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Varroa destructor in Africanized honey bees in Brazil: genetic and reproductive profile

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Abstract

The mite *Varroa destructor* is one of the most studied parasites in apiculture, and its genotype variation is a key factor for the severity of infestation in bee colonies. Here we report the genetic and reproductive profile of mites from 14 Brazilian states with different geographic and climatic conditions. We performed PCR to amplify a fragment of the COI gene and differentiate the haplotypes using restriction enzymes. The K haplotype was widely prevalent in the studied sites, while the J haplotype was found only in four municipalities. We also observed both haplotypes (*J* and *K*) coexisting in the same colony, a fact unprecedented in Brazil. Infestation levels were low (0.33 to 15.3%). The reproductive potential showed wide variation (0 to 1.5), indicating that even with the massive presence of K haplotype, environmental and biotic factors related to Africanized honeybees may be responsible for maintaining the mite under low levels in Brazil.

Introduction

The mite *Varroa destructor* (Anderson & Trueman, 2000) is one of the most studied insect parasites worldwide. Formerly, this species was considered *V. jacobsoni* Oudemans, which is only found on Asian honey bee *Apis cerana* F. (Anderson & Trueman, 2000). However, unlike *V. jacobsoni*, *V. destructor* has adapted to parasitize *Apis mellifera* L. It has quickly spread, becoming a great concern in worldwide apiculture (Anderson & Trueman, 2000; Rosenkranz et al., 2010; Thoms et al., 2019). In *A. mellifera*, *V. destructor* generally shows a wide variation in infestation dynamics and colony damage (Mortensen et al., 2016). Although there is no consensus, genetic variation of this mite and infested bee race are considered the main factors for this variation (Guzman-Novoa et al., 2012; Mortensen et al., 2016). Anderson and Trueman (2000) have evaluated the morphology and genetics of mites from several world regions, identifying 18 mitochondrial genotypes, of which only two (Korean-Russian or K and Japanese-Thai or J) could be found in *Apis mellifera.* In this same study, the authors could identify relevant differences in reproductive potential between these two haplotypes, which could explain why the mite is more virulent than others in some regions.

Besides the direct effects on bees, *V. destructor* mites are also vectors of several bee viruses (Levin et al., 2019), such as Deformed Wing Virus (DWV), which affects colony health and demands the use of acaricides to control infestations in countries such as USA and Argentina (Rosenkranz et al., 2010). In Brazil, these mites are easily found in colonies; however, infestation levels are low, causing no damage to local apiculture (De Jong & Gonçalves, 1998; Pinto et al., 2011; 2015; Guimarães-Cestaro et al., 2017a; 2017b). Previously it was believed that low levels of infestation found in Brazil are

due to the wide presence of J haplotype mites in Africanized honey bees (AHB) used in Brazilian apiculture (Anderson & Trueman, 2000). However, in a study with honey bee colonies from the South and Southeast region of the country, Garrido et al. (2003) have identified predominance of K haplotype and increasing mite reproductive rates, which urges caution about the bee health situation in the country.

In addition, local beekeepers and field researchers have reported increasing mite infestation and virus infections in Africanized honey bees. Considering the relevance of *V. destructor* on worldwide bee health, exhaustive investigations on different aspects of mite biology are necessary to infer the influence of this mite on these recent colony losses. Few records about reproductive dynamics and the genetic profile of *V. destructor* can be found in Brazil. Until now, only small studies considering specific regions have been carried out to identify genetic and biological aspects of interactions between the mite and AHB. In this context, we have evaluated the genetic and reproductive profile of *V. destructor* from 14 Brazilian states with different geographic and climatic conditions. Given the importance of this mite to worldwide apiculture, a wider genetic and reproductive profile can be highly useful to know the current situation of *V. destructor* in Brazil and understand differences in infestation rates between regions and bee races.

Methods

Sampling

Samples were collected in 58 municipalities distributed in 14 Brazilian states (Table 1). Samples from 3 colonies were obtained from 1-3 apiaries for each municipality, each consisting of a piece (3 x 10 cm) of capped worker brood comb (16th day) obtained from colonies in Langstroth nests. In addition, ~200 adult nurse bees were obtained from each colony to increase the chances of finding mites. Adult mites and offspring found in bee combs were quantified and stored in 1,5 ml microtubes with 70% ethanol until use. The DNA of three adult females obtained from different brood cells was extracted for each sample. In samples where it was not possible to find mites in combs, we used mites found on nurse bees from the same colony. Along with Brazilian samples, mites from two Argentinean regions were used only in genetic comparisons.

