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Scientific note

A scientific note on the genetic distinctness of *Varroa* mites on *Apis mellifera* L. and on *Apis cerana* Fabr. in North Vietnam

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***Varroa* / *Apis mellifera* / *Apis cerana* / diversity / mtDNA / Vietnam**

In 1995, Boot et al. [4] reported that *Varroa* mites taken from *Apis mellifera* L. colonies or *A. cerana* Fabr. colonies in Vietnam reproduced by 80% and 10%, respectively, in either bee species after they had been artificially transferred in worker brood cells. They proposed this to be due to a difference in reproductive traits of *Varroa* strains from both origins. Alternatively, the observed reproduction may have been due to host-specific influences of the source colonies on the mites. We used molecular techniques to clarify whether or not both bee species host different strains of *Varroa* mites.

We collected a total of 144 mites from three locations in North Vietnam (Vu Quang, Moc Chau and Hanoi), either by opening drone brood cells or by dusting worker bees with rice flour or powdered sugar and shaking them in a sieve. Vu Quang is situated in a remote area where no *A. mellifera* bees have so far been introduced. In Moc Chau, which was the location Boot et al. [4] had carried out their experiment, both bee species are present in the same area. We sampled *Varroa* mites from two apiaries about 6 km apart, each having one of the bee species. In Hanoi, *Varroa* mites were sampled from one colony of *A. mellifera* and one colony of *A. cerana*, which had been placed at a distance of 5 m from each other 6 weeks prior to the sampling. From the *A. mellifera* colony, we additionally sampled 11 *Varroa* mites from worker brood, and examined each for evidence of reproduction (presence of eggs and/or nymphs).

In one of the *Varroa* mites from Vu Quang, a 458 base-pair region of the cytochrome oxidase subunit (COI) was sequenced as described by Anderson and Fuchs [2]. The sequence differed in 2 base pairs from that described for the GER mite type and by 30 base pairs from that of the PNG mite type [2], which have recently been renamed the Korea haplotype and the Java haplotype, respectively [3]. Our sequence from Vu Quang was identical with that previously found in mites from Phjong (North Vietnam), but differs by at least one base pair from 17 other biotypes so far investigated, including a Japan/Thailand haplotype [3].

Based on the sequences of all known haplotypes, a discrimination scheme was worked out using three restriction endonuclease sites (*XhoI*, *Sac I*, and *BsmFI*) by which this sequence can be distinguished unambiguously from all other known haplotypes [3]. In particular, it allows to discriminate from the two haplotypes so far found in *A. mellifera* (the Korea and the Japan/Thailand haplotypes). Results are given in Table I. The 78 mites sampled from *A. mellifera* almost exclusively belonged to the Korea haplotype, which is the most common haplotype found on *A. mellifera* worldwide. Only three of these mites belonged to the Vietnam haplotype previously found on *A. cerana* in Phjong, North Vietnam. In contrast, the 66 mites sampled from *A. cerana* almost uniformly belonged to the Vietnam haplotype, with the exception of one mite which was the Korea haplotype.

It can be concluded, that indeed *Varroa* from *A. cerana* and from *A. mellifera* from different localities over North Vietnam belong to two distinct haplotypes. The haplotype found on *A. cerana* is that which is specific for the area, and is now reported from 4 locations covering a major

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Table I. *Varroa* mites of the Vietnam or the Korea haplotype in colonies of *Apis cerana* or *Apis mellifera* at different locations of North Vietnam. N = number of colonies.

Bee species	*Apis cerana*			*Apis mellifera*		
Haplotype	N	Vietnam	Korea	N	Vietnam	Korea
Sampling location and method						
Vu Quang	2					
From adult workers		1				
From drone brood		15				
Moc Chau	3			4		
From adult workers		20	1			35
Hanoi	1			1		
From adult workers		29			1	31
From worker brood						
With offspring						9
without offspring					2	

region of North Vietnam. The other found on *A. mellifera* is the Korea haplotype known for its deleterious effects in *A. mellifera* bee colonies almost worldwide, identical to the Russian genotype described in [5] and the GER genotype described in [2].

Although isolation between the haplotypes is fairly complete, single specimens of the "wrong" haplotype can be found either way. This contrasts to the situation between the Java and the Korea haplotype in Java, where the Korea haplotype has not yet been found in colonies of *A. cerana* [3], and might reflect the comparatively low genetic distance of the Vietnam haplotype from the Korea haplotype. However, even placing colonies of *A. cerana* and *A. mellifera* in the same vicinity did not markedly increase the proportion of the wrong-haplotype mites. This raises the question of the nature of the isolation, which could work by the inability of mites to enter colonies, or by the failure of mites to reproduce in them. That inability to reproduce might play a role is indicated by the fact that the only two non-reproducing mites of the 11 mites found in worker brood of *A. mellifera* were of the Vietnam type (Fisher exact, $P < 0.02$). This difference in reproduction is in accordance with the results of Boot et al. [4]. It would, however, need a more detailed investigation to determine whether this inability to reproduce is partial or absolute, and specifically whether it also applies to the reproduction in drone cells.

This is yet a further report on a situation where *A. mellifera* and *A. cerana* have been shown to host different strains of *Varroa* mites [1, 2, 3]. However, while the genetic differences between the *Varroa* mites found on *A. cerana* and *A. mellifera* in Java (Java haplotype and Korea haplotype, respectively) are quite substantial, in Vietnam the Vietnam haplotype is comparatively close to the Korea haplotype. Nevertheless the difference seems sufficient to induce effective isolating mechanisms. These findings strongly underline the necessity to be aware, in any investigation of this parasite, of the specific strain one is dealing with, and to be conservative in the generalization of results.

Note scientifique sur la différence génétique des acariens *Varroa* présents sur *Apis mellifera* et *Apis cerana*.

Eine wissenschaftliche Notiz über die genetische Unterschiedlichkeit von Varroamilben aus Völkern von *Apis mellifera* und *Apis cerana* in Vietnam.

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