TABLE 1. Mean values and standard deviations (s.d.) of potassium content in different types of honey (n = number of samples).

	Botanical origin		
	honeydew from *Metcalfa pruinosa*	multifloral	*Robinia pseudoacacia*
Mean (mg/kg)	4106	1158	282
s.d. (mg/kg)	1104	544	157
n	66	113	42

Potassium content in *M. pruinosa* honeydew honey was determined to have a Gaussian distribution ($P < 0.001$) centred around a value of 128 Bq/kg (corresponding to 4.1 g/kg stable K) (fig. 1). The mean values and the standard deviations of ^{40}K content in the different types of honey are reported in table 1; as expected, the *M. pruinosa* honeydew honey has a much greater K content than that observed in the other types of honey produced in the same area ($P < 0.01$).

By measuring electrical conductivity in *M. pruinosa* honeydew honey (Barbattini *et al.* 1991) and applying the correlation between electrical conductivity and total ash content (Accorti *et al.* 1987), the mean value of ash for *M. pruinosa* honeydew honey was estimated to be approximately 8.6 g/kg.

The K content in *M. pruinosa* honeydew honey was estimated to be about 50% of the total ash content. This value agrees with that reported by Sabatini (1991) for the Italian honeydew honeys. This investigation confirmed that K content is greater in honey made from *M. pruinosa* honeydew than in other honeys produced in northern Italy. Our test also demonstrated that gamma spectrometry is a valid method to quantify potassium in honey.

REFERENCES

The numbers given at the end of references denote entries in *Apicultural Abstracts*.

ACCORTI, M; PIAZZA, M G; PERSANO ODDO, L (1987) La conductivité électrique et le contenu en cendre du miel. *Apiacta* 22: 19–20.

BARBATTINI, R; GREATTI, M; IOB, M; SABATINI, A G; MARCAZZAN, G L; COLOMBO, R (1991) Osservazioni su *Metcalfa pruinosa* (Say) e indagine sulle caratteristiche del miele derivato dalla sua melata. *Apicoltore* No. 7: 113–135. 1226/93

BARBATTINI, R; GREATTI, M; IOB, M; SABATINI, A G; MARCAZZAN, G L; COLOMBO, R (1997) Apiarian interest of *Metcalfa pruinosa* (Say): production and physicochemical characteristics of honey derived from its honeydew. *In* Apimondia (eds) *Proceedings of the XXXIII International Congress of Apiculture, Beijing, 1993*. Apimondia Publishing House, Bucharest, Romania; pp 240–243.

GIOVANI, C; PADOVANI, R; BARBATTINI, R; FRILLI, F; IOB, M (1993) Radiocaesium distribution in Friuli-Venezia Giulia through spectrometry measurements of honey samples. Proceedings of the VII Congress Nazionale AIFB, Ancona (Italy), 1992. *Physica Medica* 9 (supplement 1): 292–294.

SABATINI, A G (1991) Il miele: origine, composizione e proprietà. *L'Italia Agricola* 128(1): 151–160.

SABATINI, A G; SPINA, D (1972) Il miele di foresta (o di melata). *Monti e Boschi* 23(4): 29–38. 945/77

ZANGHERI, S; DONADINI, P (1980) Comparsa nel Veneto di un Omottero neartico: *Metcalfa pruinosa* Say (*Homoptera, Flatidae*). *Redia* 63: 301–304.

Journal of Apicultural Research 37(1): 49–51 (1998)

CONGRUENCE OF RAPD AND MITOCHONDRIAL DNA MARKERS IN ASSESSING *VARROA JACOBSONI* GENOTYPES

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(Received 5 January 1998, accepted subject to revision 9 January 1998, accepted for publication 14 January 1998)

Keywords: *Varroa jacobsoni*, genotypes, RAPD, mitochondrial DNA, restriction endonuclease, CO 1 region

