Biochemical Genetics, Vol. 35, Nos. 9/10, 1997

DNA Evidence of the Origin of *Varroa jacobsoni* Oudemans in the Americas

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Received 15 Apr. 1997—Final 12 Sept. 1997

Randomly amplified polymorphic DNA (RAPD) was used to examine possible origin of Varroa jacobsoni *Oudemans in the Americas. Among 64 primers screened, 2 primers provided variation which was informative for this study. All* V. jacobsoni *collected from the United States had the same banding pattern to that of mites collected from Russia, Morocco, Germany, Italy, Spain, and Portugal (Russian pattern). This banding pattern was different from the pattern found for mites collected from Japan, Brazil, and Puerto Rico (Japanese pattern). The Japanese pattern lacked a 766-bp band found in the Russian pattern (OPE-07). With primer OPP-03, the Russian pattern had a distinct band at 442 bp not found in the Japanese pattern. Two bands located at 675 and 412 bp were specific to the Japanese pattern. These results suggest that the* V. jacobsoni *of the United States is probably predominantly Russian in origin (via Europe), while the* V. jacobsoni *of Brazil and Puerto Rico are probably predominantly Japanese in origin.*

KEY WORDS: *Varroa jacobsoni;* honey bees; randomly amplified polymorphic DNA; genetic variability.

INTRODUCTION

Varroa jacobsoni Oudemans is a virulent parasite of honey bees. It was first discovered in Java, Indonesia, parasitizing the eastern honey bee, *Apis cerana* F. (Oudemans, 1904). In 1909, *V. jacobsoni* was found on *A. cerana* in Japan (Crane, 1984). The first association of *V. jacobsoni* and *A. mellifera* L. probably occurred in Japan. *A. mellifera* has been in Japan since 1877 (Sakai and Okada, 1973), but

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0006-2928/97/1000-0327$12.50/0

V. jacobsoni infestation on this bee species was not observed until 1957 (Crane, 1984). However, by that time *V. jacobsoni* infestation in Japan was widespread, indicating that *A. mellifera* colonies may have been infested prior to 1957. From Japan, *V. jacobsoni* were introduced into the Western Hemisphere in 1971 on queens and brood combs transported to Paraguay. In 1972, varroa mites were introduced into Brazil via infested bees from Paraguay (de Jong and Goncalves, 1981; de Jong *et al.*, 1982). However, no colony mortality was reported with varroa infestations in South America (Moretto *et al.*, 1991).

In Euro-Asia, association between *A. mellifera* and *V. jacobsoni* probably occurred during the late 1950s when Ukrainian beekeepers brought bees into far-eastern Russia. *V. jacobsoni* infestations were first recorded on eastern Russian *A. cerana* colonies in 1952 (Crane, 1978), although it is unclear when *V. jacobsoni* first shifted to *A. mellifera* (de Jong *et al.*, 1982). With the belief that the Ukrainian *A. mellifera* were superior stocks, daughter queens and *V. jacobsoni* with them were brought back to the European USSR in the 1960s (Crane, 1978). Subsequently, the mites dispersed throughout Europe with devastating consequences. The introduction of *V. jacobsoni* into western Germany is controversial. Ruttner and Ritter (1980) and Ruttner (1983) cited two possible ways of introduction: (1) importation of *A. cerana* from Pakistan for research purposes and (2) importation of queens from Romania, the USSR, or Greece. In the United States, varroa mites were first detected in 1987 in an apiary in Wisconsin established with bee packages brought from Florida (Anonymous, 1987). Subsequently, *V. jacobsoni* collected from these two states were thought to be of South American origin since these mites were more morphologically similar to mites collected from Brazil than to mites collected from Europe and Asia (Delfinado-Baker and Houck, 1989). Since then, *V. jacobsoni* has been responsible for enormous honey bee colony losses nationwide. In 1989, *V. jacobsoni* were found infesting a swarm of honey bees in Puerto Rico. The origin of this infested swarm or *V. jacobsoni* is still unknown.

