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Original article

Temporal changes in *Varroa destructor* fertility and haplotype in Brazil

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Abstract – The relationship between fertility and haplotype was studied in *Varroa destructor* mites sampled from colonies of *A. mellifera carnica* and Africanized Honeybees (*Apis mellifera*) in Germany and Brazil respectively. Both in Germany and in Brazil, only the *V. destructor* Korea haplotype was found, though the Japan-Thailand haplotype was formerly thought to have been more abundant in Brazil. The fertility of *Varroa* mites in Brazil has increased since 1998 and is currently (2001) at European levels. Temporal changes in mite fertility and haplotype are not fully congruent.

***Varroa destructor* / *Apis mellifera* / host-parasite relationship / reproduction / COI gene / RFLP**

1. INTRODUCTION

The rate of reproduction of a parasite is an important factor determining its virulence and has a major impact on the persistence of both host and parasite. The relationship between the parasitic mite *Varroa* spp. and *Apis,* its honey bee host, exemplifies this point (Calis et al., 1999). In order to reproduce, *Varroa* females enter drone or worker *Apis* brood shortly before the larva is sealed with a wax capping. Approximately 70 h after cell capping, the mite lays the first egg (Steiner et al., 1994). However, a certain percentage of *Varroa* females do not reproduce after invading a brood cell, and *Varroa* fertility can be defined on the basis of whether a female lays an egg or not (Rosenkranz and Engels, 1994; Rosenkranz, 1999). The levels of *Varroa* mite fertility differ depending on host subspecies of *Apis mellifera*, the mite's new host. In Europe and the US, honeybees are highly susceptible to the parasite and colonies do not survive without acaricidal treatment. Moreover, the fertility of *Varroa* females is similarly high in drone and worker brood, ranging between 80 and 90% (Martin, 1994; 1995; Rosenkranz and Engels, 1994). In contrast, Africanized *A. mellifera* (AHB) in Brazil have survived with *Varroa* without acaricidal treatment for the last 20 years (Rosenkranz, 1999). Therefore, AHBs represent the best documented example for *Varroa* tolerance in *A. mellifera.* The low fertility of female mites in AHB worker brood (about 50%, also defined whether a female lays an egg or not; Rosenkranz, 1999) was the most obvious factor for this phenomenon. In European honey bees at the same study site, *Varroa* fertility was about 80% (Rosenkranz, 1999). This difference indicates that tolerance has a host component.

Marked genetic variation among *Varroa* populations has been described (Anderson and Fuchs, 1998; Anderson, 2000; de Guzman and

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Rinderer, 1999; de Guzman et al., 1999; Anderson and Trueman, 2000), variation which was also thought to correlate with different virulence of mites on subspecies of *A. mellifera* (de Guzman, 1999). Indeed, on the basis of their mt-DNA CO-I sequences Anderson and Trueman (2000) divided *Varroa jacobsoni* into two species: *Varroa jacobsoni* and *Varroa destructor*. *Varroa jacobsoni sensu stricto* was thought to be limited to the Indonesian/Malaysian region and to be unable to reproduce on *A. mellifera* (Anderson and Fuchs, 1998; Anderson and Trueman, 2000). *Varroa destructor*, on the other hand, was found on the Asian mainland in association with *A. cerana* and is the species that has become distributed world-wide with *A. mellifera*.

Variation in COI sequence exists even within *V. destructor*, and Anderson and Trueman (2000) have described two COI haplotypes, the Korea haplotype and the Japan-Thailand haplotype, that have become widely distributed with *A. mellifera*. The Korea haplotype is predominant in unbalanced host-parasite systems, as exist in Europe, while in stable systems, as in Brazil, the Japan-Thailand haplotype formerly predominated (Anderson and Trueman, 2000). Therefore, in addition to host susceptibility described above, *Varroa* tolerance could also reflect the more benign or malignant behaviour of different parasite haplotypes.

Indeed, a central question concerning *Varroa* parasitism is the extent to which existing differences in reproduction among host honey bee species and subspecies are due to characteristics of the host or the parasite. A highly relevant study site for this question is Brazil, where mite fertility was formerly low (Rosenkranz, 1999) but is obviously now increasing (Corrêa-Marques, 2000; Corrêa-Marques et al., 2003) and where both Korea and Japan-Thailand haplotypes of *V. destructor* have recently been reported (Anderson and Trueman, 2000).

