Apidologie 37 (2006) 699–708
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DOI: 10.1051/apido:2006044

Original article

Molecular characterization and population structure of *Apis mellifera* from Madeira and the Azores*

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Received 23 June 2005 – Revised 31 January 2006 – Accepted 3 March 2006

Abstract – Mitochondrial and microsatellite variation were analyzed in honeybee populations from the Madeiran and Azorean archipelagos. The most frequent mitochondrial haplotypes corresponded to the A_{III} subset of the African evolutionary lineage of *A. mellifera*. Genetic variability of these island populations was analyzed in relation to Canarian and continental (Morocco, Portugal and southern Spain) honeybee populations. Island and continental populations were genetically differentiated. Microsatellite analyses supports (i) the distinctness of the Macaronesian honeybee populations, and (ii) the close relationship between Macaronesian and NW African populations. Recent introgression events due to apicultural practices were detected, possibly changing the genetic structure of locally adapted populations.

***Apis mellifera* / Madeira / Azores / mtDNA / microsatellites / biogeography / population genetics**

1. INTRODUCTION

Morphological and molecular studies have been carried out on different island populations of the honeybee *Apis mellifera* L. (Balearic Islands: Radloff et al., 2001; De la Rúa et al., 2001b, 2003; Canary Islands: Padilla-Alvarez et al., 1997; De la Rúa et al., 1998, 2001a, 2002; Greek Islands: Garnery et al., 1993; Malta: Sheppard et al., 1997; Sardinia and Corsica: Franck et al., 2000; Sicily: Sinacori et al., 1998; Franck et al., 2000). Genetic analysis of the Canarian honeybee populations revealed that these populations (De la Rúa et al., 1998, 2001a, 2002) were differentiated from continental populations and showed a closer relationship to Moroccan than to Iberian populations, although morphological analyses demonstrate the affinity between Canarian and Iberian populations (Ruttner, 1988; Padilla-Alvarez et al., 1997). De la Rúa et al. (2001a) suggested that the honeybees from the Canary Islands were originally derived through early founder events from a stock having an African origin and, thereafter underwent genetic differentiation due to isolation from continental populations. The same can be inferred from the rough data of Franck et al. (2001, Fig. 1) about the honeybees from Cape Verde Islands, which bear mitochondrial haplotypes similar to those found in the neighbouring African coast.

The colonisation of Canarian and Cape Verde archipelagos possibly occurred during late Pleistocene when honeybees reached the Atlantic coast of Africa (Ruttner, 1988; Garnery et al., 1992; Arias and Sheppard, 1996). By that time, Madeira and the Azores had probably attained their present configuration (Galopin de Carvalho and Brandão, 1991; Borges, 1992; Carracedo, 1994). However, it is uncertain whether these last two archipelagos

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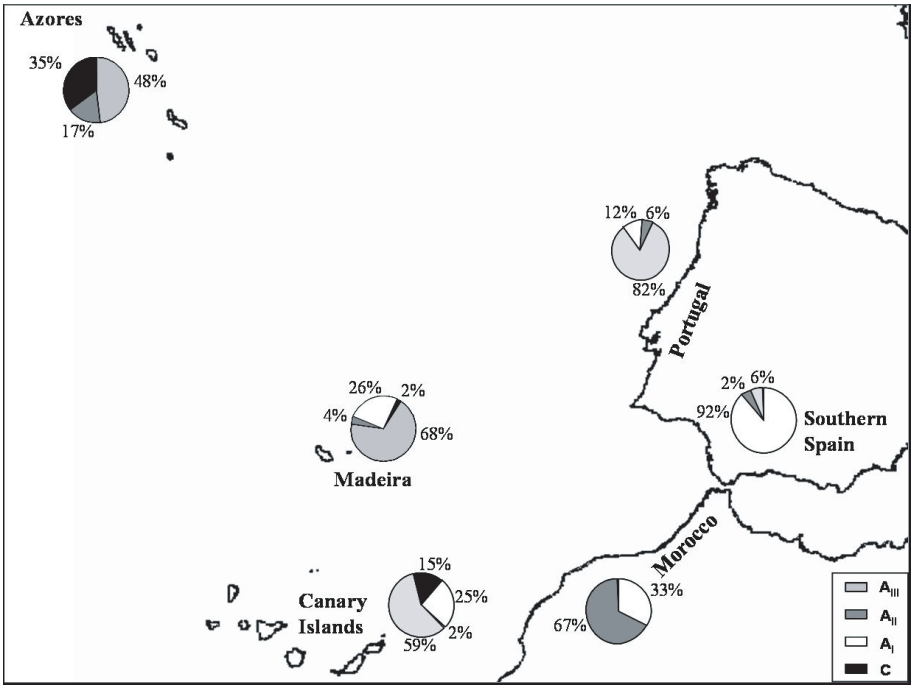