Griffiths *et al.* (1983) postulated the possible existence of more than one varroa species. In 1987, *V. underwoodi* was discovered infesting *A. cerana* by Delfinado-Baker and Aggarwal in Nepal. A third species, *V. rindereri*, was identified recently by de Guzman and Delfinado-Baker (1996) associated with *A. koschevnikovi* in Borneo.

Among *V. jacobsoni* populations worldwide, detectable variation depends upon the characteristics and populations studied. Studying morphological characters, Grobov *et al.* (1980) observed differences among *V. jacobsoni* from the USSR, Japan, and Germany. This observation was confirmed by later studies using similar techniques (Delfinado-Baker and Houck, 1989). Delfinado-Baker (1988) identified three biotypes of *V. jacobsoni* based on the damage they inflict on the bee hosts and behavior of the mites. Further studies using allozymes in *V. jacobsoni* collected from Brazil and Germany also showed frequency differences

in the MDH_1 and MDH_2 [*sic*] loci (Issa, 1989; Rosenkranz *et al.*, 1989). However, with a genetic identity of $I = 0.87$, this method was not diagnostic. Similar techniques employed by Biasiolo (1992) showed no variation among *V. jacobsoni* collected from *A. mellifera* in 12 apiaries in European countries and 1 apiary in China. Using cuticular hydrocarbons, no detectable variation between mites from Italy and mites from Florida was observed (Nation *et al.*, 1992). However, genetic variability among *V. jacobsoni* populations was observed using the randomly amplified polymorphic DNA (RAPD) technique (Kraus and Hunt, 1995). Therefore, we used this same technique to investigate the origin of *V. jacobsoni* in the Americas.

MATERIALS AND METHODS

Adult females of *V. jacobsoni* were used for DNA analyses. Mites from the United States were collected from Louisiana, Iowa, Florida, Minnesota, and Wisconsin. *V. jacobsoni* from Louisiana were collected from seven apiaries in southern Louisiana. Some of these mites were starved for 2 days and others were not, to determine any variation arising from undigested honey bee hemolymph. All samples were kept frozen until used. Mite samples from Cresco, Iowa (one apiary), Gainesville, Florida (one apiary), St. Paul, Minnesota (one apiary), and Madison, Wisconsin (one apiary), were collected in 70% alcohol. *V. jacobsoni* from Russia were collected in liquid nitrogen from the Primorsky Territory in 1995. European mites were collected from Italy (seven apiaries), Spain (two apiaries located in two towns), one apiary in Portugal, and one apiary in Germany. With the exception of German mites, all European *V. jacobsoni* were obtained from frozen honey bees collected in 1992. Mites from Germany (Oberursel) were collected alive in 70% ethanol, air-shipped to the United States, and frozen upon arrival. Mites from Morocco were also obtained from frozen bees collected in 1992 from one apiary. *V. jacobsoni* from Brazil were sampled from honey bees collected in 1990 (five apiaries located in five towns of the state of Rio de Janeiro) and from three apiaries from four towns in the state of Rio de Janeiro in 1993. Bees with mites collected in 1990 were put in 70% ethyl alcohol after collection and then placed in liquid nitrogen after several hours; 1993 samples were collected in liquid nitrogen. Puerto Rican samples were also collected in liquid nitrogen. *V. jacobsoni* were collected from two apiaries of *A. mellifera* colonies. One apiary was located in the humid west coast at the foot of the mountain and the other apiary in the dry south coast of the country. Japanese mites were collected from both *A. mellifera* and *A. cerana japonica* colonies from four apiaries located in Tokyo and Shikoku Island. All mites from *A. cerana* and some mites from *A. mellifera* were frozen, while some mites from *A. mellifera* were collected in 70% ethyl alcohol and sent to the United States. All alcohol samples were washed with deionized water, blotted dry, and frozen until used.

DNA Analyses

DNA was extracted using a 10% Chelex solution (Bio-Rad) as described by Rowe *et al.* (1997) with few modifications. Each individual mite was ground in liquid nitrogen and an aliquot of 150 µl Chelex solution was added to the tube. The tube was then vortexed for 10 sec, incubated at 56°C for 30 min, vortexed for 10 sec, then spun at 12,000 rpm for 3 min. Template DNA was stored at −20°C until used.