Genetic profile of V. destructor mites

For genetic identification, a fragment of COI gene from *V. destructor* mites was amplified using the primers F 5'-TACAAAGAGGGAAGAAGCAGCC-3' e R 5'-GCCCCTATTCTTAATACATAGTGAAAATG-3', described by Navajas et al. (2002). The PCR conditions were as follows: (1) 92°C for 4 min, one cycle; (2) 92°C for 60 s, 52°C for 90 s, 72°C for 90 s, 35 cycles; and (3) 72°C for 90 s, one cycle. Amplified products were separated by electrophoresis and photo-documented under UV transillumination. After fragment amplification, to distinguish the mite species and haplotypes, 5 µL-aliquot of each amplified PCR product was used in two separated reactions with the enzymes *XhoI* to distinguish *V. destructor* from *V. jacobsoni*; and *SacI* for differentiation between haplotypes K and J of *V. destructor* (Anderson & Fuchs, 1998). Upon incubation for 3 h at 37ºC, the digested products were also separated by electrophoresis and photo-documented under UV transillumination. According to the results of restriction enzymes, PCR products of samples related to each haplotype (K and J) were purified and sequenced by the Sanger method. The sequences were edited and organized by the Lasergene® software suite. The edited sequences were subsequently identified and compared with sequences from both haplotypes present on Genbank® by NCBI's BLAST® tool (National Center for Biotechnology Information). Lastly, the amount of divergence between *V destructor* haplotypes (K and J) and *V. jacobsoni* was calculated using the software MEGA V 6.0.

Infestation rates and reproductive potential

To evaluate mite reproductive potential and infestation levels, 100 capped worker brood cells were inspected per comb piece, and all adult mites and their offspring were quantified (De Jong et al., 1982; Dietemann et al., 2013). With comb inspections, it was evaluated (per sample) the number of adult female mites (total), infested brood cells (%), brood cells infested with offspring (%), viable offspring (%), and reproductive potential (number of viable offspring/number of adult females) (Table1). To identify the viability of offspring, it was considered the age of the analyzed honey bee brood (brown eyes pupa, 16th day) and the time till they reach the adult phase. This information made it possible to establish that only female mite offspring from the deutonymph phase could be considered viable offspring (Martin, 1994).

Data analysis

To evaluate the location effect on mite infestation rates and reproductive potential, the municipalities and states were used such as explanatory variables and the five evaluated parameters (% of infested cells, % of infested cells with offspring, total number of adults, viable offspring, and reproductive potential) were considered as response variables. All parameters were analyzed through "Generalized Linear Models" (GLM) with standard errors (Crawley, 2002), followed by ANOVA ($\alpha < 0.05$). All statistical analyses cited above were conducted using R software (R Development Core Team, 2012).

All analyses were carried out in the Laboratory Specialized in Beekeeping Health.

Results

Genetic profile of V. destructor mites

Among the 158 samples analyzed, 474 mites presented PCR products with approximately 376 bp (Table 1 and Fig 1). All samples presented cleavage site on the presence of *Xho* I,

generating two fragments of ~150 and ~226 bp, which indicates that all belong to *V. destructor* species (Fig 2). In the presence of *Sac* I, 152 samples did not present any cleavage, which indicates the K haplotype (Fig 2). In turn, six samples presented cleavage in the presence of *Sac* I generating two fragments of 124-128 and 252-256 bp indicating J haplotype. Fernando de Noronha Island (Pernambuco) was the sole location where only the J haplotype could be found. Both haplotypes could be found in Bananal (São Paulo) and Areias (São Paulo) samples but in different colonies. In turn, both haplotypes could be found in the same colony in samples from Rio Claro (Rio de Janeiro). Two other samplings were carried out in 6 months in the same colonies to confirm this coexistence. Ten adult mites per colony were individually analyzed for each sampling, totaling 90 evaluated mites on the three evaluated colonies. In all samplings and colonies, it was possible to find both haplotypes in the same colonies, but not sharing the same brood cell (Fig 1)

