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Original article

Differentiation of *Varroa jacobsoni* Oud populations by random amplification of polymorphic DNA (RAPD)

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Summary — Specimens of *Varroa jacobsoni* collected from *Apis mellifera* L colonies in California, Texas and Germany and specimens collected from *A cerana* Fab colonies in Malaysia were compared by means of random amplification of polymorphic DNA (RAPD). A high percentage of monomorphic bands indicated low genetic variability among and within populations of the parasite. It was not possible to distinguish mites collected in California and mites collected in Texas. Mites collected in the USA were distinguishable from mites collected in Germany by 3 specific markers. Mites collected from *A mellifera* in the USA and Germany were distinguishable from mites collected from *A cerana* in Malaysia by 27 and 24 specific markers. RAPD proved to be a valuable tool especially for further interpopulation studies of *V jacobsoni*.

***Varroa jacobsoni* Oud / *Apis mellifera* L / *Apis cerana* Fab / RAPD marker / genetic variability / geographical variability**

INTRODUCTION

The parasitic mite *Varroa jacobsoni* Oud is today's most important honey bee parasite and causes colony losses almost world wide. Originally a parasite of the Asian honeybee *Apis cerana* Fab the mite was detected on *A mellifera* in the 1960s (Delfinado, 1963; De Jong *et al,* 1982). Importation of commercial *A mellifera* colonies into areas with an *A cerana* population brought the previously allopatric bee species into contact (Ruttner and Maul, 1983) and allowed *V jacobsoni* to switch to the new host. While the populations of the parasite reach only a small size within colonies of *A cerana* (Koeniger *et al,* 1983) and do not damage the colony, infested *A mellifera* colonies die. According to new models of host–parasite coevolution the mite can

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develop towards less virulence or extirpate its new host as well (Levin and Pimentel, 1981; Anderson and May, 1982). Numerous studies have been conducted which deal with factors influencing the virulence of *V jacobsoni* such as climate (De Jong *et al,* 1984; Ritter and De Jong, 1984; Moretto *et al,* 1991) and bee race (Moritz and Hänel, 1984; Moretto *et al,* 1991; Otten, 1991). Unfortunately, little is known about the population structure of *V jacobsoni* from different countries and continents. In a study conducted with allozymes it was not possible to distinguish European and Asian mites (Biasolo, 1992) and in another study conducted with cuticular hydrocarbons it was not possible to distinguish European and North American mites (Nation *et al,* 1992). Multivariate morphometric techniques revealed some geographic variation in *V jacobsoni* (Grobov *et al,* 1980; Delfinado-Baker and Houck, 1989). In order to test a model of coevolution of *A mellifera* and *V jacobsoni* a method is required that allows reliable differentiation between mites of different origin. The objective of the present study was to test RAPD (random amplification of polymorphic DNA) as a method to reveal such differences.

MATERIALS AND METHODS

Mite material

The adult females used in this study were collected from *A mellifera* colonies in California, Weslaco (Texas) and Oberursel (Germany) and from *A cerana* colonies in Tenom (Borneo, Malaysia). The Californian population included samples of 5 mites each from 14 feral honey bee colonies and 11 pesticide-treated commercial colonies located within the Californian Central Valley or at the Californian Central Coast (San Francisco area, Monterey). The host colonies were located within an area 100 km north, 400 km south, 100 km west and 200 km east of Sacramento. Mites were, if possible, obtained from commercial colonies located within the same area as feral honey bee colonies used as mite sources. Nineteen mites obtained from Texas originated from 11 colonies. Nine mites collected in Germany originated from one colony. Twenty-four mites obtained from Malaysia originated from 3 colonies. Samples collected in the USA were kept on liquid nitrogen immediately after removing the parasite from its host bee with a paint brush and subsequently kept at –70°C. Samples collected in Germany or Malaysia were kept in ethanol (95%).

