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## Occurrence of two genotypes of *Varroa jacobsoni* Oud. in North America

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**Abstract** – Both the Russian and Japanese genotypes of *Varroa jacobsoni* were present in the United States and Canada. Only the Russian genotype was found in Mexico. Our results suggest that North America, and especially the United States has had more than one introduction of *Varroa jacobsoni*. Differences in virulence among strains of *V. jacobsoni* may be the basis for reports of regional variation in honey bee resistance to *Varroa*. Thus, further studies on the interaction between *Varroa* and honey bee genotypes are needed to fully resolve the issue. © Inra/DIB/AGIB/Elsevier, Paris

*Varroa jacobsoni* / genotype / RAPD / North America / USA / Canada / Mexico / Russia / Europe

### 1. INTRODUCTION

Variation in *Varroa jacobsoni* populations has been reported by several researchers. Grobov et al. [9] observed morphological differences among *V. jacobsoni* mites from USSR, Japan and Germany. These observations were later confirmed by Delfinado-Baker and Houck [8] using similar procedures. Without any genetic bases, Delfinado-Baker [7] established three biotypes of *V. jacobsoni* based on the mite's

damage to its host bees and the behavior of the mites as influenced by the bees. In 1989, the isozyme structures of mite populations collected from Brazil and Germany revealed detectable differences [13, 21]. However, this technique failed to find differences among *V. jacobsoni* collected from *A. mellifera* colonies from 12 European apiaries and one apiary from China [2]. Also, no differences were detected between mites from Italy and Florida based on cuticular hydrocarbons [19]. Using random amplified poly-

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morphic DNA (RAPD), Kraus and Hunt [14] reported different banding patterns of mites collected from Malaysia (*Apis cerana*) to those mites collected from the United States and Germany (*A. mellifera*). Kraus and Hunt also showed that US (Texas and California) and German mites are distinguishable.

Our earlier results indicate two *V. jacobsoni* genotypes [10]: 1) a genotype thought to originate in Russia which is found in far-eastern Russia, Europe and five states in the United States, and 2) a genotype thought to originate in Japan which is found in Japan, Brazil and Puerto Rico. These surveys only detected one type of mite in each of the listed countries or area. Consistent diagnostic patterns were found in the RAPD banding patterns of these two types of mites using two different RAPD primers.

Recently, partial mitochondrial DNA (mtDNA) sequencing of German mites showed differences from homologous sequences of mites collected from Papua New Guinea (PNG) [1]. Anderson and Fuchs also reported the presence of a *Xho* I restriction endonuclease site in German *V. jacobsoni* which was absent in the sequence of PNG mites, and a *Sac* I (also known as *Sst* I) restriction site in the PNG but not in the German mite sequence. The amplification of the mtDNA CO I region with site specific primers and digestion of the PCR product with *Sst* I provided results identical to those produced by the RAPD markers [11]. Thus, this technique serves as another tool in distinguishing between the Russian and Japanese types [10] and also between the German (the same as Russian) and PNG genotypes [1].

## 2. MATERIALS AND METHODS

Using RAPD, we analyzed *V. jacobsoni* collected from 15 states in the United States, three provinces in Canada and from central Mexico. DNA was extracted from  $3 \pm 2$  mites (mean  $\pm$  SD; range 1–20) per colony. DNA extraction

and amplification conditions were as described by Kraus and Hunt [14] and de Guzman et al. [10].

## 3. RESULTS AND DISCUSSION

*Table 1* shows that both the Russian and Japanese mite genotypes [10, 11] were present in the United States and Canada. Only the Russian genotype was found in Mexico. Thus far, only the United States and Canada have been shown to have both genotypes of mites.