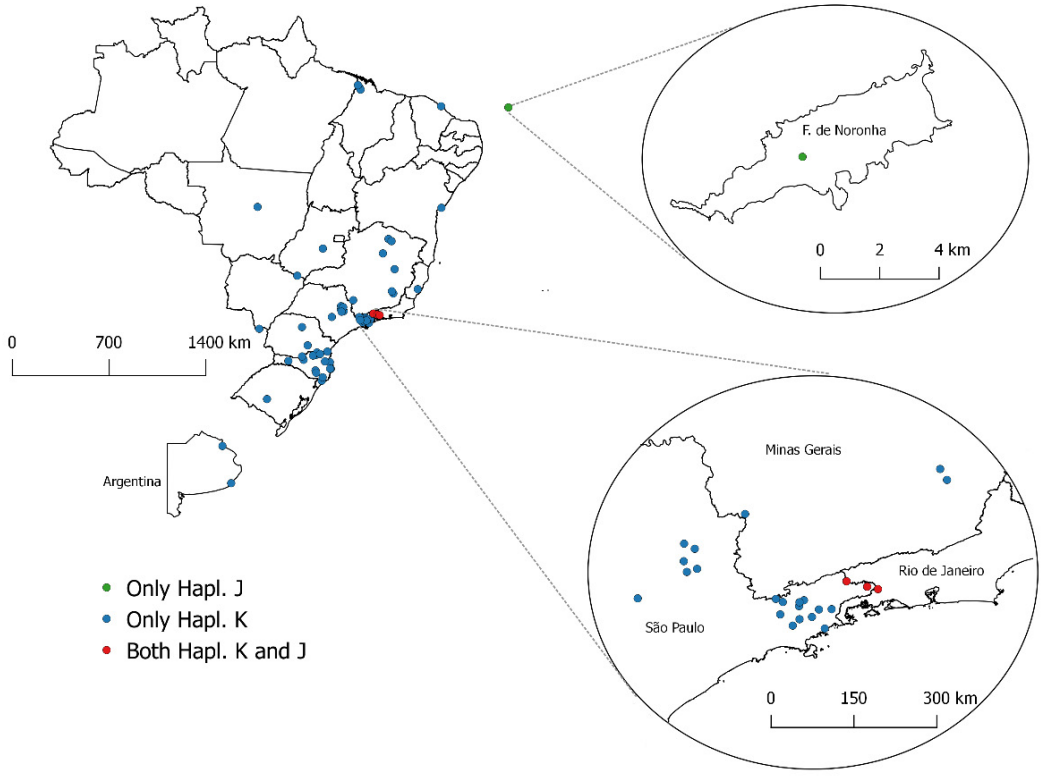

Fig 1. Representative map from evaluated regions. Dots represent each municipality where samples were taken to genetic characterization of mites. Blue dots represent places where only K haplotype was found. Green dot represents where only J haplotype was found. Red dots represent where both haplotypes were found coexisting in a same colony or in a same sampling site.

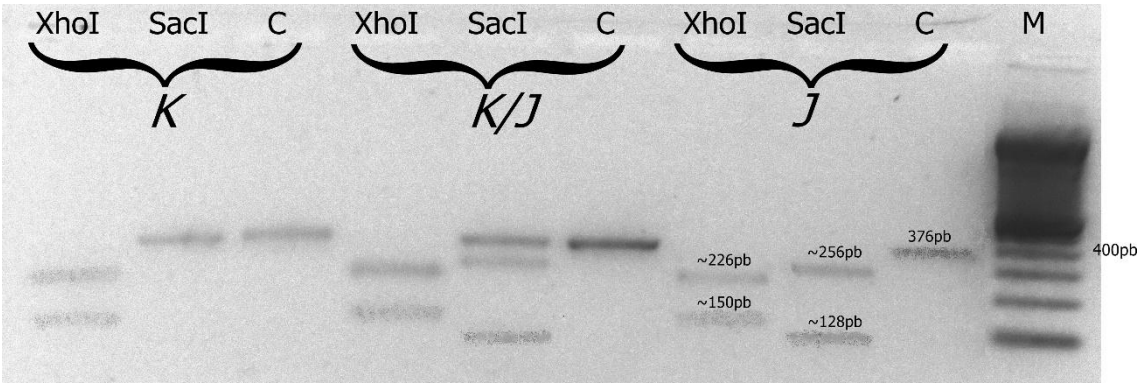

Fig 2. Results from restriction fragment analysis (PCR-RFLP) of partial COI region (376 bp). M = marker; C = Control (PCR result without any enzyme); *SacI* = restriction enzyme with cleavage site for haplotype differentiation (K and J); *XhoI* = restriction enzyme with cleavage site for *V. destructor* differentiating it from *V. jacobsoni*.

Table 1. Reproductive and genetic parameters of worker brood cells infested with *Varroa destructor* (mean ± sd): AF – adult female mite (total), IC – infested brood cells (%), CO – brood cells infested with offspring (%), VO – viable offspring (total), RP – reproductive potential (total) and Hap – haplotype.