A total of 64 primers from Operon Inc. was screened using two or three samples each. However, 62 primers did not show any variation. Only two primers, OPE-07 (5′AGATGCAGCC) and OPP-03 (5′CTGATACGCC) (Kraus and Hunt, 1995), provided the variation which was analyzed in this study.

Samples were prepared for amplification in a 12.5 µl volume (Kraus and Hunt, 1995). The reaction contained 10 m*M* Tris–HCl, 50 m*M* KCl, 2 m*M* $MgCl_2$, 0.1 m*M* each dATP, dCTP, dTTP, and dGTP, 0.2 m*M* primer, 0.5 U of Taq polymerase (Promega) and 2.5 µl of DNA template. Amplification was done for 48 cycles of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C. PCR products were electrophoresed in a 1.5% agarose gel. Products were visualized using ethidium bromide staining (Maniatis *et al.,* 1982). Photographs of the gels were taken and bands were measured against a standard 100-bp ladder (GIBCO-BRL). Bands were digitized and scored using USDA-DNA, a gel digitizing program developed at this laboratory (available on request to L.d.G.).

RESULTS

Using OPE-07, 210 samples of *V. jacobsoni* collected from the United States, Russia, Morocco, Germany, Italy, Spain, and Portugal (Group 1; Table I) showed three distinct bands at 866, 766, and 671 bp (Russian pattern) (Fig. 1). Bands at 866 and 671 bp were shared by 110 samples of *V. jacobsoni* collected from Japan, Brazil, and Puerto Rico (Group 2; Table I) but the PCR products from these mites lacked the 766-bp fragment (Japanese pattern). With RAPD primer OPP-03, Group 1 *V. jacobsoni* had a distinct band at 442 bp, which was not shared by Group 2 mites (Fig. 2). Conversely, Group 2 mites had two distinct bands at 675 and 412 bp which were not present in the mites of Group 1.

The banding patterns of *V. jacobsoni* starved for 2 days and unstarved mites from the U.S. population showed identical patterns using both primers. This observation corroborates the findings of Kraus and Hunt (1995) indicating that only DNA from the mites, and not the bee hemolymph, was analyzed. Likewise, mite samples from Japan and the United States collected alive in alcohol revealed the same banding pattern as the frozen mites, suggesting that alcohol did not interfere in our analyses, an observation also reported by Kraus and Hunt (1995).

Table I. *Varroa jacobsoni* Collection and Analysis Parameters

Country source and date of collection	Number of mites analyzed	Number of source colonies
Brazil (1990, 1993)	28	12
Germany (1996)	6	1
Japan (1995, 1996)		
A. mellifera	47	8
A. cerana japonica	5	3
Italy (1992)	19	6
Morocco (1992)	7	5
Portugal (1992)	3	1
Puerto Rico (1994)	30	27
Russia (1995)	31	14
Spain (1992)	34	8
United States (1994–1996)		
Louisiana	67	20
Iowa	5	1
Florida	27	8
Minnesota	6	1
Wisconsin	5	3

DISCUSSION

Our results are consistent with reports by de Jong and Goncalves (1981) that *V. jacobsoni* in Brazil originated from Japan via Paraguay. They also suggest that *V. jacobsoni* from Puerto Rico may have come from Brazil or Japan via swarms in ships or shipment of queens and bees. In addition, our results show that *V. jacobsoni* from at least five U.S. population of mites are likely to have come from Europe. Based on cuticular hydrocarbons, Nation *et al.* (1992) showed that mites collected from Italy and Florida are similar. Using RAPD analysis, we observed that *V. jacobsoni* collected in Italy and Florida had the same banding pattern (Russian pattern).

The similarity of the banding pattern of the *V. jacobsoni* from all European countries with *V. jacobsoni* collected from Russia (Primorsky Territory) is consistent with the reports of Crane (1978) that European mites originated from this region via European Russia. This observation also suggests that *V. jacobsoni* may have been introduced into Germany via neighboring European countries such as Romania, the USSR, and or Greece (Ruttner and Ritter, 1980), and not from Pakistan as had been assumed (Ruttner, 1983). However, more samples from Germany and Pakistan should be analyzed to support this claim further.