In this study we examined the current (2001 and 2002) fertility and haplotypes of *V. destructor* in Germany (*A. mellifera carnica*) and Brazil (Africanized *A. mellifera*, AHB) to provide insight into this host-parasite relationship. Moreover, we gathered data (own and from the literature) on mite fertility and haplotype from former years in Brazil to determine the temporal pattern of change in both and thereby ascertain the extent to which variation in *V. destructor* tolerance is a product of differences in host or parasite.

2. MATERIALS AND METHODS

2.1. Study sites and test colonies

2.1.1. Germany

Samples of mites for genetic analysis were taken in 2001 (3 colonies) and 2002 (4 colonies) from colonies at the Hohenheim University Campus and nearby Swabian Alb, Germany. Reproductive data presented here were collected in 2002 from 16 colonies at the same study sites.

2.1.2. Brazil

Data on *Varroa* incidence and reproduction were collected from AHB colonies in Ribeirão Preto (São Paulo State, 17 colonies) and Florianópolis (Santa Catarina State, 12 colonies) in November and December 2001. In Ribeirão Preto, reproducing and non-reproducing mites were collected and stored separately as we expected to find both Korea and Japan-Thailand haplotypes of *V. destructor*. In May 2001 AHB colonies in Estrela (Rio Grande do Sul State, 3 colonies) were sampled for genetic analysis. An additional number of mites attached to frozen AHBs from 1996 (samples from 4 colonies in Ribeirão Preto, Brazil) were also genetically examined.

2.2. Mite infestation and rates of fertility

The infestation rate was determined as the percentage of cells containing one or more *V. destructor* females. Fertility of mite females was measured by opening sealed drone and worker brood and determining whether they were infested with the parasite. Only cells infested by a single mite and in which the host was at the white eyed or older pupal stage were taken into consideration (Rembold and Kremer, 1980; Rosenkranz and Engels, 1994). Every female which had laid at least one egg was considered fertile.

2.3. mt-DNA haplotype analysis

V. destructor females for genetic analysis were transferred to ethanol (96%) and stored at 4 °C.

Table I. Relative fertility of *Varroa destructor* mites from singly infested cells in drone and worker brood of the examined colonies. Each mite female laying at least one egg was considered fertile.

	Number of colonies	Worker brood			Drone brood	
		total examined cells	n single infested cells	Fertility [%]	total examined cells	n single infested cells
Germany						
A. m. carnica	10	1270	148	86	451	83
Brazil						
AHB						
Ribeirão Preto	10	1820	143	82	290	64
Florianópolis	4	420	9	89	100	7

DNA extraction from mites used a high salt protocol (Paxton et al., 1996). The mt-DNA COI gene was amplified by the PCR and PCR products digested by the restriction enzymes Sac I and Xho I as described in Anderson and Fuchs (1998). The resulting products were resolved in 1.5% agarose gels with a commercial size marker (50–1500 bp) and visualised under UV light after ethidium bromide staining.

3. RESULTS

3.1. Fertility of *V. destructor* females

3.1.1. *Germany*

V. destructor infestation was high in Germany; 14% of worker brood cells and 37% of drone brood cells were infested (10 colonies). In brood cells infested by a single mite, 86% of the mites in worker brood laid eggs (n = 148 singly infested cells examined) and 84% in drone brood (n = 83, Tab. I).

3.1.2. *Brazil*

Mite infestation was low in AHB worker brood (8%) but nearly as high as in Germany in drone brood (33%, 14 colonies). Mite fertility in singly infested worker brood cells was 82% (n = 143 singly infested cells examined) and 77% in drone brood (n = 64). The high fertility was observed both in Ribeirão Preto and Florianópolis (Tab. I), though the sample size was very low at the latter location. This represents a significant increase in fertility at Ribeirão Preto over previously published data from 1985 to 1997 (Fig. 1; Chi-Square = 8,480, d.f. = 1, $P < 0.01$).

3.2. *V. destructor* haplotypes

3.2.1. *Germany*

Only the *V. destructor* Korea haplotype was found (n = 19 mites from 5 colonies).

3.2.2. *Brazil*

All samples taken in the year 2001 corresponded to the *V. destructor* Korea haplotype (n < 38 mites from 12 colonies). Although we did not find any mites with the Japan-Thailand haplotype, from the binomial theorem we can state with 95% confidence that less than 8% of the *V. destructor* population in Ribeirão Preto in 2001 had this haplotype.

Of the 6 mites found on the frozen AHB samples from 1996, only 5 could be haplotyped. One corresponded to the *V. destructor* Japan-Thailand haplotype, whilst the other four were of the Korea haplotype (Tab. II).