Figure 1. Map showing the location of the sampled islands and the distribution of the evolutionary lineages and sublineages. Data for the Canary Islands (De la Rúa et al., 2001a), Morocco, Southern Spain and Portugal (Franck et al., 1998) are also included.

were also naturally colonised by honeybees because of their distance to the mainland. Madeira is 668 km from the African coast and includes four islands of volcanic nature originated from the Middle Atlantic Ridge in the early Tertiary (50–60 MY). The Azores are 1280 km from Portugal, are not older than 8 MY and consist of nine main and about a dozen small islands also originating from the volcanic activity of the Middle Ridge. Current knowledge indicates that Macaronesian archipelagos are related in their flora and fauna (Borges, 1992; Machado, 1995).

Many of the molecular studies of the honeybee have been based on the analysis of the mitochondrial DNA molecule, especially the region between the $tRNA^{leu}$ and COII genes that contains a non-coding sequence with two sequence elements: P, showing several forms (P, P_0 and P_1) and Q (Garnery et al., 1993). The arrangement of these elements and the sequence polymorphism yielded to the description of more than 60 mitochondrial haplotypes from honeybee populations around the Old World (Garnery et al., 1993, 1995, 1998; Franck et al., 1998, 2000; De la Rúa et al., 2001a, b). Most of the evolutionary lineages defined by Ruttner (1988) on morphological grounds are also characterized by the presence and frequency of different mitochondrial haplotypes. However, this differentiation is not addressable in the case of the morphological O lineage (including *A. m. caucasica*), that is not differentiated from C lineage subspecies as *A. m. ligustica*, based on this same mitochondrial analysis. The mitochondrial African A lineage (corresponding to almost all African subspecies) has been further divided in three different sublineages (Franck et al., 2001), according to the presence and frequency of particular haplotypes and their geographical distribution. The haplotypes of the African sublineage A_{III} show a high frequency on the honeybee populations from the Canary Islands (De la Rúa et al., 1998), and have also been reported in Portugal (Franck et al., 1998).

Table I. Haplotype frequency (%) and unbiased estimates of haplotype diversity (D) and sampling variance in the honeybee populations of the Azores and Madeira (N is the sample size).

Island	N	African Lineage										East European	D
		A_I			A_{II}		A_{III}					C	
		A1	A2	A4'	A9	A10	A11	A14	A15	A16	A20	C1	
Azores	50				4	13		42		6		35	0.69 ± 0.04
Madeira	48	20	4	2		4	6	2	48	10	2	2	0.73 ± 0.05

The aim of this study was to determine the distribution of mitochondrial haplotypes of honeybee populations from the Azores and Madeira, and to investigate their genetic structure through the analysis of microsatellite markers. The comparison of these results with those obtained in continental populations (Iberian Peninsula, Morocco) and the Canary Islands, may help in understanding the history of the honey bee colonization on the Macaronesian archipelagos.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 98 colonies were sampled in 2001 from 32 apiaries on Madeira (Madeira) and 16 apiaries on São Miguel (Azores) (Fig. 1, Tab. I). Bees were killed by immersion in absolute ethanol and kept at –20 °C until they were processed in the laboratory. One worker bee per colony was analyzed for both mitochondrial and microsatellite markers.

2.2. Molecular analyses

DNA isolation was performed following the Chelex method (Walsh et al., 1991) with slight modifications. One μL of this solution was used for the PCR amplification.

The $tRNA^{leu}$-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 in a total volume of 12.5 μL. The size of the PCR-amplified products was determined after the electrophoretic separation on 1.5% agarose gels.