This consistent RAPD banding pattern observed in all European mites may also explain the similarity of *V. jacobsoni* collected from *A. mellifera* in 12 apiaries from European countries and one apiary from China using allozymes (Biasiolo, 1992). Populations of mites which differ in RAPD analyses may be

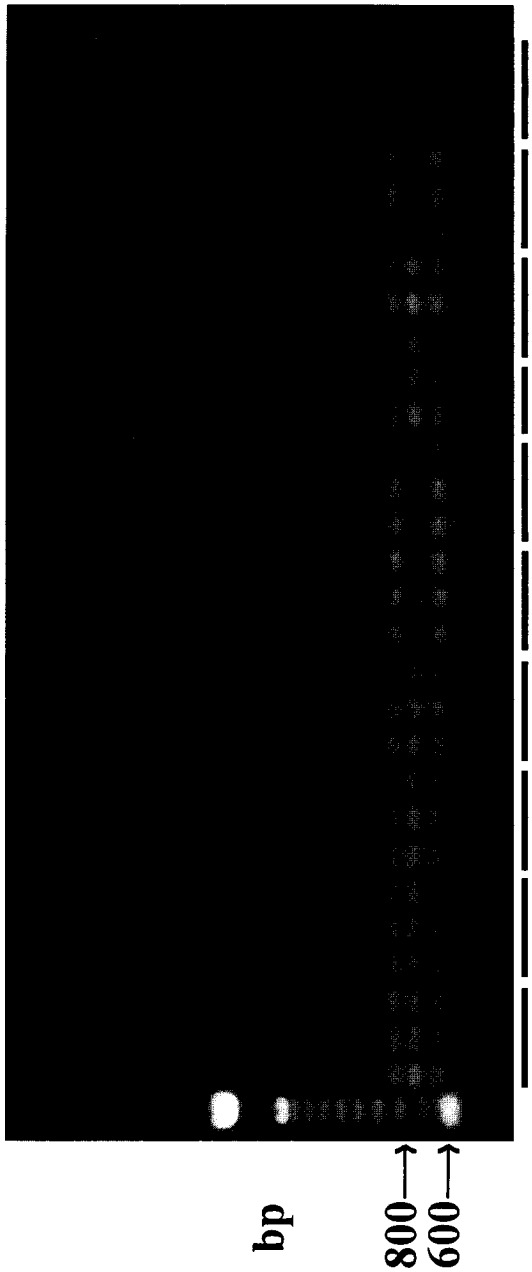

Fig. 1. RAPD banding patterns of *Varroa jacobsoni* collected from different countries using primer OPE-07. (A) Germany; (B) Spain; (C) Russia; (D) United States; (E) Japan; (F) Puerto Rico; (G) Morocco; (H) Italy; (I) Brazil; (J) Portugal. The first lane is the 100-bp ladder.