Different genotypes of varroa have been described which may explain reported differences in the degree of virulence on infested hosts. Delfinado-Baker (1988) designated three biotypes of varroa based solely on reports of injuries caused by the mites and mite behaviour. Molecular variation in varroa populations showed differences between mites collected from Brazil and Germany based on isozyme structure (Issa, 1989, Rosenkranz *et al.*, 1989). Kraus and Hunt (1995) showed that German mites can be distinguished from US mites, and that both western groups of mites could be distinguished from Malaysian mites using random amplified polymorphic DNA (RAPD). Recent research suggests that the genotype of the *Varroa jacobsoni* that has spread nearly worldwide is not the type that was first described from Java by Oudemans (1904). The mtDNA CO 1 gene sequences of earlier collections of mites from *Apis mellifera* and *A. cerana* in Java were found to be identical to the sequence of varroa collected from Papua New Gunea (PNG), but different from the sequence of mites from Germany (Anderson & Fuchs, 1998). German mites have similar RAPD banding patterns to those mites collected from Russia, Morocco, other parts of Europe and the USA (Russian pattern), while mites collected from Japan, Brazil and Puerto Rico are of another genotype (Japanese pattern) (Guzman et *al.*, 1997).

We investigated genotypes of *V. jacobsoni* using two molecular techniques. DNA extracts from mites collected from the USA, Europe, Morocco, Russia, Brazil and Puerto Rico from *A. mellifera* colonies, and Japan from *A. mellifera* and *A. cerana japonica* colonies were analysed using two procedures. First, OPE-07 was used to establish the two genotypes of varroa as described by Guzman et *al.* (1997). Then, using the same DNA extracts, a region of the mtDNA cytochrome oxidase subunit 1 (CO 1) gene was amplified and the PCR products were then digested with *Xho I* and *Sst I* restriction endonucleases, and visualized in 1.5% agarose gels (Anderson & Fuchs, 1998).

The two procedures showed consistent differences between the Japanese and Russian types of varroa established by Guzman et *al.* (1997) (table 1). Using the RAPD marker OPE-07, varroa populations from Japan, Brazil and Puerto Rico lacked a 766 bp band found in mites from Russia, Morocco, Europe and the USA (fig. 1). Amplification of the CO 1 region of the mitochondria showed differences between the two types only when digested with *Sst I*. Using *Xho I*, both Russian and Japanese types have two bands at 269 and 300 bp. *Sst I* produced two bands at 236 and 338 bp in the Japanese type, similar to the pattern established by Anderson and Fuchs (1998) for PNG mites using *Sac I*, which is also known as *Sst I*. The Russian type had a single band located at 519 bp, a pattern similar to the German mites using *Sst I* and to PNG mites using *Xho I* (Anderson & Fuchs, 1998). Thus, the Japanese type can be distinguished from PNG mites with *Xho I* but not with *Sst I*.

These findings indicate that there are at least three types of varroa (Japanese, Russian, PNG) which can