Municipality	Country/State	Nº samples	AF	IC	CO	VO	RP	Hap
Buenos Aires	ARG/B. A	0 £	-	-	-	-	-	*K*
Mar Del Plata	ARG/B. A	0 £	-	-	-	-	-	*K*
Salvador	BRA/BA	3	7.3±4.0	6.7±2.9	6.7±2.9	10.0±4.4	1.4±0.2	*K*
Fortaleza	BRA/CE	3	11.0±8.2	9.6±6.0	8.0±6.0	5.7±2.0	0.3±0.3	*K*
Domingos Martins	BRA/ES	1	6.0	6.0	2.0	0.0	0.0	*K*
Goiânia	BRA/GO	1	8.0	7.0	6.0	12.0	1.5	*K*
Maracaçumé	BRA/MA	3	10.3±14.4	9.3±12	6.7±9	5.7±6.2	0.7±0.3	*K*
Maranhãozinho	BRA/MA	3	8.6±5	8.7±5	8.7±5	13.7±8.5	1.5±0.2	*K*
Junco do Maranhão	BRA/MA	3	3.3±0,8	3.3±0,8	3.0±0.0	3.0±0.0	9.0±0,3	*K*
Muzambinho	BRA/MG	3	8.0±6.0	8.0±6.0	7.0±5.5	14.6±15.6	1.3±1.1	*K*
São João Evangelista	BRA/MG	3	2.0±2.6	2.0±2.6	1.7±2.9	0.7±1.2	0.1±0.2	*K*
Viçosa	BRA/MG	3	10.7±12.0	8.3±8.0	6.7±7.4	8.3±11.0	0.8±0.3	*K*
Guaraciaba	BRA/MG	3	7±4,4	6,7±3,8	6,3±3,2	7,3±4,2	1,1±0,4	*K*
Janaúba	BRA/MG	3	1.3±1.5	1.3±1.5	1.3±1.5	2.0±2.0	1.1±1.0	*K*
Bocaiuva	BRA/MG	3	1.0	1.0	1.0	2.0±2.0	0.3±0.6	*K*
R. Machados	BRA/MG	3	1.3±2.3	1.3±2.3	0.7±1.1	0	0	*K*
Sete Quedas	BRA/MS	1	21.0	19.0	17.0	24.0	1.1	*K*
Campo Grande	BRA/MS	3	3.0±3.6	2.7±3.0	2.3±2.5	3.7±3.5	1.0±1.0	*K*
Sinop	BRA/MT	3	1.7±1.5	1.3±1.5	1.3±1.5	1.0±1.0	0.4±0.5	*K*
Fernando de Noronha	BRA/PE	0 £	-	-	-	-	-	*J*
Irati	BRA/PR	3	19,6±13.2	15.3±9.2	3.0±2.6	4.7±4.5	0.3±0.4	*K*
Tamarana	BRA/PR	3	20.0±8.0	13.6±5.3	2.3±2.3	6.3±3.2	0.4±0.3	*K*
Rio Claro	BRA/RJ	3	4.3±2.5	4.3±2.5	3.3±1.1	0.7±1.1	0.2±0.3	*K*/*J***
São Gabriel	BRA/RS	3	13.7±3.5	12.3±4.0	14.0±3.6	17.0±5.3	1.2±0.2	*K*
Balneário	BRA/SC	3	17.0±5.5	14.0±4.5	9.6±4.0	5.7±2.0	0.3±0.2	*K*
Bom retiro	BRA/SC	3.0	1.3±1.5	1.0±1.0	1.0±1.0	2.0±2.7	0.9±0.8	*K*
Caçador	BRA/SC	1.0	0.6±0,5	1.0	0,6±0,5	0	0	*K*
Criciúma	BRA/SC	3.0	6.0±1.7	5.6±1.5	5.0±1.7	5.0±1.0	0.9±0.5	*K*
Garuva	BRA/SC	0 £	-	-	-	-	-	*K*
Florianópolis	BRA/SC	3.0	17.6±10.0	15.0±7.0	13.0±6.5	11.3±2.9	0.7±0.3	*K*
Itainópolis	BRA/SC	3.0	7.3±7.5	5.0±3.4	3.0±4.3	0.0	0.0	*K*
Matos Costa	BRA/SC	3.0	5.6±4.0	5.3±3.5	4.0±4.3	4.0±4.0	0.9±1.0	*K*
Mafra	BRA/SC	1.0	1.0	1.0	1.0	1.0	1.0	*K*
Orleans	BRA/SC	3.0	0.3±0.5	0.33±0.55	0.3±0.5	0.3±0.6	0.3±0.6	*K*
Papanduva	BRA/SC	1.0	53.0	16.0	0.0	0.0	0.0	*K*
Rio Negrinho	BRA/SC	3.0	4.0±0.0	3.5±0.7	1.5±0.7	1.5±2.1	0.4±0.5	*K*
São José	BRA/SC	3.0	5.0±4.2	5.0±4.2	3.5±2.1	2.5±0.7	0.7±0.4	*K*
Urupema	BRA/SC	2.0	0.5±0.71	0.0	0.5±0.71	0.0	0.0	*K*
Xanxerê	BRA/SC	3.0	3.3±5.7	3.0±5.1	3.0±5.1	1.3±2.3	0.1±0.2	*K*
Bocaina do sul	BRA/SC	3.0	2.7±1.2	2.7±1.2	0.7±1.2	0.3±0.6	0.1±0.1	*K*
Araras	BRA/SP	2.0	1.0	1.0	1.0	1.5±0.7	1.5±0.7	*K*
Areias	BRA/SP	3.0	10.0±6.0	9.3±5.5	2.3±3.2	3.7±6.3	0.3±0.5	*K*/*J**
Bananal	BRA/SP	3.0	7.0±10.4	6.6±9.8	5.3±8.3	3.3±5.7	0.2±0.3	*K*/*J**
Botucatu	BRA/SP	3.0	2,3±0,5	2.0±1.0	1.0±1.0	1.0±1.7	0.3±0.6	*K*
Corumbataí	BRA/SP	3.0	2.3±2.3	2.3±2.3	1.6±1.1	2.0±1.0	1.4±1.3	*K*
Cunha	BRA/SP	3.0	7.3±2.8	8.3±4.0	5.3±5.8	2.3±1.5	0.3±0.2	*K*

Table 1. Reproductive and genetic parameters of worker brood cells infested with *Varroa destructor* (mean ± sd): AF – adult female mite (total), IC – infested brood cells (%), CO – brood cells infested with offspring (%), VO – viable offspring (total), RP – reproductive potential (total) and Hap – haplotype. (Continuation)