Ten μL aliquots of the PCR products were digested with five units of the *Dra*I enzyme. The restriction reactions were kept in a waterbath at 37 °C for 4–12 h and the resulting fragments were visualized on 8% acrylamide gels (*Dra*I digestion) and stained with ethidium bromide or silver (Merril et al., 1981). Samples of each different haplotype found were sequenced to confirm its identity to haplotypes already published (De la Rúa et al., 1998; Franck et al., 2001). In these cases, both strands of the PCR fragments were sequenced on an automated DNA sequencer (Applied Biosystem) at the sequencing service of the CSIC (Consejo Superior de Investigaciones Científicas, Madrid).

Six polymorphic microsatellite loci were analysed: B124, A113, A7, A24, A28 and A8 (Estoup et al., 1995; Franck et al., 1998). Multiplex PCR reactions were performed when the annealing temperature and the $MgCl_2$ concentration coincided. The reactions were done with fluorescent-labelled 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).

2.3. Data analysis

Unbiased estimates and standard deviations of mtDNA gene diversity (Nei and Tajima, 1981) were calculated using the ARLEQUIN version 2.0 software (Schneider et al., 1997). The Excel Microsatellite Toolkit (http://animalgenomics.ucd.ie/sdepark/ms-toolkit/) was used to obtain population parameters and the files for the statistical packages. Summaries of the allelic pattern were done by the GeneAlex package (Peakall and Smouse, 2005). 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).

To gain additional information about the genetic relationships of the Macaronesian honeybee populations with respect to continental populations, data

from the Canary Islands (De la Rúa et al., 2001a) and Portugal, Southern Spain and Morocco (Franck et al., 1998) were included in the analyses.

Pairwise FST values based on the microsatellite variation were calculated as a short-term genetic distance between pairs of island and continental populations of *A. mellifera*. The distance method of pairwise difference with 10100 permutations as implemented by the ARLEQUIN software was used for these purposes (Schneider et al., 1997). The neighbor-joining method (Saitou and Nei, 1987) and the chord distance of Cavalli-Sforza and Edwards (1967) were used to obtain an unrooted tree with bootstrap values over 2000 iterations of the microsatellite data set (Hedges, 1992) using the PHYLIP package (version 3.5c, Felsenstein, 1993).

3. RESULTS

3.1. Haplotype and evolutionary lineages description, distribution and frequency on Madeira and the Azores

The *Dra*I analysis revealed 11 haplotypes in the honeybee populations of Madeira and the Azores (Tab. I). Ten of these haplotypes belong to the African lineage as they have the P_0 or P_1 sequences with 68 and 51 bp (P_1 has a 17 bp deletion in the 3'end of sequence P, see De la Rúa et al., 1998 for more details), respectively. Haplotypes of three African sublineages (A_I, A_{II} and A_{III}) as described by Franck et al. (2001) were found in these samples. Haplotypes A1, A2 and A4' with one, two and three Q sequences, respectively, correspond to the A_I sublineage as they display a restriction site at the beginning of the first Q sequence. Haplotypes A9 and A10 with two and three copies, respectively, of the Q region are members of the AII sublineage. Haplotypes A20 (with one Q), A11 and A14 (with two Qs) and A15 and A16 (with three Qs), are characteristic of the A_{III} sublineage as they bear the P_1 sequence in the intergenic region. Haplotype C1, the most widespread in the eastern European subspecies, was found in both archipelagos. No differences from the published sequence data were found in the sequenced samples.

The frequency and distribution of each haplotype and sublineage per archipelago are summarised in Table I and Figure 1. Ten haplotypes corresponding to three African sublineages were observed on Madeira; A15 is the most frequent (almost 50% of the colonies). In the Azorean population, we found only four different haplotypes of two African sublineages. Haplotypes A14 and A16 occurred in an overall frequency of 48%. The A15 haplotype commonly found on Madeira and the Canaries was not found on Azores. However, the population from the Azores showed a high frequency of colonies (35%) with the eastern European C haplotype, particularly on the southeast part of São Miguel, where the proportion of C1 was 55%.

The unbiased estimates of haplotype diversity and the sampling variance for the studied island populations was calculated for each archipelago, including those bees with the East European haplotype (Tab. I). The values were similar to those found on the Canaries (De la Rúa et al., 2001a), and Sicily (Franck et al., 2000) and slightly lower than those reported for continental Iberian and Moroccan colonies (Franck et al., 1998) in relation to a similar sample size.