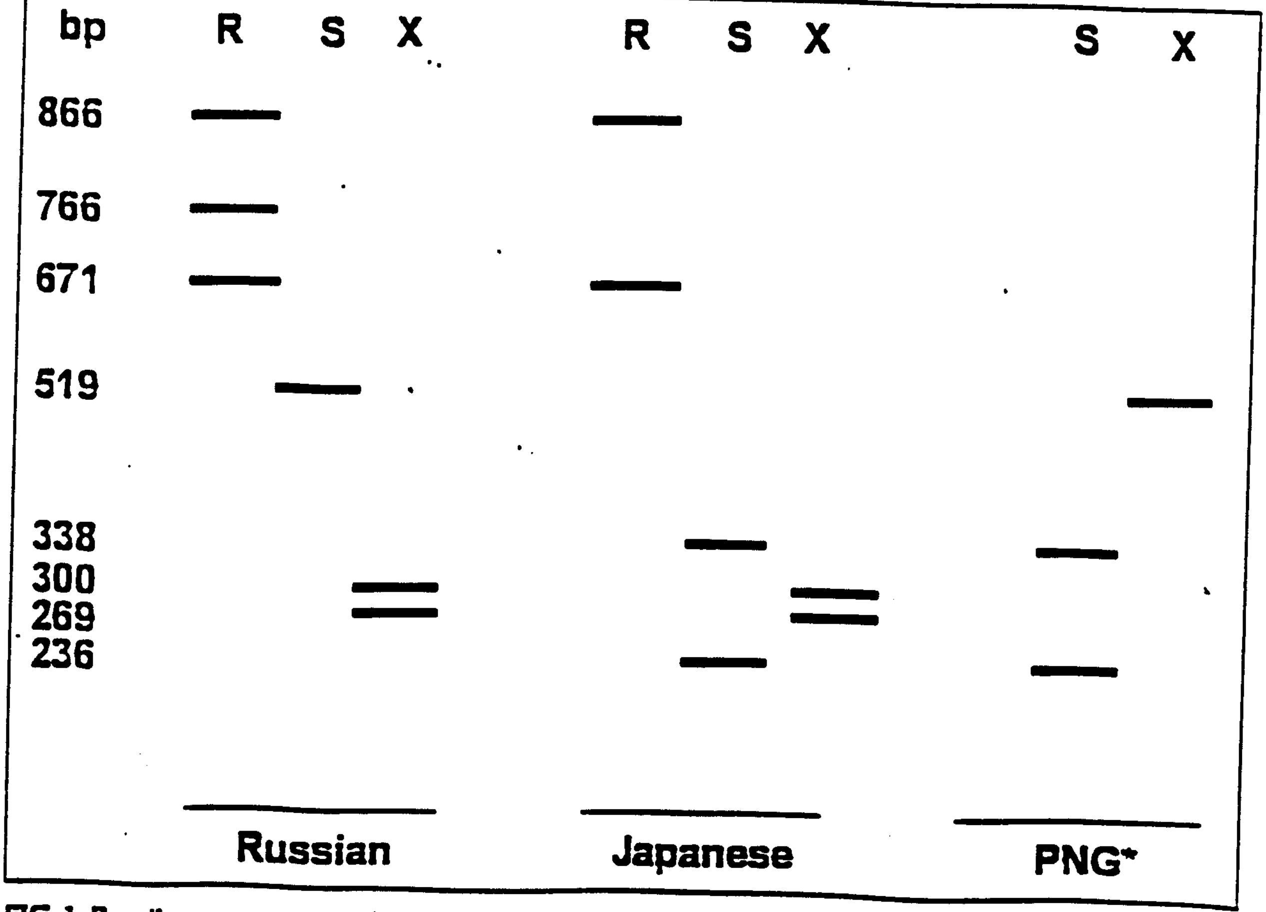

FIG. 1. Banding patterns produced when RAPD marker OPE-07 (R) was used and fragment of the mtDNA cytochrome oxidase subunit I gene of the same mites was amplified and digested with *Sst I* (S) and *Xho I* (X). *Pattern reported by Anderson and Fuchs (1998) using *Sac I* also known as *Sst I*.

TABLE 1. Genotypes of varroa collected from different countries revealed by using RAPD marker OPE-07 and the *Sst I* restriction endonuclease sites of the mitochondrial CO 1 region.

Country source	Number of mites analysed	Varroa genotype
Brazil	10	Japanese
Germany	6	Russian
Japan	10	Japanese
Italy	10	Russian
Morocco	7	Russian
Portugal	3	Russian
Puerto Rico	10	Japanese
Russia	10	Russian
Spain	10	Russian
USA:		
Florida	10	Russian
Iowa	5	Russian
Louisiana	10	Russian
Minnesota	6	Russian
Wisconsin	5	Russian