Based on our limited samples, we observed single-genotype infestation with the Japanese genotype in Santa Cruz island (California, USA). Single-infestation with the Russian genotype was observed in samples from Louisiana, Maryland, Minnesota, Oregon and Wisconsin. Mixed infestations (both genotypes were present in a colony or both genotypes were found in separate colonies in one location) were recorded from Arizona, Connecticut, Florida, Georgia, Iowa, Nebraska, Texas and Virginia. In states where mixed infestations were found, there were more colonies infested with the Russian genotype than with the Japanese genotype. In addition, the Russian genotype was also found in higher frequencies in single colonies with mixed genotypes.

Our results suggest that North America, and especially the United States, has had more than one introduction of *V. jacobsoni*. Since there were more colonies infested with the Russian genotype (160) than with the Japanese genotype (9), and the Russian genotype has a wider distribution, it is likely that the Russian genotype was introduced into the country first. This introduction was most likely from Europe and not from Brazil as inferred by Delfinado-Baker and Houck [8].

In Canada, the Japanese genotype was found only in Ontario. The immediate origin of Canadian *V. jacobsoni* mites is not known. However, it is very likely that mites in Canada may have originated from the

**Table I.** Genotypes of *Varroa* mites collected from different locations in the United States, Canada and Mexico.

Country source	Total no. of colony sources	No. colonies with Japanese type	No. colonies with Russian type	No. colonies with mixed type	Total no. of mites analyzed
United States					
Arizona	87	2	84	1	157
California	3	3			20
Connecticut	2			2	11
Florida	11		8	3	48
Georgia	1			1	20
Iowa	1			1	5
Louisiana	24		24		57
Maryland	11		11		29
Minnesota	1		1		9
Nebraska	8		6	2	17
Ohio	5	1	1	3	16
Oregon	1		1		10
Texas	21	2	18	1	67
Virginia	9	1	3	5	28
Wisconsin	3		3		9
Total	188	9	160	19	503
Canada					
B. Columbia	8		8		18
Nova Scotia	2		2	2	14
Ontario	6	3	1		29
Total	16	3	11	2	61
Mexico	4		4		23

United States which has both genotypes. A more intense survey may reveal a wider distribution of the Japanese genotype, the possible occurrence of hybrids between the two genotypes or perhaps other genotypes of *V. jacobsoni*. These possibilities are magnified by interstate movement of colonies for overwintering or pollination and by shipment of queens.

The existence of at least two genotypes of *V. jacobsoni* in the United States raises questions concerning their differential virulence on honey bee colonies. There are conflicting reports on the virulence of *V. jacobsoni* on *A. mellifera*. *V. jacobsoni* infestations cause severe colony losses of European honey

bees (EHB) in Europe [15, 20] and in the United States [12] while no colony mortality is reported in Brazil despite the presence of mites in the colonies [3]. The nonvirulence of *V. jacobsoni* in Brazil, where Africanized honey bees (AHB) are abundant, has been attributed to the increased resistance of AHB [16, 17] and to climate [5, 18]. However, some EHB imported from the United States are also resistant to mites in Brazil [6] suggesting that honey bee genotype does not underlie the lack of *V. jacobsoni* virulence. Climate may be the cause but another hypothesis arises. Recently, DNA analysis showed that mites from Brazil have a different genotype from the mites

found in Europe and Russia [10]. It appears that this genotype originated from Japan, which corroborates the reports of de Jong and Gonçalves [4]. Whether or not differences in *V. jacobsoni* genotype play the critical role in producing the apparent mite resistance by AHB and EHB in South America is not known. Differences in virulence among strains of *V. jacobsoni* may be the basis for reports of regional variation in honey bee resistance to mites, although further studies that incorporate genetic data for bees and mites together with climatic information are needed to fully resolve the issue.

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### Résumé – Présence de deux génotypes de *Varroa jacobsoni* en Amérique du Nord.