Municipality	Country/State	Nº samples	AF	IC	CO	VO	RP	Hap
Descalvado	BRA/SP	3.0	4.3±5.8	3.3±4.9	0.6±0.1	0.7±1.1	0.1±0.1	*K*
Lagoinha	BRA/SP	3.0	5.6±3.0	4.6±2.5	3.0±2.6	1.0±1.7	0.1±0.2	*K*
M. Lobato	BRA/SP	3.0	10.0±4.5	9.0±4.0	4.0±3.6	4.7±4.1	0.5±0.5	*K*
Paraibuna	BRA/SP	3.0	9.6±8.3	9.0±7.2	3.0±4.3	3.3±5.0	0.2±0.2	*K*
Pindamonhangaba	BRA/SP	3.0	5.0±1.0	5.0±1.0	1.3±1.5	1.0±1.7	0.2±0.4	*K*
Pirassununga	BRA/SP	3.0	4.3±3.5	3.6±2.5	3.0±3.0	1.7±1.5	0.3±0.2	*K*
Redenção	BRA/SP	3.0	2.6±1.5	2.6±1.5	1.0±1.7	0.0	0.0	*K*
Rio claro	BRA/SP	3.0	5.0±0.0	4.6±0.5	3.0±1.0	4.0±1.0	0.8±0.2	*K*
São Francisco Xavier	BRA/SP	3.0	6.3±2.9	5.3±2.5	4.3±2.5	2.3±1.5	0.4±0.1	*K*
São Luiz do Paraitinga	BRA/SP	3.0	5.3±3.8	5.0±3,4	4±2,6	2,3±2,3	0,6±0,4	*K*
São José dos Campos	BRA/SP	3.0	4.6±0.6	4.6±0.5	2.3±2.3	1.0±1.0	0.2±0.2	*K*
Taubaté	BRA/SP	3.0	3.0±0	3.0	1.0±1.7	0.0	0.0	*K*
Tremembé	BRA/SP	3.0	5.3±3.2	5.0±2.6	2.3±4.0	0.7±1.1	0.1±0.1	*K*
Ubatuba	BRA/SP	3.0	5.0±0.0	4.6±0.5	4.3±0.5	5.7±1.1	1.1±0.2	*K*

£ = There was no brood comb samples, but only mites collected from adult bees for genetic evaluations.
*K/J** = Both haplotypes found in a same city, however on different apiaries.
K/J** = Both haplotypes found in a same samples/colony

The Mitochondrial sequences related to the K haplotype were identical to the complete sequence of the mitochondrial genome published by Navajas et al. (2002) (mites from Avignon, France; EMBL accession number AJ493124). In turn, sequences related to J haplotype were 98% identical to J haplotype sequences from Taiwan and USA (NCBI *accession number* AJ784872; Solignac et al. (2005). The alignment of K and J sequences with *V. jacobsoni* sequence (NCBI *accession number* AF106909.1; Anderson &Trueman 2000) presented nucleotide divergences of 12.7 and 10.9%, respectively. In turn, both sequences presented a nucleotide divergence of 1.3% between each other (Table 2).

Infestation rates and reproductive potential

The aspects of *V. destructor* mites infestation were evaluated in 154 colonies. The levels of infested honey bee brood cells varied from 0.33 ± 1.55 % to 15.3 ± 9.2 %. Infested cells hosting offspring ranged from 0 to 13 %. In turn, considering only viable offspring, the reproductive potential varied from 0 to 1.5 offspring per female mite. Significant differences were observed on five parameters analyzed between municipalities evaluated (Table 1). There was no difference in reproductive potential compared to the haplotype pattern in colonies with both haplotypes in the municipality of Rio Claro-RJ ($p > 0.05$), with low reproductive levels regardless of the genetic profile of the mite. Significant differences were observed between 58 municipalities distributed in 14 states of Brazil in all parameters evaluated regarding the infestation by the mite V. destructor in brood combs. This study covered municipalities with regional climatic differences both in temperature and rainfall located in a tropical zone, where varroa infestations are less harmful (De Jong et al., 1984) and have different infestation rates, higher in the colder regions (Moretto et al., 1991). The climate has a strong influence on the rate of mite infestation. (De Jong et al., 1984; Moretto et al., 1991; Mondragon et al., 2006). Unfortunately, it is impossible to make an objective comparison with several studies on mite reproduction due to the lack of uniformity of the variables. In Brazil, mite infestation rates are considered low and may be related to defense mechanisms carried out by Africanized bees, such as hygienic behavior (Carneiro et al., 2014). No differences in infestation and reproductive parameters related to mite haplotypes were observed.

Table 2. Level of divergence (in percentage) between the different *Varroa destructor* COI haplotypes and *Varroa jacobsoni*. This table includes the Korean haplotype (widely found in this study), the Japanese haplotype (found only in four Brazilian municipalities) and the *V. jacobsoni* sequence (Vj, accessions AF106909.1).