Mite DNA

DNA was isolated according to the modified method of Hunt and Page (1992). A single individual was ground in a microcentrifuge tube; 50 μl of lysis buffer (1 μg/ml proteinase K, 1% hexadecyltrimethyl ammoniumbromide, 0.75 M NaCl, 10 mM EDTA, 50 mM Tris-HCl, pH 8) were added while grinding. The homogenate was incubated at 60°C for 1 h and 30 μl of lysis buffer containing 1.5 M NaCl were added. After that the homogenate was extracted twice by an equal volume of phenol/chloroform/octanol (25:24:1) and chloroform/octanol (24:1). The phases were separated by spinning 10 min and 2 min at full speed in a microcentrifuge. The DNA was precipitated by adding 110 μl ethanol and 10 μl 3 M NaAc, pH 5. The DNA was dried and resuspended in 10 μl TE (10 mM Tris-HCl, pH 8, 1 mM EDTA). Quantification was conducted using a fluorometer (Hoefer Instruments model TKO-100). DNA was dissolved in TE (10 mM Tris-HCl, pH 8, 0.3 mM EDTA) to achieve a concentration of 1 ng DNA per μl.

The banding patterns of bee samples and mite samples were compared with 2 primers. Mite samples and bee samples shared no bands. This result demonstrates that only DNA from the *Varroa* mite but no DNA from the hemolymph of the host was analyzed. The banding pattern of mites kept in ethanol at room temperature for a period of 10 months was not distinguishable from the banding pattern of fresh material kept on liquid nitrogen for 24 h.

Primers

The primers used (Operon Inc) were 10 nucleotides in length and approximately 60% G/C

in content. Nomenclature for RAPD loci indicates the primer kit designation given by Operon Inc and the molecular weight of the band in kilobases. A total of 224 primers were screened with 3 samples each; 72 primers with a clear banding pattern were chosen for further screening with 2 samples from feral honey bee colonies and 2 samples from pesticide-treated commercial colonies. Since no polymorphisms were detected primers were chosen again for a clear banding pattern which means several highly reproducible bright bands and only few dim bands. All mite samples collected in California were examined with the following primers: A3, A6, B14, C12, D2, G1, H1, J13, J16, K11, M11, P3, R5, T2, W3, X4. Twenty-four primers with a clear banding pattern were chosen for further screening with 2 samples each from California, Germany and Malaysia. Those primers revealing the highest number of polymorphic bands were chosen for further studies. All mite samples collected in Texas, Germany and Malaysia were examined with the following primers: A3 (5'AGTCAGCCAC), A6 (5'GGTCCCTGAC), B14 (5'TCCGCTCTGG), C12 (5'TGTCATCCCC), H1 (5'GGTCGGAGAA), J13 (5'CCACACTACC), J16 (5'CTGCTTAGGG), K11 (5'AATGCCCCAG), P3 (5'CTGATACGCC), W3 (5'GTCCGGAGTG), X4 (5'CCGCTACCGA).

DNA amplification

RAPD marker reactions were conducted according to the modified method of Williams *et al* (1990). Reaction volumes of 12.5 µl contained 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM each dATP, dCTP, dTTP and dGTP, 0.2 µM primer, 0.5 units of Taq DNA polymerase and 1 ng DNA-template. Amplifications were performed in a thermal cycler (Perkin-Elmer Cetus model 480) programed for 48 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C. The products were resolved by electrophoresis. Gels contained 1% Synergel and 0.7% ultrapure agarose (BRL). After staining with ethidium bromide, gels were photographed with polaroid film.

With the exception of primer P3, only the absence or presence of bands that were monomorphic within a population was scored. Presence (absence) of a band means that the band is present (absent) in all examined individuals of same origin. Bands referred to as specific markers are bands which were present in all samples of one origin and absent in all samples of another origin. Only in case of P3 were differences in brightness scored.

Statistical analysis

As a measure of the strength of association between different populations Jaccard's index was calculated (Ludwig and Reynolds, 1988). This index is equal to the proportion of the bands shared by 2 populations out of all bands scored. The index reaches a maximum of 1 when populations are not distinguishable.

Table I. Absence and presence of RAPD markers in different populations of *V jacobsoni.*

Band (kb)	*USA*	*Germany*	*Malaysia*
B14-1.3	+	+	–
B14-1.4	+	+	–
B14-2.0	+	+	–
B14-2.2	–	–	+
C12-0.92	+	–	–
C12-1.2	+	+	–
C12-1.4	–	–	+
H01-0.49	–	–	+
H01-0.92	+	+	–
J13-0.22	–	–	+
J13-0.72	+	+	–
J13-2.0	+	–	–
J13-2.2	–	–	+
J13-2.3	+	–	–
J13-2.4	+	+	–
J16-0.47	–	–	+
J16-1.3	–	–	+
J16-1.7	+	+	–
J16-1.9	+	+	–
K11-0.73	+	+	–
P03-0.67	+	+	–
W03-0.46	+	+	–
W03-0.49	–	–	+
W03-0.60	+	+	–
W03-1.2	–	–	+
X04-1.1	+	+	–
X04-1.8	+	+	–

Only primers which revealed differences between mites of different origin are listed. + = present; – = absent.

