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

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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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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 MDH₁ and MDH₂ [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’CTGATAACGCC) (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 mM Tris–HCl, 50 mM KCl, 2 mM MgCl₂, 0.1 mM each dATP, dCTP, dTTP, and dGTP, 0.2 mM 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

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

## 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 MDH1 and MDH2 [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.

Dellinado-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.

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REFERENCES


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