999, submitted; Cánovas *et al.*, 2002, unpubl. res.; Hernández-García *et al.*, 2002) is corroborating the hypothesis firstly put forward by Smith *et al.* (1991) based on the study of a few samples roughly representing a latitudinal transect of Iberia. De la Rúa *et al.* (submitted) have suggested that this geographic pattern must be the result of a natural process, as it is found across the whole Iberian Peninsula and could be hardly explained merely by recent human introductions of colonies from North Africa.

The percentage of the colonies bearing the A2 haplotype is 81.25 percent, the highest value within Iberian and Balearic honeybee populations (Garnery *et al.*, 1998a; De la Rúa *et al.*, 2001a). The fact that this haplotype is common in Iberia and some Mediterranean islands as far away as Greece (Garnery *et al.*, 1993) but is not common nowadays in North Africa, suggests that there was an ancient colonization of the Southern Mediterranean Basin by honeybees bearing the A2 haplotype. Afterwards, other colonizing waves from Africa might have increased the diversity of African haplotypes, as more than 10 haplotypes of this evolutionary lineage have been reported in the Iberian Peninsula to date (Franck *et al.*, 2001; De la Rúa *et al.*, unpubl. res.). Another interesting result is the finding of the haplotype A14, that was reported from Canarian populations (De la Rúa *et al.*, 1998, 2001b) and has also been found in the other Macaronesian archipelagos (De la Rúa *et al.*, in press). This haplotype and the A15 are characteristic of these Macaronesian archipelagos but it could

not be determined whether they are autochthonous or had a peninsular origin. The fact that they are found in Huelva, a province closely related to migration movements since the discovery of America, is a good support to the second hypothesis.

The presence of two colonies in Huelva with one haplotype (M7) belonging to the West European lineage is possibly due to recent beekeeping practices, as transhumance has become a frequent activity in the last twenty years in many Spanish regions, as well as the purchase of colonies to replace those ones destroyed by *Varroa destructor*. Castilla-León and Valencia are regions that rear and sell queens to other Spanish regions, and there the frequency of West European haplotypes is higher than 50 percent (De la Rúa *et al.*, submitted; Cánovas *et al.*, unpubl. res.).

The genetic variation in West Andalusia at the microsatellite *loci* is similar to that reported in African populations (Franck *et al.*, 1998), and higher than that found in other Spanish populations (Franck *et al.*, 1998; Garnery *et al.*, 1998b; De la Rúa *et al.*, 2001a, 2003). Also, the allelic diversity is higher in the Andalusian populations and similar to that of African subspecies. These observations are possibly due to the marked migratory behavior (swarming) of African populations (Franck *et al.*, 2001). This character has been thoroughly selected by Spanish beekeepers as a way to multiply the number of colonies in the apiaries, which is a common practice in Andalusia. The honeybee samples from Cadiz show a significant departure of the Hardy-Weinberg equili-

brium, although they are homogeneous at the mitochondrial level (90 percent of the analyzed colonies bear the A2 haplotype). This situation is perhaps due to a recent introduction of honeybee queens from other origin(s), having the same haplotype but different microsatellite alleles than those of the established colonies. Another possibility (that does not exclude the former) is that new alleles have been introgressed through the drones of transhumant colonies, located close to the established colonies during the mating season.

In conclusion, both mitochondrial and nuclear molecular markers indicate that honey bee populations of Western Andalusia have a clear genetic affinity to North African populations. While mitochondrial haplotypes are useful for indicating past evolutionary events (they are geographically stable because

of its maternal inheritance and the replacement of the old queen by her daughter), microsatellite *loci* are notably variable, and hence useful for showing recent population events.

ACKNOWLEDGEMENTS

This work was supported by the RZ00-013 (Spanish National Institute of Agricultural Investigation, INIA) and BABE (EVK-2000-00628, European 5th Framework) projects. P. de la Rúa has been supported by the COBICE program and Fundación Séneca. We thank all the beekeepers and Beekeeper Associations that have generously contributed with samples to perform this study. Also A.B. Jensen is acknowledged for her help in the handling of the ALF377 sequencer.

REFERENCES

- Cánovas, F., P. De la Rúa, J. Serrano y J. Galián. 2002. Variabilidad del ADN mitocondrial en poblaciones de *Apis mellifera iberica* de Galicia (NW España). *Arch. Zootec.*, 51: 441-448.
- Cornuet, J.-M. et J. Fresnaye. 1989. Étude biométrique de colonies d'abeilles d'Espagne et du Portugal. *Apidologie*, 20: 93-101.
- De la Rúa, P., J. Galián y J. Serrano. 1999. Variabilidad del ADN mitocondrial en poblaciones de abejas de la miel (*Apis mellifera* L.) de la Región de Murcia. *Invest. Agr: Prod. San. Anim.*, 14: 41-49.
- De la Rúa, P., J. Galián, J. Serrano and R.F.A. Moritz. 2001a. Molecular characterization and population structure of the honeybees from the Balearic Islands. *Apidologie*, 32: 417-427.
- De la Rúa, P., J. Galián, J. Serrano and R.F.A. Moritz. 2001b. Genetic structure and distinctness of *Apis mellifera* L. populations from the Canary Islands. *Mol. Ecol.*, 10: 1733-1742.
- De la Rúa, P., J. Galián, J. Serrano and R.F.A. Moritz. 2002. Microsatellite analysis of non-migratory colonies of *Apis mellifera iberica* from south-eastern Spain. *J. Zool. Syst. Evol. Res.*, 40: 1-5.
- De la Rúa, P., J. Galián, J. Serrano and R.F.A. Moritz. 2003. Genetic structure of Balearic honeybee populations based on microsatellite variation. *Gen. Sel. Evol.*, 35: 339-350.
- De la Rúa, P., J. Serrano and J. Galián. 1998. Mitochondrial DNA variability in the Canary Islands honeybees (*Apis mellifera* L.). *Mol. Ecol.*, 7: 1543-1547.
- Franck, P., L. Garnery, A. Loiseau, H.R. Hepburn, M. Solignac and J.-M. Cornuet. 2001. Genetic