	K haplotype	J haplotype	*V. jacobsoni*
K haplotype		1,3%	12,7%
J haplotype	1,3%		10,9%
V. jacobsoni	12,7%	10,9%	

Discussion

Our genetic screening of *V. destructor* in different Brazilian regions could identify the massive predominance of the K haplotype. The high prevalence of this haplotype could also be identified in other South-American countries where the mite is considered a great threat in apiculture.

In a related study in Argentina, 100% of mites from six provinces were identified as K haplotype (Maggi et al., 2012), and ten other provinces were recently sampled (Muntaabski et al., 2020). Other studies have also identified related results with more than 95% of mites belonging to K haplotype in Chile (Solignac et al., 2005) and 100% in different areas of Uruguay (Mendoza et al., 2020).

Previously it was believed that the J haplotype, which was generally found in areas with low infestation rates (Anderson & Trueman, 2000), was the most prevalent genotype of the mite in Brazil. However, with multiple entries of colonies from different regions of the world to South America, the genetic profile of mites could be changed. Garrido et al. (2003) identified that the J haplotype, widely found in samples from the 1990s in Ribeirão Preto (São Paulo) and Florianopolis (Santa Catarina), was excluded from mite populations after 2001. As in our results, Strapazzon et al. (2009) has also found only K haplotype in municipalities from Santa Catarina, South of Brazil, reinforcing this haplotype replacement. Recently, the K haplotype was also observed in samples collected in the Vale do Ribeira region, including municipalities bordering the state of São Paulo and the state of Paraná (Guimarães-Cestaro et al., 2017a).

On the other hand, the J haplotype apparently could be found in specific isolated areas where geographic barriers or the absence of migratory beekeeping prevents the entry of the K haplotype. Thus, the geographic isolation can explain the exclusive presence of haplotype J in Fernando de Noronha Island (De Jong & Soares, 1997; Strapazzon et al., 2009). Colonies of European honey bees were introduced to this island in the 1970s (Malagodi et al., 1986) when only haplotype J mites were established in South America. Besides Fernando de Noronha, haplotype J mites were also found in colonies from municipalities of the extreme east of São Paulo and south of Rio de Janeiro (Fig 1). Considering the proximity of municipalities where J haplotype mites were found, we believe this is a specific endemic region where the remnant populations can still be found. Besides the isolation characteristics of this region (apiaries surrounded by fragments of Atlantic Rain Forest), it is not clear why the J haplotype is still present and coexisting with the K haplotype in some sites, even at the same colony. Such an event is reported for the first time in Brazil. Solignac et al. (2005) admitted the possibility of the coexistence of both haplotypes. However, due to reproductive advantages and isolation factors, a strong tendency to K prevalence and J exclusion could be expected. Such mechanisms could explain the wide prevalence of the K haplotype found in this study, which gradually could have excluded the J haplotype from honeybee colonies after its introduction in Brazil.

Our study could not detect mites from different haplotypes sharing the same brood cell, and reproduction possibilities between haplotypes were not identified. In a study with K/J infested colonies, Solignac et al. (2005) identified heterozygosis between haplotypes but in a very low percentage of mites due to the necessity of multiple infestations in brood cells to achieve cross reproduction. As observed here, the infestation levels of *V. destructor* in AHB are generally low, and multiple infestations in a single cell are rare events (Fuchs & Langenbach, 1989; Martin, 1995). Moreover, all colonies where we found both haplotypes were monitored for six months and, even confirming the coexistence, we could not find K and J mites in the same brood cell and offspring with heterozygosis.

Considering the necessity of an operculated brood for evaluations, all samplings were obtained during months with high pollen availability, a factor that stimulates brood production. In this colony stage, female mites finish the forethic period and invade brood cells to reproduce and increase the mite population. Then, the infested colony faces a weakening in the following months when food availability is low (Rosenkranz et al., 2010). However, in tropical/subtropical countries such as Brazil, the dynamics of mite infestation tend to be different. Due to the absence of well-defined seasons, rain occurrence and its effect on food availability may be more related to infestation rates. Each Brazilian region has specific rainfall characteristics throughout the year, which drastically affects bee flora and brood production, consecutively affecting mite reproductive dynamics (Moretto et al., 1997; Pinto et al., 2011; Correia-Oliveira et al., 2018). Besides environmental effects, a variation in biological features on bees among studied regions may also affect the relation between host and parasite. Due to crossbreeding between *A. mellifera scutellata* with European races, there is great genetic variability among bee populations in Brazil (Moretto et al., 1991; Sheppard et al., 1999; Collet et al., 2006). For example, bees from South and Southeast states, where several samples were taken for this study, have highly distinctive genotypes (Collet et al., 2006). Due to this, genetically based behaviors directly involved in *V. destructor* control, such as hygienic behavior and grooming, also present great variation between colonies (Spivak, 1996; Ibrahim & Spivak, 2006; Guzman-Novoa et al., 2012; Pinto et al., 2012; Oddie et al., 2021).