4. DISCUSSION

In Germany, both mite fertility and haplotype corresponded to our expectations. The fertility was at a consistently high level of 86% and 84% in worker and drone brood respectively, and we could find only the *V. destructor* Korea haplotype. These data agree with previous studies (Anderson and Fuchs,

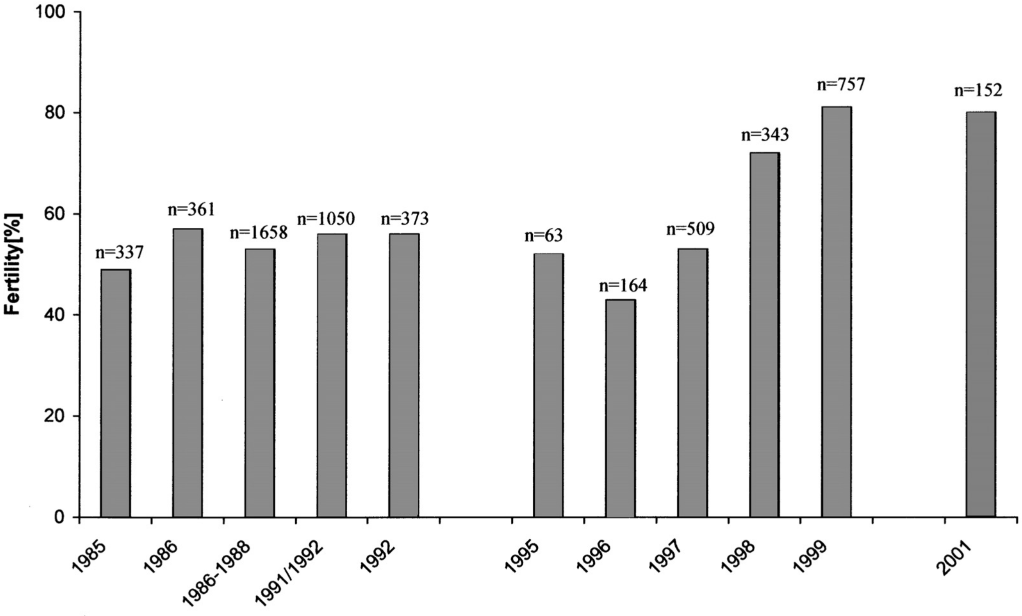

Figure 1. Changes in rates of fertility in Ribeirão Preto. n = total number of examined cells. Data from 1985–1997 reviewed in Rosenkranz (1999), data from 1998 and 1999 taken from Corrêa-Marques (2000); fertility data were not recorded in 1993/94 and 2000.

Table II. *Varroa* species and haplotypes sampled in different host colonies.

	Varroa jacobsoni	*Varroa destructor* Korea haplotype	*Varroa destructor* Japan-Thailand haplotype
Germany			
A. m. carnica	--	19	--
Brazil			
AHB			
Ribeirão Preto 1996	--	4	1
Ribeirão Preto 2001	--	12	--
Florianópolis 2001	--	12	--
Estrela 2001	--	14	--

1998; Anderson and Trueman, 2000; Martin, 1994, 1995; Rosenkranz and Engels, 1994).

Our most notable result is the increase of the proportion of fertile mites in Brazil, where previous studies had demonstrated ca. 50% average mite fertility (Fig. 1, see also Rosenkranz, 1999). The change to higher fertility appears to have occurred very quickly around the period from 1997 to 1998. We have data on *V. destructor* reproduction in Ribeirão Preto for a period spanning nearly 20 years (Rosenkranz, 1999), the last data taken from our group in 1997, with a fertility of 53% in that year. Corrêa-Marques (2000) and Corrêa-Marques et al. (2003) reported a fertility of *V. destructor* females of 72% in Ribeirão Preto already in 1998, similar to the 82% we now report from the same location in 2001. As the low fertility of mites in AHB worker brood has been discussed as one of the most important factors contributing to *V. destructor* tolerance in this host-parasite system, our data demand further attention.

In Brazil the patterns of *V. destructor* genetic variation have apparently changed since the Anderson and Trueman (2000) study. All of our recent mites corresponded to the Korea haplotype, independent of whether the mites had reproduced or not. Indeed, only one Japan-Thailand haplotype of *V. destructor* was found in the 5 mites we analysed from 1996.