MOLECULAR CHARACTERIZATION OF WEST-ANDALUSIAN HONEYBEES

- diversity of the honeybee in Africa: micro-satellite and mitochondrial data. *Heredity*, 86: 420-430.
- Franck, P., L. Garnery, M. Solignac and J.M. Cornuet. 1998. The origin of West European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data. *Evolution*, 52: 1119-1134.
- Garnery, L., P. Franck, E. Baudry, D. Vautrin, J.M. Cornuet and M. Solignac. 1998a. Genetic biodiversity of the West European honeybee (*Apis mellifera mellifera* and *A. m. iberica*). I. Mitochondrial DNA. *Gen. Sel. Evol.*, 30: 31-47.
- Garnery, L., P. Franck, E. Baudry, D. Vautrin, J.M. Cornuet and M. Solignac. 1998b. Genetic biodiversity of the West European honeybee (*Apis mellifera mellifera* and *A. m. iberica*). II. Microsatellite loci. *Gen. Sel. Evol.*, 30: 49-74.
- Garnery, L., E.H. Mosshine and J.M. Cornuet. 1995. Mitochondrial DNA variation in Moroccan and Spanish honey bee populations. *Mol. Ecol.*, 4: 465-471.
- Garnery, L., M. Solignac, G. Celebrano and J.M. Cornuet. 1993. A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structures of *Apis mellifera* L. *Experientia*, 49: 1016-1021.
- Goetze, G.K.L. 1964. Die Honigbiene in natürlicher und Künstlicher Zuchttauselese. Paul Parey, Hamburg.
- Hernández-García, R., B. Fernández, P. De La Rúa, J. Galián and J. Serrano. 2002. The effect of transhumance on the genetic variability of *Apis mellifera iberica* from Murcia (Southeast Spain). In: Jones, R (ed) Bees without frontiers. pp. 96-103. IBRA pub., Cardiff.
- Izquierdo, J., A. Domínguez, J. Albornoz y E. Santiago. 1985. Discriminación entre poblaciones de abejas (*Apis mellifera*) de Asturias y la Submeseta Norte. *Bol. C. Nat. IDEA*, 35: 87-101.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci., USA*, 70: 3321-3323.
- Orantes-Bermejo F.J. and P. García-Fernández. 1995. Morphological variability of *Apis mellifera iberica* in different apiaries of southern Spain. *J. Apic. Res.*, 34: 23-30.
- Padilla Álvarez, F., R. Hernández-Fernández, J. Reyes López, F. Puerta Puerta, J.M. Flores Serrano y M. Bustos. 1998. Estudio morfológico de las abejas melíferas del Archipiélago Canario (Gran Canaria, Tenerife, La Palma, Gomera). *Arch. Zootec.*, 47: 451-459.
- Padilla Álvarez F., M.J. Valerio Da Silva, F. Campano Cabanes, E. Jiménez Vaquero, J.M. Flores Serrano, F. Puerta Puerta y M. Bustos Ruiz. 2001. Discriminación entre poblaciones de abejas (*Apis mellifera* L.) del sur de España, centro de Portugal y Madeira. *Arch. Zootec.*, 50: 79-89.
- Ruttner, F. 1988. Biogeography and taxonomy of honeybees. pp: 163-257. Springer-Verlag, Berlín.
- Schneider, S., J-M. Kueffer, D. Roessli and L. Excofier. 1997. Arlequin: a software for population genetic data analysis, Version 1.1. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.
- Sheppard, W.S., T.E. Rinderer, M.D. Meixner, H.R. Yoo, J.A. Stelzer, N.M. Schiff, S.M. Kamel and R. Krell. 1996. *HinfI* variation in mitochondrial DNA of old world honey bee subspecies. *J. Hered.*, 87: 35-40.
- Smith, D.R., M.F. Palopoli, B.R. Taylor, L. Garnery, J.M. Cornuet, M. Solignac and W.M. Brown. 1991. Geographical overlap of two mitochondrial genomes in Spanish honeybees (*Apis mellifera iberica*). *J. Hered.*, 82: 96-100.
- Walsh, P.S., D.A. Metzger and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, 10: 506-513.

Recibido: 19-2-04. Aceptado: 27-9-04.