Despite the high variation in infestation rates between the regions evaluated (Table 1), the values were lower than those found in European honey bees during summer/spring without acaricide treatment: Bulgaria, 18-49% (Kanchev et al., 1989); southern England, 15-40% (Martin, 1994); and the United Kingdom, 6-42% (Martin, 2001). However, closer values were found when comparing the results obtained in other southeast regions of Brazil: 3.3-11% in the Mata Mineira zone, Minas Gerais state (Bacha-Júnior et al., 2009); 1.1 to 11.6% and 5.0 to 14.0% in the Vale do Ribeira region, São Paulo state (Pinto et al., 2011 and Guimarães-Cestaro et al., 2017a, respectively); 2.83 to 9.48% in the central-eastern region of São Paulo state (Guimarães-Cestaro et al., 2017b) and 7.2 % ± 3.3% in southeastern region of the country (Peixoto et al., 2021). Although the levels of infestation were generally low, they were high in some southern regions, such

as the states of Paraná (19.6±13.2 and 20.0±8.0) and Santa Catarina (17.0±5.5 and 17.6±10.0) (Table 1). In the USA, where the mite causes great economic losses every year, and acaricide tolerance starts to show off, a recent selection of *V. destructor*-tolerant bee lineages has been shown promising results (Gupta et al., 2014). Thus, considering the high number of *V. destructor*-tolerant AHB colonies in Brazil, a selection program focused on bee health and production could provide great results, avoiding future problems. In the same way as the other infestation parameters, mite fertility also presented great variation between regions, but low levels were generally found. This result was expected considering the direct relationship between infestation levels and reproductive potential, as observed in several studies (Martin, 1994; Martin & Medina, 2004; Rosenkranz et al., 2010). These variations and low levels found in AHB are generally attributed to high levels of offspring mortality, including the only male produced by the first laid egg (Mondragon et al., 2006). In addition, this fact, together with the rarity of multiple invasions in a single cell, may be responsible for the high number of infertile mites found in most of our samples.

A few years ago, increasing *V. destructor* reproductive potential was reported in some regions, but due to the absence of proper monitoring, it isn't easy to track possible causes (Garrido et al., 2003; Carneiro et al., 2014). In municipalities of São Paulo state, where we also found contrasting values, Garrido et al. (2003) found increases of 52 to 83% in the number of fertile females during evaluations between 1998 and 2001. It is unknown if reproductive changes can be attributed to the mite's genetic profile. However, once considered the main one responsible for high infestation levels, haplotype K also presented great variation between the studied regions.

The set of geographic characteristics and honey bee genetic variability in Brazil promotes this wide variation of the dynamics of *V. destructor* infestation. The Korean-Russian haplotype presented wide prevalence and great variation in reproductive potential, indicating that other factors apart from genotype could be responsible for mite tolerance in AHB. In turn, we observed that haplotype J, once highly prevalent in Brazil, has been drastically affected by the K haplotype's introduction and is now restricted to only a few isolated areas.

Authors contribution

FAP: investigation, writing-original draft, writing-final draft.
EWT: investigation, formal analysis, resources, writing-original draft, writing-final draft.
LGC: investigation, formal analysis, writing-original draft, writing-final draft.
MFM: methodology, formal analysis, writing-original draft, writing-final draft.
MLTMFA: investigation, formal analysis, writing-original draft, writing-final draft.
DM: supervision, writing-original draft, writing-final draft.

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Conflict of interest

The authors declare that they have no potential conflict of interest concerning the study in this paper.

References

Anderson, D.L. & Fuchs, S. (1998). Two genetically distinct populations of *Varroa jacobsoni* with contrasting reproductive abilities on *Apis mellifera.* Journal of Invertebrate Pathology, 37: 69-78. doi: 10.1080/00218839.1998.11100957

Anderson D.L. & Trueman, J.W.H. (2000). *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Experimental and Applied Acarology, 24: 165-189. doi 10.1023/A:1006456720416

Bacha-Júnior, G.L., Felipe-Silva, A.S. & Pereira, P.L.L. (2009). Taxa de infestação por ácaro *Varroa destructor* em apiários sob georreferenciamento. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 61: 1471-1473.

Carneiro, F.E., Barroso, G.V., Strapazzon, R. & Moretto, G. (2014). Reproductive ability and level of infestation of the *Varroa destructor* mite in *Apis mellifera* apiaries in Blumenau, State of Santa Catarina, Brazil. Acta Scientiarum. Biological Sciences, 36: 109-112. doi: 10.4025/actascibiolsci.v36i1.20366

Collet, T., Ferreira K.M., Arias, M.C., Soares, A.E.E. & Del Lama, M.A. (2006). Genetic structure of Africanized honeybee populations (*Apis mellifera* L.) from Brazil and Uruguay viewed through mitochondrial DNA COI–COII patterns. Heredity, 97: 329-335. doi: 10.1038/sj.hdy.6800875

Correia-Oliveira, M. E., Mercês, C.C., Mendel, R. B., Neves, V.S.L., Silva, F.L., Carvalho, C.A.L. (2018). Can the Environment Influence Varroosis Infestation in Africanized Honey Bees in a Neotropical Region? Florida Entomologist, 101: 464-469.

Crawley M.J. (2002). Statistical computing – An introduction to data analysis using s-plus. London: John Wiley and Sons, 772 p.