Nos résultats antérieurs indiquent l'existence de deux génotypes de *Varroa jacobsoni*: i) un génotype que l'on estime originaire de Russie et qui est présent en Russie extrême-orientale, en Europe et dans cinq états des États-Unis [9, 11] et ii) un génotype que l'on estime originaire du Japon et que l'on trouve au Japon, au Brésil et à Porto-Rico. Nous avons déterminé la répartition de ces deux génotypes à l'aide de la méthode RAPD. Les acariens ont été prélevés dans 15 états des États-Unis, dans trois provinces du Canada et dans le centre du Mexique.

Le *tableau 1* montre que les deux génotypes russe et japonais de *V. jacobsoni* sont présents aux États-Unis et au Canada. Au Mexique, seul le génotype russe a été trouvé. Nous avons observé une infestation par un

seul génotype, le japonais, sur l'île de Santa Cruz (Californie). Une mono-infestation, par le génotype russe, a été observée dans des échantillons provenant de Louisiane, du Maryland, du Minnesota, de l'Oregon et du Wisconsin. Les deux génotypes ont été trouvés en Arizona, au Connecticut, en Floride et en Géorgie, dans l'Iowa, le Nebraska et le Texas et en Virginie. Ces observations suggèrent qu'il n'y a pas eu qu'une seule introduction de *V. jacobsoni* en Amérique du Nord, et en particulier aux États-Unis. Puisqu'il y a eu plus de colonies infestées par le génotype russe ( $n = 160$ ) que par le génotype japonais ( $n = 9$ ) et que le génotype russe a une plus grande répartition (*tableau 1*), il est probable qu'il ait été introduit le premier aux États-Unis. Cette introduction s'est faite probablement à partir de l'Europe, plutôt qu'à partir du Brésil comme l'avaient conclu Delfinado-Baker et Houck [2].

L'existence d'au moins deux génotypes de *V. jacobsoni* aux États-Unis soulève la question de leur plus ou moins grande virulence vis-à-vis des colonies d'abeilles mellifères. Les infestations par *V. jacobsoni* causent des pertes sévères aux colonies d'abeilles européennes (EHB) en Europe [12, 13] et aux États-Unis [14], alors qu'on ne signale aucune mortalité de colonies au Brésil malgré la présence de *V. jacobsoni* [15]. La non virulence de *V. jacobsoni* au Brésil, où l'abeille africanisée (AHB) est abondante, a été attribuée à une résistance accrue de l'AHB [16, 17] et au climat [18, 19]. Néanmoins certaines EHB importées des États-Unis sont elles aussi résistantes à *V. jacobsoni* [20], ce qui laisse penser que le génotype n'est pas à la base de l'absence de virulence de *V. jacobsoni*. Le climat peut en être la cause, mais une autre hypothèse est suggérée. Des analyses d'ADN ont récemment montré que les acariens *V. jacobsoni* du Brésil avaient un génotype différent de celui des acariens trouvés en Europe et en Russie [9]. Ce dernier est originaire du Japon, ce qui confirme les conclusions de de Jong et Gonçalves [21]. Que les différences de génotype jouent ou non un rôle

critique en produisant la résistance apparente de l'AHB et de l'EHB à l'acarien en Amérique du Sud, on l'ignore. Il se peut que les différences de virulence parmi les souches de *V. jacobsoni* soient à la base des variations régionales mentionnées portant sur la résistance de l'abeille à *V. jacobsoni*, mais des études complémentaires, qui prennent en compte les données génétiques des abeilles et de *V. jacobsoni* ainsi que les données climatiques, sont néanmoins nécessaires pour donner une solution complète au problème. © Inra/DIB/AGIB/Elsevier, Paris

### **Varroa jacobsoni / génotype / Amérique du Nord / RAPD / virulence**

**Zusammenfassung – Vorkommen von zwei Genotypen von Varroa jacobsoni in Nordamerika.** Frühere Untersuchungen führten zur Beschreibung von zwei Genotypen bei *Varroa jacobsoni*: 1) ein Genotyp, der vermutlich aus Russland stammt, kommt im fernöstlichen Russland, in Europa und fünf Staaten der Vereinigten Staaten von Amerika vor [9, 11], und 2) ein Genotyp, der vermutlich in Japan entstanden ist, wird in Japan, Brasilien und Puerto Rico gefunden. Wir bestimmten die Verteilung dieser zwei *Varroa* Genotypen in Nordamerika mit Hilfe der RAPD (random amplified polymorphic DNA). Die *Varroa* Milben wurden in 15 Staaten der USA, drei Provinzen in Kanada und im zentralen Mexiko gesammelt.