3.2. Honeybee population structure on Madeira and the Azores

Genetic analyses of the honeybee workers collected from the Azores and Madeira revealed considerable microsatellite polymorphism as shown in Table II. The number of alleles per microsatellite locus ranged from 2 to 9. The average gene diversity varied between 0.34 ± 0.11 found on the Azores and 0.47 ± 0.11 on Madeira. The allele variation values were higher on the Azores (4.33 ± 2.73) than on Madeira (4.17 ± 0.11).

Nine significant departures from Hardy-Weinberg equilibrium were detected among 12 (6×2) locus per population combinations, when only one was expected by chance at the 5% level. Five significant tests were observed on Madeira and four on the Azores. The Hardy-Weinberg equilibrium was analyzed per population on each island. In the case of the

Table II. Number of alleles per locus (Na) and estimates of the observed (Ho) and expected (He) heterozygosities for six microsatellite loci in the honeybee populations from Madeira and the Azores.

Population	B124	A113	A7	A24	A8	A28	Average
Madeira							
Na	3	6	3	6	2	5	4.17 ± 1.72
Ho	0.714	0.391	0.217	0.136	0.000	0.300	0.29 ± 0.04
He	0.643	0.644	0.302	0.704	0.071	0.341	0.47 ± 0.11
Azores							
Na	6	9	3	2	2	4	4.33 ± 2.73
Ho	0.500	0.538	0.242	0.023	0.000	0.042	0.22 ± 0.03
He	0.634	0.663	0.216	0.022	0.198	0.289	0.34 ± 0.11

Azores, the population with the highest frequency of C1 haplotype showed the largest deviation of the Hardy-Weinberg equilibrium. On Madeira the largest deviation was detected in the population with many samples bearing haplotypes characteristic of Portuguese populations (A11 and A16).

Exact test for linkage disequilibrium resulted in one significant value ($P = 0.005 \pm 0.00049$, loci A8-A28 on the Azores) out of 15 pairwise comparisons, which was expected to occur by chance at the 5% level. The probability values given by the Fisher's method for each locus pair across all populations resulted in one significant value out of 9, corresponding to the combination of the loci A8 and A28. Fisher's exact test for multilocus genic and genetic differentiation indicates that differentiation between the island populations was highly significant.

3.3. Genetic relationships among the Macaronesian and continental populations

We assume that the colonies bearing the C lineage haplotype are recently founded populations derived from imported queens of the *A. m. ligustica* subspecies and the Buckfast strain. This hypothesis is based on the assumption that bees of the C lineage did not naturally exist either on these archipelagos or in the putative Iberian sources (i.e., the Portuguese colonies from which bees were imported since the XIV century, when the archipelagos became inhabited). In fact, C haplotypes are very rare in the Iberian Peninsula (De la Rúa et al., 2005; Cánovas et al., unpubl. data; 0.26% of 2445 investigated colonies covering the whole Peninsula), and their occurrence is often associated to verbal reports of importations by beekeepers. For this reason, colonies bearing the C haplotype are considered inadequate to investigate the historical relationships between the honeybee populations of Madeira and the Azores and those from the Canary Islands, North Africa and the Iberian Peninsula. Hence, we have excluded them from the analyses that investigate these relationships.

Microsatellite pairwise Fst values were calculated among the Azores, Madeira and the Canary archipelagos and the mainland: Morocco, Southern Spain and Portugal, for assessing the extent of genetic differentiation among the Macaronesian islands and continental honeybee populations (Tab. III). From all possible comparisons, two population pairs (Madeira-Azores and Southern Spain-Portugal) were not significantly different. The Fst values, as an estimate of genetic distance, revealed a weak differentiation between Madeira and Azores, Portugal and Southern Spain. The distance values obtained between the island populations and Morocco were lower than to the Portuguese and Southern Spanish populations. Similar population relationships were depicted from the neighbor-joining algorithm and the Cavalli-Sforza and Edwards distance based on the microsatellite variation (data not shown).

4. DISCUSSION

This study shows that the honeybee populations on the Macaronesian islands are

Table III. Population pairwise F_{ST} based on the microsatellite variation (*not significant at 0.05 level). Data for the Canary Islands (De la Rúa et al., 2001a), Morocco, Southern Spain and Portugal (Franck et al., 1998) are also included.