easily be identified using both the *Sst I* and *Xho I* restriction endonucleases and RAPD marker OPE-07 (fig. 1.). The existence of different varroa genotypes may explain reported differences in colony losses due to varroa parasitism. Varroa continues to kill colonies in Europe and the USA (Ritter et *al*., 1984; Kulincevic & Rinderer, 1988; Hoff & Willet, 1994). In contrast, no colony mortality had been reported in Brazil despite the absence of chemical treatment to control varroa, tracheal mites and various bee diseases (De Jong et *al*., 1984; De Jong, 1996; De Jong & Soares, 1997). Our observations indicate the need for further examination of the taxonomic classification of varroa genotypes since a much wider survey may reveal more genotypes of varroa throughout the world. Our observations also raise the questions of an apparent cyto-nuclear disequilibrium between populations of mites. It may be that there is a lack of gene flow or an asymmetrical gene flow between the mite types. However, mite biology restricts the opportunities for outcrossing and gene flow. Gene flow may occur freely given appropriate opportunities. Regardless of these considerations, our results indicate that mite genotype should be identified when selecting honey bees for resistance to varroa mites.

Acknowledgements

We thank G Delatte, K Kimura, N Koeniger, D Pesante, K Rosenlund, M Spivak, S Sheppard, H A Sylvester and T Yoshida for their help in collecting varroa samples, and K Medveczky for technical help. This research is completed in co-operation with the Louisiana Agricultural Experiment Station.

REFERENCES

The numbers given at the end of references denote entries in *Apicultural Abstracts*.

ANDERSON, D L; FUCHS, S (1998) Genetically distinct populations of *Varroa jacobsoni* with contrasting reproductive abilities on the European honey bee, *Apis mellifera*. *Journal of Apicultural Research* (in press).

DE JONG, D (1996) Africanized honey bees in Brazil, forty years of adaptation and success. *Bee World* 77(2): 67–70. 1303/96

DE JONG, D; GONÇALVES, L S; MORSE, R A (1984) Dependence on climate of the virulence of *Varroa jacobsoni*. *Bee World* 65(3): 117–121. 958/85

DE JONG, D; SOARES, A E E (1997) An isolated population of Italian bees that has survived *Varroa jacobsoni* infestation without treatment for over 12 years. *American Bee Journal* 137: 742–745.

DELFINADO-BAKER, M (1988) Variability and biotypes of *Varroa jacobsoni* Oudemans. *American Bee Journal* 128(8): 567–568. 591/89

GUZMAN, L I DE; RINDERER, T E; STELZER, J A (1997) DNA evidence of the origin of *Varroa jacobsoni* Oudemans in the Americas. *Biochemical Genetics* 35: 327–335.

HOFF, F L; WILLETT, L S (1994) *The USA beekeeping industry*. Agricultural Economic Report No. 680; Economic Research Service, USDA, Washington, DC, USA; 69 pp. 153/95

ISSA, M R C (1989) Enzyme patterns in *Varroa* and *Apis* from Brazil and Germany. *Apidologie* 20(6): 506–508. 1287/90

KRAUS, B; HUNT, G (1995) Differentiation of *Varroa jacobsoni* Oud. populations by random amplification of polymorphic DNA (RAPD). *Apidologie* 26(4): 283–290. 1046/96

KULINCEVIC, J M; RINDERER, T E (1988) Breeding honey bees for resistance to *Varroa jacobsoni*: analysis of mite population dynamics. *In* Needham, G R; Page, R E Jr; Delfinado-Baker, M; Bowman, C E (eds) *Africanized honey bees and bee mites*. Ellis Horwood Limited; New York, USA; pp 434–443. 958/89

OUDEMANS, A C (1904) Note III. On a new genus and species of parasitic acari. *Notes Leyden Museum* 20: 216–222.

RITTER, W; LECLERQ, E; KOCH, W (1984) Observations on bee and varroa mite populations in infested honey bee colonies. *Apidologie* 15: 389–400.

ROSENKRANZ, P; ISSA, M; RACHINSKY, A; STRAMBI, A; STRAMBI, C (1989) Honeybee-varroa relationships: a comparison of africanized and carniolan colonies. *In* Cavalloro, R (ed) *Present status of varroatosis in Europe and progress in the varroa mite control. Proceedings of a meeting of the EC Experts' Group, Udine, Italy, 28–30 November 1988*. Office for Official Publications of the European Communities; Luxembourg, pp 193–198.