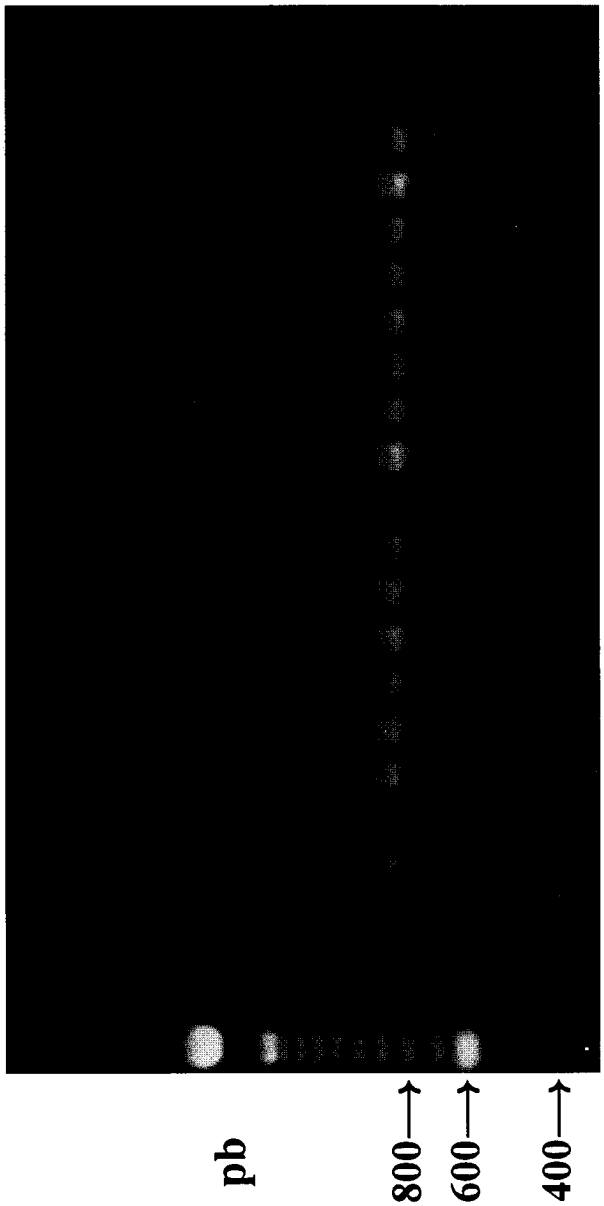

Fig. 2. RAPD banding patterns of *Varroa jacobsoni* collected from different countries using primer OPP-03. (A) Italy; (B) Brazil; (C) Russia; (D) Japan; (E) Spain; (F) Puerto Rico; (G) Germany; (H) United States; (I) Portugal; (J) Morocco. The first lane is the 100-bp ladder.

more likely to differ in allozyme structure. Allozyme frequency differences were observed in the MDH_1 and MDH_2 [*sic*] loci by Issa (1989) and Rosenkranz *et al.* (1989) using groups of *V. jacobsoni* collected from Brazil and Germany. Using RAPD analysis, we observed consistent diagnostic differences in the banding patterns of these two *V. jacobsoni* populations using two primers. This genetic variation between these two mite populations may be correlated with differences in their virulence on their bee hosts.

Delfinado-Baker (1988) had identified three biotypes of *V. jacobsoni* based on the damage done to the host bee species and mite behavior. Likewise, Kraus and Hunt (1995) suggested that differences may exist between *V. jacobsoni* of the same population parasitizing different bee species. Kraus and Hunt (1995) found bands that were shared by U.S. and German *V. jacobsoni* (all from *A. mellifera*) but not present in mites collected from Malaysia (from *A. cerana*) using different RAPD primers. However, our results showed no differences in the banding patterns of *V. jacobsoni* collected from *A. mellifera* and *A. cerana japonica* in Japan using the two primers. This disparity may be a consequence of a small sample size of mites from *A. cerana* or perhaps different types of *A. cerana* may harbor different types of *V. jacobsoni.*

These results support the conclusion that the United States has at least one population of *V. jacobsoni* which appears to have been imported from Europe and not Brazil. A more intense survey may discover *V. jacobsoni* in North America with an origin in South America or elsewhere. In addition, a worldwide genetic survey of *V. jacobsoni* from *A. cerana* and *A. mellifera* may provide information concerning genetic differences which correlate with differences in the virulence of *V. jacobsoni* on their bee hosts.

ACKNOWLEDGMENTS

We thank R. Whiteside and R. Gipson for searching mites from our honey bee collections and R. Gipson for DNA extraction. We thank the following for their help in the collection of mite samples: G. Delatte, R. Danka, and V. Kuznetsov (Russia), A. Sylvester and T. Yoshida (Japan), W. Gressler (Brazil), D. Pesante (Puerto Rico), S. Sheppard (Morocco, Portugal, and Spain), N. Koeniger (Germany), M. Spivak (Minnesota), A. Phibbs (Wisconsin), and K. Rosenlund (Florida). E. Smith provided some RAPD primers. This research is completed in cooperation with the Louisiana Agricultural Experiment Station.

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