RESULTS

Differentiation of V jacobsoni *of different origin*

Mites sampled from *A cerana* in Malaysia showed 9 bands not present in mites sampled from *A mellifera* in Germany or the USA (table I). Mites obtained from Germany or the USA showed 15 bands not present in mites from Malaysia. Three bands were only found in mites collected in the USA while all bands present in mites obtained from Germany and Malaysia were present in the samples collected in the USA. All bands shared between mites obtained from the USA and Malaysia were also present in mites collected in Germany.

No specific bands were found in mites collected from Californian feral honey bee colonies compared to mites collected from Californian pesticide-treated commercial colonies, or mites collected in Texas (table II). Mites obtained from the USA were distinguishable from mites collected in Germany by 3 specific markers. Mites obtained from the USA and Germany were distinguishable from mites collected in Malaysia by 27 and 24 specific markers. Some 76% of all scored bands (n = 111, 11 primers) were found to be common to all populations.

Primer P3 revealed the same banding pattern at 0.41, 0.45 and 0.52 kilobases in all samples from Germany, Malaysia and 5% of the samples from feral honey bee colonies (table III, fig 1). All samples from Texas and 94% of the samples from California showed the same banding pattern, which differed

Table II. Degree of association between populations of *V jacobsoni.*

Population	*Number of individuals*	*Jaccard's index*	*Number of bands (number of primers)*
California feral–California commercial	75–55	1.00	133 (16)
California–Texas	130–19	1.00	102 (11)
USA–Malaysia	149–23	0.76	111 (11)
USA–Germany	149–9	0.97	102 (11)
Germany–Malaysia	9–23	0.78	109 (11)

Table III. Frequency of bands of different brightness revealed by primer P3 in different populations of *V jacobsoni.*

Band	*California*			*Texas*	*Germany*	*Malaysia*
	118 out of 125 samples total	*4 out of 75 samples from feral colonies*	*3 out of 50 samples from commercial colonies*			
P03.0.41	Bright	Faint	Bright	Bright	Faint	Faint
P03.0.45	Faint	Bright	Bright	Faint	Bright	Bright
P03.0.52	Faint	Faint	Bright	Faint	Faint	Faint

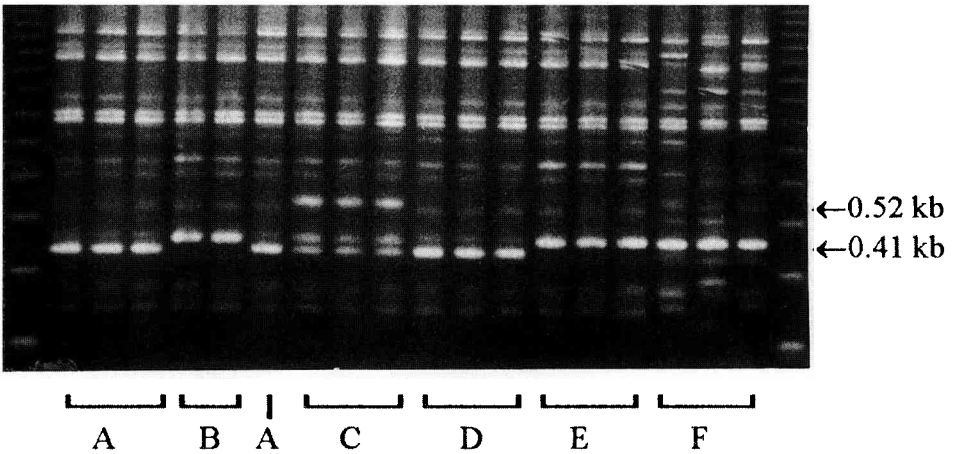

Fig 1. RAPD banding pattern among *V jacobsoni* samples of different origin, primer P3 (table III). Samples A, 4 individuals with banding pattern common in California. Samples B, 2 individuals from feral colonies with rare banding pattern. Samples C, 3 individuals from commercial colonies with rare banding pattern. Samples D, 3 individuals from Texas. Samples E, 3 individuals from Germany. Samples F, 3 individuals from Malaysia. The ladder is a 123 kb ladder.