Taking into account that we found only the Korea haplotype in Brazil in 2001 and that Anderson and Trueman (2000) described a mixture of Korea and Japan-Thailand haplotypes in 1997, these patterns suggest that AHBs in Brazil were formerly highly tolerant to *V. destructor* due to the lower fertility of the Japan-Thailand haplotype on *A. mellifera*. However, our results do not fully fit this hypothesis.

At least in Ribeirão Preto, the Japan-Thailand haplotype did not appear to be predominant in 1996, a time when *V. destructor* still displayed low fertility. The proportion of Korea haplotypes in 1996 was high in our study, yet the proportion of fertile mites in worker brood was still only 43% at the same time and location (Rosenkranz, 1999). However, genetic analysis of greater numbers of mites for Brazil both before and during the change in mite fertility (1993–1999) would help clarify the link between the temporal dynamics of fertility and haplotype change. Use of genetic markers with finer resolution than mt-DNA RFLPs may also reveal more details about differences in *V. destructor* populations.

Additionally, mites sampled in Brazil have been shown to have a higher fertility when transferred into colonies of European *A. mellifera* races (Rosenkranz and Engels, 1994). This indicates that host effects do influence the host-parasite relationship.

Another indication for host effects is the still stable host-parasite situation with AHBs in Brazil (de Jong, pers. communication), despite the increased fertility and the apparently changed haplotype of mites.

AHBs first appeared in Mexico in 1986 and *V. destructor* infestation was first detected in 1992. The degree of mite tolerance in Mexico seems to be lower compared to that of AHBs in Brazil (Guzmán-Novoa et al., 1999), but the system is also stable (Vandame et al., 1999). However, the fertility of mites has been found to be slightly higher than in Brazil (88% Medina and Martin, 1999; 83–96% Medina et al., 2002; 82–97% Vandame et al., 1999). Moreover, Corrêa-Marques et al. (2003) found a lower number of viable female offspring produced by the invading mites in Brazil compared to Mexico. As the degree of "Africanization" was considered the same in Mexico and Brazil (Vandame et al., 2000), the mite haplotype has been discussed as a cause for the former differences in mite fertility between Brazil and Mexico (Medina and Martin, 1999; Vandame et al., 2000). Mites reported from Mexico have also been reported as belonging to the *V. destructor* Korea-haplotype (Anderson and Trueman, 2000; de Guzman and Rinderer, 1999). Thus, the fact that in Brazil and Mexico the host-parasite situation is stable despite the presence of the Korea haplotype shows that tolerance is not entirely dependent on the mites' haplotype. For further research on tolerance mechanisms, a re-evaluation of the various tolerance factors and possible synergistic effects of host and parasite traits is required.

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Résumé – **Modification au cours du temps de la fertilité et de l'haplotype de *Varroa destructor* au Brésil.** Un certain pourcentage d'acariens *Varroa destructor* qui ont pénétré dans une cellule de couvain d'abeilles ne pond aucun œuf et est donc considéré comme infertile (Rosenkranz et Engels, 1994). Le pourcentage de femelles de *V. destructor* infertiles varie beaucoup selon l'hôte et représente un facteur essentiel de la stabilité de l'équilibre hôte-parasite. L'Abeille africanisée, en Amérique du sud, est un exemple pour cette variation (Rosenkranz, 1999). Outre la tolérance de l'hôte, des caractéristiques propres au parasite pourraient être responsables de cet état de fait. Anderson et Trueman (2000) ont montré, sur la base d'analyses de l'ADNmt, qu'il existait à côté de *Varroa jacobsoni* une autre espèce (*V. destructor*) avec plusieurs haplotypes. Parmi ceux-ci l'haplotype « Corée » prédomine largement dans les systèmes hôte-parasite en déséquilibre, tandis que l'haplotype « Japon-Thaïlande » domine dans les systèmes stables.
Nous avons étudié la fertilité et les haplotypes des acariens *Varroa* sp. en Allemagne (sur *A. m. carnica*) et au Brésil (sur l'Abeille africanisée). Le pourcentage d'acariens femelles fertiles a été

déterminé dans les cellules de couvain mono-infestées et renfermant des nymphes aux yeux foncés. L'identification génétique a été faite par RFLP sur le gène COI de l'ADNmt (Anderson et Fuchs, 1998).