Unsere Ergebnisse zeigen, daß beide, der russische und der japanische Genotyp, in den USA und in Kanada vorkommen. In Mexiko wurde nur der russische Genotyp gefunden. Auf der Insel Santa Cruz (Kalifornien, USA) fanden wir nur Infektionen mit dem japanischen Genotyp. Infektionen ausschließlich mit dem russischen Genotyp wurde in jeweils einer Probe von Louisiana, Maryland, Minnesota, Oregon und Wisconsin nachgewiesen. Beide Genotypen wurden in Proben aus Arizona, Connecti-

cut, Florida, Georgia, Iowa, Nebraska, Texas und Virginia registriert. Diese Befunde sprechen dafür, daß in Nordamerika und speziell in den USA eine Einschleppung der *Varroa* Milben mehr als einmal stattgefunden hat. Da es insgesamt mehr Völker mit dem russischen Genotyp (160 Völker) als mit dem japanischen Typ (9 Völker) gab, und der russische Genotyp eine größere Verbreitung hatte, ist wahrscheinlich der russische Typ zuerst eingeschleppt worden. Diese Einschleppung erfolgte mit hoher Wahrscheinlichkeit aus Europa und nicht aus Brasilien, wie von Delfinado-Baker and Houck [2] angenommen wurde.

Das Vorkommen von mindestens zwei Genotypen von *Varroa* in den Vereinigten Staaten wirft Fragen über ihre unterschiedliche Virulenz für die Bienenvölker auf.

*V. jacobsoni* Infektionen verursachen hohe Völkerverluste bei den europäischen Honigbienen (EHB) in Europa [12, 13] und in den Vereinigten Staaten [14], während aus Brasilien keine Verluste gemeldet werden, obwohl es Milben in den Völkern gibt [15]. Die geringere Virulenz von *V. jacobsoni* in Brasilien, wo die afrikanisierten Honigbienen (AHB) häufig ist, wurde einer zunehmenden Resistenz der AHB [16, 17] und dem Klima [18, 19] zugeschrieben. Jedoch erwiesen sich einige EHB, die aus den USA eingeführt wurden, ebenfalls resistent gegen die Milben in Brasilien [20]. Das legt nahe, dass das Fehlen der Virulenz nicht auf den Genotyp der Bienen zurückzuführen ist. Das Klima mag eine Rolle spielen, aber es entsteht eine andere Hypothese. Kürzlich zeigten DNA Analysen, daß Milben von Brasilien einen anderen Genotyp aufweisen als Milben von Russland und Europa [9]. Es scheint als ob dieser Genotyp aus Japan stammt, was mit den Berichten von de Jong und Goncalves [21] übereinstimmt. Ob die Unterschiede im Genotyp der *V. jacobsoni* die kritische Bedeutung bei der offensichtlichen Milbenresistenz der AHB und EHB spielen oder nicht, ist noch ungeklärt. Jedoch könnte die Basis für die Berichte

von verschiedenen regionalen Variationen in den Unterschieden in der Virulenz der *V. jacobsoni* Linien liegen. Weitere Untersuchungen, die zusätzlich zu den genetischen Daten von Bienen und Milben auch die Informationen über das Klima aufnehmen, sind nötig, um dieses Problem vollständig zu klären. © Inra/DIB/AGIB/Elsevier, Paris

***Varroa jacobsoni* / Genotyp / RAPD / Nordamerika / USA / Kanada / Mexiko / Russland / Europa**

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