from the banding pattern of mites from Germany and Malaysia. Six percent of the samples obtained from pesticide-treated commercial colonies showed a banding pattern only found in those samples. The distribution of these bands in the feral population and commercial colonies in California was significantly different (2 x 3 contingency $\chi^2 = 64.5$, $p < 0.001$).

Genetic variability within populations of V jacobsoni

Ten clearly scorable bands were found to be polymorphic within 125 Californian samples compared to 133 scored monomorphic bands (7%, 0.056% per individual). In 23 samples from Malaysia 8 bands were found to be polymorphic compared to 94 scored monomorphic bands (9%, 0.39% per individual). In 19 samples from Texas 4 bands were found to be polymorphic compared to 102 scored monomorphic bands (4%, 0.21% per individual). In 9 samples from Germany 1 band was found to be polymorphic compared to 99 scored monomorphic bands (1%, 0.11% per individual).

DISCUSSION

V jacobsoni samples collected from 25 different sources within California and all sources in Texas were found to generate highly similar banding patterns. The genetic variability of the parasite among different populations also was found to be very low. Absence or presence of specific bands that were monomorphic within populations, clearly distinguished mites from different sources.

V jacobsoni collected in California were not distinguishable from mites collected in Texas. The identity of banding patterns from mites collected from 2 geographic regions in the USA shows the reliability of the banding patterns scored. Mites obtained from Germany were distinguishable from *V jacobsoni* obtained from Texas or California with specific markers but the populations shared

a high percentage of bands. The high similarity of German mites and North American mites might suggest European origin of North American mites but might also only reflect low genetic variability in *V jacobsoni*. A final answer to the question of the origin of *V jacobsoni* in the USA is only possible by an examination of mites obtained from South America. Mites collected in Malaysia from *A cerana* proved to be clearly different from mites collected from *A mellifera* in Europe or the USA. This result might be caused by strong differences between Malaysian mites and mites in areas where European and South American mites originated from, and/or clear differences between *V jacobsoni* parasitizing different bee species. Delfinado-Baker and Houck (1989) suggested that mites parasitizing the 2 species represent different biotypes. Sample sizes used in this study are too low to allow conclusions concerning general differences between Varroa mites from California, Texas, Germany and Malaysia. However, the desribed differences in banding patterns of mites of the examined specific origins demonstrate the value of the method.

The detected differences in frequency of bands (primer P3) in the feral honey bee population and the population of pesticide-treated commercial colonies in California can be explained in several ways. Possible explanations are for example differences between *V jacobsoni* in both bee populations, a geographic effect or deviations from common allele frequencies (Hardy–Weinberg law) caused by high levels of inbreeding in colonies with a small mite population.

The results of the present study prove that random amplification of polymorphic DNA is a valuable tool for interpopulation studies and intrapopulation studies on *V jacobsoni*. The high percentage of monomorphic bands shared by mites of different origin causes difficulties in finding differences, especially when intrapopulation studies are conducted, but detection of specific markers is also possible in this species. A disadvantage of the RAPD method is that bands shared between populations may have no genetic identity. However, in the future sequencing of bands and crosses have to be conducted to overcome this problem.

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Résumé — Différenciation des populations de *Varroa jacobsoni* Oud au moyen de la technique des RAPD. L'acarien *Varroa jacobsoni* Oud est actuellement le parasite le plus dangereux pour l'abeille. En utilisant une technique d'analyse enzymatique, il a été possible de différencier des acariens originaires d'Asie de ceux provenant d'Europe (Biasolo, 1992). En revanche, en analysant la composition en hydrocarbures cuticulaires, il n'a pas été possible de distinguer les acariens européens de ceux d'Amérique du Nord (Nation *et al,* 1992). Dans le but, d'une part, de proposer un modèle de coévolution de l'abeille *Apis mellifera* et de *V jacobsoni* et, d'autre part, de comparer les résultats des différents travaux portant sur la recherche d'abeilles résistant aux acariens, il est nécessaire de mettre au point une méthode permettant la caractérisation d'acariens d'origines différentes. Ce travail présente les résultats d'une étude réalisée au moyen de la technique des RAPD ; il porte sur 14 colonies d'abeilles sauvages et 11 colonies commerciales traitées par un acaricide de Californie (5 acariens dans chaque