En Allemagne, nous avons trouvé, comme attendu, des taux de fertilité élevés dans le couvain d'ouvrières (86 %) et dans celui de mâles (84 %) (Tab. I). Tous les acariens correspondaient à l'haplotype « Corée » de *V. destructor* (Tab. II). Au Brésil, un taux élevé d'acariens fertiles dans le couvain de mâles (77 %) et dans celui d'ouvrières (82 %, Tab. I) a été trouvé, en opposition avec les données d'études antérieures (Rosenkranz, 1999). Ceci est surprenant puisque, au même endroit, les taux de fertilité étaient restés à un niveau constant bas entre 1983 et 1997 (Rosenkranz, 1999, Fig. 1). Tous les acariens prélevés en 2001 correspondaient à l'haplotype « Corée » de *V. destructor* (Tab. II). Dans un échantillon plus ancien datant de 1996, nous n'avons pu déterminer qu'un acarien de l'haplotype « Japon-Thaïlande ».

Nous ne pouvons donc pas élucider si l'augmentation de la fertilité de l'acarien *Varroa* s'accompagne d'une modification génétique au sein de la population d'acariens. Il faut en tout cas retenir que la situation de *Varroa* au Brésil, malgré la dominance de l'haplotype « Corée » et malgré le taux élevé de fertilité, reste stable comme auparavant.

***Varroa destructor* / *Apis mellifera* / relation hôte-parasite / reproduction / gène COI / RFLP**

Zusammenfassung – Zeitliche Änderungen der Fertilität und des Haplotyps von *Varroa destructor* in Brasilien. Ein bestimmter Anteil der in die Brutzellen von Honigbienen eingedrungenen *Varroa*-Milben legt überhaupt keine Eier und wird demzufolge als „infertil" bezeichnet (Rosenkranz und Engels 1994). Der Anteil an infertilen *Varroa*-Weibchen ist in den verschiedenen Parasit-Wirt-Systemen sehr unterschiedlich und offensichtlich ein wesentlicher Faktor für die Stabilität des Parasit-Wirt-Gleichgewichts. Ein Beispiel hierfür sind die afrikanisierten Bienen Südamerikas (Rosenkranz, 1999).

Hierfür könnten neben einer Toleranz des Wirtes auch Eigenschaften des Parasiten verantwortlich sein. Anderson und Trueman (2000) wiesen auf der Basis von mt-DNA-Analysen nach, dass neben *Varroa jacobsoni* noch eine weitere Art (*V. destructor*) mit mehreren Haplotypen existiert, von denen der "Korea-Haplotyp" vorwiegend in unbalancierten Systemen vorkommt, während der "Japan-Thailand-Typ" in stabilen Systemen dominiert.

Wir untersuchten die Fertilität und den Haplotyp von *Varroa*-Milben in Deutschland (*A. m. carnica*) und Brasilien (afrikanisierte Bienen). Der Anteil fertiler *Varroa*-Weibchen wurde in einfach befallenen Brutzellen mit dunkeläugigen Puppen bestimmt. Die genetische Typisierung erfolgte über RFLP am mt-DNA COI-Gen (Anderson und Fuchs, 1998).

In Deutschland wurden wie erwartet hohe Fertilitätsraten in Arbeiterinnenbrut (86 %) und Drohnenbrut (84 %, Tab. I) gefunden. Alle Milben entsprachen dem *V. destructor* "Korea-Haplotyp" (Tab. II).

In Brasilien wurde im Gegensatz zu früheren Untersuchungen (Rosenkranz, 1999) ein hoher Anteil fertiler Milben in der Drohnen- (77 %) und Arbeiterinnenbrut (82 %, Tab. I) ermittelt. Dies ist erstaunlich, da die Fertilitätsraten an diesem Untersuchungsort zwischen 1983 und 1997 auf einem konstant niedrigem Niveau lagen (Rosenkranz, 1999, Abb. 1). Alle im Jahr 2001 gesammelten Milben entsprachen dem *V. destructor* "Korea-Typ" (Tab. II), in einer alten Probe aus dem Jahr 1996 konnten wir lediglich eine Milbe vom "Japan-Thailand-Typ" feststellen. Damit können wir nicht eindeutig klären, ob die signifikante Zunahme der *Varroa*-Fertilität mit einer genetischen Veränderung innerhalb der Milbenpopulation einherging. Festzuhalten bleibt, dass die *Varroa*-Situation in Brasilien trotz der Dominanz des "Korea-Haplotyps" und trotz hoher Fertilitätsraten nach wie vor stabil ist.

***Varroa destructor* / *Apis mellifera* / Wirt-Parasit-Beziehung / Reproduktion / COI-Gen / RFLP**

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