	Azores	Madeira	Morocco	S. Spain	Canaries
Madeira	0.011*				
Morocco	0.059	0.042			
S. Spain	0.084	0.067	0.022		
Canaries	0.081	0.070	0.042	0.068	
Portugal	0.084	0.070	0.024	0.016*	0.069

characterised by a predominance of mitochondrial haplotypes belonging to the African sublineage A_{III} (Franck et al., 2001), namely A11, A14, A15, A16 and A20. These haplotypes bear the P_1 sequence and were first described in honeybee colonies from the Canary Islands (De la Rúa et al., 1998, 2001a); lately they have been found in the Atlantic coast of Portugal (Frank et al., 1998; Garnery et al., 1998). The geographic distribution of this sublineage was possibly wider than previously thought, as Franck et al. (2001) found the A14 haplotype in *Apis mellifera adansonii* populations from Namibia. Likewise, we found the A15 haplotype in a few samples from southern Morocco and the Cape Verde archipelago (De la Rúa et al., unpubl. data). The high frequency of this haplotype relates Madeira and the Canary Islands (these two archipelagos also share the A20 haplotype), whereas Madeira and the Azores share the A16 haplotype with Portugal. The Archipelago of Madeira is farther (668 km) from the mainland than the Canary Islands (104 km), but there are not significant differences between them in climate, ecological diversity or volcanism, and both archipelagos probably attained their present-day configuration during the late Pleistocene when *Apis mellifera* had already colonised Africa (Ruttner, 1988; Garnery et al., 1992; Arias and Sheppard, 1996). Thus, haplotype differences between Madeira and the Canaries are better explained by both natural and man-made influences, as each archipelago has likely undergone a different history of hive importation after the respective Portuguese and Spanish occupation of these islands in mid XV century. Samples of Portuguese honeybee populations were carried to Madeira and the Azores whereas the Canaries were enriched with hives of Spanish (possibly Andalusian) populations transported by colonizers.

Overall, Macaronesian honeybee populations are characterized by lower mitochondrial variability compared to continental populations of similar size (data from Franck et al., 1998). The finding of particular mtDNA haplotypes in high frequencies suggests an ancient colonization of Madeira and the Canaries with a moderate degree of local differentiation on each archipelago. The occurrence of posterior colonization events is suggested by the finding of haplotypes belonging to other sublineages, but in low frequencies. Alternatively both observations could be the result of genetic drift.

The relationships among the Macaronesian honeybee populations based on mtDNA haplotypes and the results of the microsatellite analyses (Fst values and N-J tree) support a hypothesis of a natural colonisation of the Canarian and Madeiran archipelagos from continental African stocks. However, the possibility of an Iberian origin of some distinctive haplotypes (A14, A15) found in these Macaronesian archipelagos cannot be completely rejected as they have been also found in southern Spain (De la Rúa et al., 2004). It should be also noted that in the case of the Azores, these islands are 1280 km from the European mainland, making natural colonisation by feral honeybees difficult. Biometrical analyses carried out by Padilla-Alvarez et al. (2001) showed that Madeiran populations were closely related to others located at the head of the Guadalquivir Basin in Southern Spain (Cazorla, NE Andalusia), and, to a lesser extent, to some Portuguese samples. Likewise, Afonso et al. (1990) proposed that Macaronesian populations of *Drosophila subsoscura* are more related to European than to

African populations. In the case of the bumblebee, *Bombus terrestris*, Widmer et al. (1998) showed that Madeiran populations were more similar to continental populations than those from the Canaries.

Recent importations are shown by the finding of the C mitochondrial haplotype in our samples. This haplotype occurs in most European populations, but is characteristic of honeybee subspecies from the northern Mediterranean through the eastern range of the species, including the commercially important and widely spread *A. m. ligustica*, *A. m. carnica* and the Buckfast strain. This is consistent with the Hardy–Weinberg results based on the microsatellite variation, as deviations of the equilibrium could be due to the introduction of individuals coming from other populations and/or the pooling of two different populations. Native beekeepers of the archipelagos indicated us that they bought mated Italian queens for getting less aggressive and more productive colonies; they also suspect that foreign beekeepers newly arrived to the islands brought bees with them that they had been working with in their native countries. This human influence may have affected the survival of locally adapted honeybee populations, whose gene pools could be mixed with those of imported queens. The impact of this introgression has been analyzed in detail in the honeybee population of Tenerife (De la Rúa et al., 2002), which showed the need of regulating this practice to protect local ecotypes.