colonie), 1 colonie d'Allemagne (9 acariens), 3 colonies d'*A cerana* de Malaisie (24 acariens). La variabilité génétique de l'acarien est très faible. Pour les acariens de Californie, seulement 7% des 143 bandes observées étaient polymorphiques. De même chez les acariens d'*A cerana*, seulement 9% des 102 bandes étaient polymorphiques. On a trouvé 3 marqueurs spécifiques qui permettent de distinguer les varroas provenant des États-Unis de ceux provenant d'Allemagne. Les varroas provenant d'*Apis mellifera* ont été dinstingués de ceux provenant d'*A cerana* par 27 (Malaisie/États)Unis) et 24 (Malaisie/Allemagne) marqueurs. Aucune différence n'a été trouvée entre les varroas collectés au Texas et ceux collectés en Californie. La méthode des RAPD représente donc un outil performant en particulier pour les études interpopulations chez *V jacobsoni*.

***Varroa jacobsoni* Oud / *Apis mellifera* L / *Apis cerana* Fab / marqueurs RAPD / variabilité génétique / variabilité géographique**

Zusammenfassung — Unterscheidung von Populationen der Milbe *Varroa jacobsoni* Oud mittels RAPD. Die Milbe *Varroa jacobsoni* Oud ist der momentan weltweit ökonomisch wichtigste Bienenparasit. Es ist bisher weder mit Hilfe von Allozymen (Biasolo, 1992) noch durch Analyse von Kohlenwasserstoffen (Nation *et al*, 1992) gelungen, aus verschiedenen Erdteilen stammende Varroamilben zu unterscheiden. Für Studien zur Wirt-Parasitkoevolution von *A mellifera* und *V jacobsoni* ebenso wie beim Vergleich der Ergebnisse von Studien zur Varroaresistenzzucht bei der Honigbiene ist es von großer Wichtigkeit, Milben unterschiedlicher Herkunft unterscheiden und ihren Verwandschaftsgrad bestimmen zu können. In der vorliegenden Untersuchung wurden jeweils 5 Milben aus 14 wildlebenden Honigbienenvölkern und 11 pestizidbehandelten kommerziellen Völkern aus Kalifornien, 19 Milben aus Texas (11 Völker), 9 Milben aus der Bundesrepublik Deutschland (1 Volk) und 24 vom ursprünglichen Wirt *A cerana* abgesammelte Milben (3 Völker) mittels random amplification of polymorphic DNA (RAPD) untersucht. Die genetische Variabilität des Parasiten erwies sich als sehr gering. Bei den aus Kalifornien stammenden Milben traten bei insgesamt 143 ausgewerteten Banden nur 7% polymorphe Banden auf. Auch bei den vom ursprünglichen Wirt *A cerana* abgesammelten Milben traten bei nur 9% der insgesamt 102 ausgewerteten Banden Polymorphismen auf. In den Vereinigte Staaten gesammelte Milben unterschieden sich von den aus der Bundesrepublik Deutschland stammenden Milben durch 3 spezifische Marker. Die Proben aus den Vereinigte Staaten und der Bundesrepublik Deutschland waren durch 27 bzw 24 spezifische Marker von Milben zu unterscheiden, die vom ursprünglichen Wirt *A cerana* stammten. Milben aus Texas und Kalifornien waren nicht unterscheidbar. Die RAPD-Methode erwies sich als wertvolle Methode, insbesondere bei Interpopulationsstudien an *V jacobsoni*. Die geringe genetische Variabilität des Parasiten macht mit dieser Methode gefundene Unterschiede zwischen Milbengruppen relativ aussagekräftig und erlaubt daher Aussagen selbst bei geringer Probengröße.

***Varroa jacobsoni* Oud / *Apis mellifera* / *Apis cerana* Fab / RAPD Methode / genetische Variabilität**

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