In summary, although the existence of ancient honeybee populations in the Macaronesian archipelagos (except on the Azores) is supported by their distinctive haplotype composition, historical and recent importations from Portugal and Spain and from other races probably changed the former situation. This evolutionary scenario should be better assessed with a detailed analysis of honeybee populations along the Atlantic coast of Portugal, southwest Spain and Morocco. Further analyses will improve our knowledge of the gene pool of Macaronesian honeybee populations, allow us to gain a better understanding of their evolution and biodiversity, and establish the genetic basis to support a productive beekeeping compatible with the protection of adapted ecotypes.

ACKNOWLEDGEMENTS

We thank the beekeepers who kindly provided samples and to E. González who managed the sampling. Thanks are also due to F. Cánovas, B. Fernández, R. Hernández and A.B. Jensen for helping during the lab work. This work has been supported by projects HP-2004-88 and Programa Ramón y Cajal (Ministerio de Educación y Ciencia). P. De la Rúa has been supported by the COBICE program and Fundación Séneca.

Résumé – Caractérisation moléculaire et structure des populations d'*Apis mellifera* de Madère et des Açores. L'histoire de l'évolution de l'Abeille domestique (*Apis mellifera* L.) en Méditerranée occidentale et sur les archipels macaronésiens est probablement le résultat d'événements complexes de colonisation, d'extinction et d'influences humaines au cours du Pléistocène, lorsque *A. mellifera* a agrandi sa répartition le long de 2 ou 3 voies depuis le Moyen-Orient vers l'ouest (Ruttner, 1988 ; Garnery et al., 1992 ; Arias et Sheppard, 1996). De ce point de vue l'une des questions les plus importantes est l'origine des populations d'abeilles que l'on trouve actuellement sur les archipels macaronésiens. On sait que les populations indigènes des Canaries exploitaient déjà les abeilles lorsque les conquérants espagnols sont arrivés sur les îles au début du 16^{e} siècle, ce qui renvoie à une origine naturelle de ces populations d'abeilles. De la Ruà et al. (2001a) ont montré que les abeilles des Canaries formaient une sous-lignée bien définie de la lignée évolutive africaine et qu'elles étaient plus étroitement apparentées aux populations marocaines qu'aux ibériques. On en a conclu que les abeilles des Canaries avaient probablement une origine africaine avec un certain degré de différenciation dû à l'isolement par rapport aux populations continentales. Notre étude visait à obtenir de nouvelles données sur l'histoire évolutive des abeilles macaronésiennes au moyen d'une analyse moléculaire de la composition génétique et de la structure de ces populations. L'haplotype mitochondrial et la variation à 6 microsatellites ont été analysés chez 98 ouvrières issues de 48 colonies réparties en 32 ruchers sur Madère et de 50 colonies réparties en 16 ruchers sur l'île principale des Açores. 69 % des colonies de Madère et 48 % celles des Açores appartenaient à la sous-lignée africaine A_{III}. Les haplotypes les plus courants trouvés à Madère (A15, 48 %) et sur les Açores (A14, 42 %) (Tab. I) ont été trouvés également à une fréquence élevée

aux Canaries, tandis que l'haplotype A16, trouvé sur les 2 archipels, a été observé au Portugal. La structure des populations, déduite de la variation des microsatellites, suggère qu'elles ont subi des introgressions à l'époque historique et récemment par des abeilles de diverses origines. Ceci concorde avec les données mitochondriales, qui montrent que 35 % des abeilles des Açores portent l'haplotype mitonchondrial caractéristique des sous-espèces appartenant à la lignée évolutive C, et avec la présence d'haplotypes caractéristiques des populations d'abeilles portugaises. Les données moléculaires publiées pour les Canaries, le sud de l'Espagne, le Portugal et le Maroc ont été utilisées pour établir les relations entre populations au sein des îles macaronésiennes, entre elles et avec les populations continentales proches. Les résultats suggèrent une relation étroite entre les populations macaronésiennes et les africaines, probablement due à 2 événements (ou plus) de colonisation à partir du continent. Mais ces événements sont partiellement masqués par les introductions faites par l'Homme depuis le Portugal pour Madère et les Açores et depuis l'Espagne pour les Canaries.

***Apis mellifera* / biogéographie / génétique des populations / microsatellite / ADNmt / Madère / Açores**

Zusammenfassung – Molekulare Charakterisierung und Populationsstruktur der auf Madeira und den Azoren vorkommenden Honigbienen, *Apis mellifera*. Die Entwicklungsgeschichte der Honigbienen in westlichen Mittelmeergebieten und auf der makaronesischen Inselkette ist vermutlich das Ergebnis komplexer Kolonisierungsereignisse, begleitet sowohl vom Aussterben von Populationen als auch menschlich bedingten Eingriffen im Pleistozän, als sich *Apis mellifera* vom Nahen Osten aus in drei Richtungen ausbreitete (Ruttner, 1988; Garnery et al., 1992; Arias und Sheppard, 1996). Eine der wichtigsten Fragen in diesem Zusammenhang ist der Ursprung der Honigbienenpopulationen auf der makaronesischen Inselkette. Auf den kanarischen Inseln wurden Honigbienen bereits lange vor dem Eintreffen der Spanier zu Beginn des XVI Jahrhunderts ausgebeutet. Dies weist auf einen natürlichen Ursprung dieser Honigbienenpopulationen hin. De la Rúa et al. (2001a) zeigten, dass die kanarischen Honigbienen eine gut definierte Unterlinie der afrikanischen Evolutionslinie darstellen und dass sie enger mit den marokkanischen als mit den iberischen Populationen verwandt sind. Daraus wurde auf einen afrikanischen Ursprung der kanarischen Honigbienen geschlossen, gefolgt von einer isolationsbedingten genetischen Differenzierung von den kontinentalen Populationen. Ziel dieser Studie war die Gewinnung neuer Einsichten in die Entwicklungsgeschichte der makaronesischen Honigbienen mittels einer molekularen Analyse der genetischen Zusammensetzung und der Strukturierung der auf Madeira und den Azoren vorkommenden Populationen. Der mitochondriale Haplotyp und die Variabilität an sechs Mikrosatellitenloci wurde an 98 Arbeiterinnen (von 48 Völkern aus 32 Bienenständen auf Madeira und von 50 Völkern aus 16 Bienenständen auf den Azoren) gefunden. Die afrikanische Unterlinie A_{III} wurde in 69 % der Völker von Madeira und bei 48 % der Völker von den Azoren gefunden. Die häufigsten Haplotypen auf Madeira (A15, 48 %) und auf den Azoren (A14, 42 %) (Tab. I) wurden auch mit hohen Frequenzen in Bienenproben der Kanaren gefunden, wohingegen der auf der Inselkette ebenfalls vorkommende Haplotyp A16 vor allem in Portugal zu finden ist. Die aus den Mikrosatelliten erschlossene Populationsstruktur weist sowohl auf weit zurückliegende als auch solche neuere Introgressionsereignisse von Honigbienen unterschiedlichen Ursprungs hin. Damit übereinstimmend sind mitochondriale Daten, die zeigen dass 35 % der Honigbienen auf den Azoren einen mitochondrialen Haplotyp aufweisen, der für Unterarten der C-Entwicklungslinie charakteristisch ist, ebenso wie das Vorkommen von Haplotypen, die portugiesische Honigbienen auszeichnen. Wir verwendeten bereits veröffentlichte molekulare Daten, um die Populationsbeziehungen zwischen den makaronesischen Inseln, sowie die dieser Inselpopulationen mit den benachbarten Festlandpopulationen aufzuklären. Die Ergebnisse weisen auf eine enge Verwandschaft zwischen den makaronesischen und den afrikanischen Populationen hin, die vermutlich auf zwei (oder mehr) Kolonisierungsereignisse vom Festland aus zurückgehen. Diese Ereignisse sind jedoch teilweise überdeckt durch menschlich bedingte Einfuhren von Bienen verschiedenen Ursprungs: im Fall von Madeira und den Azoren kamen sie aus Portugal und im Fall der Kanarischen Inseln kamen sie aus Spanien.

***Apis mellifera* / Madeira / Azoren / mtDNA / Mikrosatelliten / Biogeographie / Populationgenetik**

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