De Jong, D., Gonçalves, L.S & Morse, R.A. (1984). Dependence on Climate of virulence of *Varroa jacobosoni*. Bee World, 65: 117-121. doi: 10.1080/0005772X.1984.11098789

De Jong, D. & Gonçalves, L.S. (1998). The Africanized bees of Brazil have become tolerant to Varroa. Apiacta, 33: 67-70.

De Jong, D., Morse, R.A. & Eickwort, G.C. (1982). Mite pests of honey bees. Annual Review of Entomology, 27: 229-252. doi: 10.1146/annurev.en.27.010182.001305

De Jong, D. & Soares, A.E.E. (1997). An isolated population of Italian bees that has survived *Varroa jacobsoni* infestation without treatment for over 12 years. American Bee Journal, 137: 742-745

Dietemann, V., Nazzi, F., Martin, S., Anderson, D.L., Locke, B., Delaplnae, K.S., Wauquiez, Q., Tannahill, C., Frey, E., Ziegelmann, B., Rosenkranz, P. & Ellis, J.D. (2013). Standard methods for *Varroa* research. Journal of Apicultural Research, 52: 1-54. doi: 10.3896/IBRA.1.52.1.09

Fuchs, S. & Langenbach, K. (1989). Multiple infestation of *Apis mellifera*. Brood cells and reproduction in *Varroa jacobsoni* Oud. Apidologie, 20: 257-266. doi: 10.1051/apido: 19890308

Garrido, C., Rosenkranz, P., Paxton, R.J. & Gonçalves, L.S. (2003). Temporal changes in *Varroa destructor* fertility and haplotype in Brazil. Apidologie, 34: 535-541. doi: 10.1051 / apido: 2003041

Guimarães-Cestaro, L., Serrão, J.E., Alves, M.L.T.M.F., Message, D. & Teixeira, E.W. (2017a). A scientific note on occurrence of pathogens in colonies of honey bee *Apis mellifera* in Vale do Ribeira, Brazil. Apidologie, 48: 384-386. doi: 10.1007s13 592-016-0481-3

Guimarães-Cestaro, L., Alves, M.L.T.M.F., Message, D., Silva, M.V.G.B. & Teixeira, E.W. (2017b). Honey bee (*Apis mellifera*) health in stationary and migratory apiaries. Sociobiology, 64: 42-49. doi: 10.13102/sociobiology.v64i1.1183

Gupta, R.K., Glenn, T. & Glenn, S. (2014). Genetics and selection of bees: Breeding for healthy and vigorous honeybees, in: R.K. Gupta, W. Reybroeck, J.W Van Veen & Gupta, A. (Eds.), Beekeeping for poverty alleviation and livelihood security (pp. 247-280). Dordrecht: Springer Netherlands

Guzman-Novoa, E., Emsen, B., Unger, P., Espinosa-Montaño, L.G. & Petukhova, T. (2012). Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees (*Apis mellifera* L.). Journal of Invertebrate Pathology, 110: 314-320. doi: 10.10 16/j.jip.2012.03.020

Ibrahim, A. & Spivak, M. (2006). The relationship between hygienic behavior and suppression of mite reproduction as honey bee (*Apis mellifera*) mechanisms of resistance to *Varroa destructor*. Apidologie, 37: 31-40. doi: 10.1051/apido:2005052

Kanchev, B., Gurgulova, K. & Stoimenov, V. (1989). Defective bee and varroatosis. Rio de Janeiro: International Congress of Apiculture (ed) Program and abstract reports., pp 145-146.

Levin, S., Sela, N., Erez, T., Nestel, D., Pettis, J., Neumann, P. & Chejanovsky, N. (2019). New viruses from the ectoparasite mite *Varroa destructor* infesting *Apis mellifera* and *Apis cerana*. Viruses, 11: 1-15. doi: 10.3390/v11020094

Maggi, M., Medici, S., Quintana, S., Ruffinengo, S., Marcángeli, J., Martinez, P.G., Fuselli, S. & Eguaras, M. (2012). Genetic structure of *Varroa destructor* populations infesting *Apis mellifera* colonies in Argentina. Experimental and Applied Acarology, 56: 309-318. doi: 10.1007/s10493-012-9526-0

Malagodi, M., Kerr, W.E. & Soares, A.E.E. (1986). Introdução de abelhas na Ilha de Fernando de Noronha. População de *Apis mellifera ligustica*. Ciência e Cultura, 38: 1070-1074.

Martin, S.J. (1994). Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. Experimental and Applied Acarology, 18: 87-100. doi: 10.1007/BF00130823

Martin, S.J. (1995). Reproduction of *Varroa jacobsoni* in cells of *Apis mellifera* containing one or more mother mites and the distribution of these cells. Journal of Apicultural Research, 34: 187-196. doi:10.1080/00218839.1995.11100904

Martin, S.J. (2001). *Varroa destructor* reproduction during the winter in *Apis mellifera* colonies in UK. Experimental and Applied Acarology, 25, 321-325. doi: 1023/A:1017943824777

Martin, S.J. & Medina, L. (2004). Africanized honeybees have unique tolerance to varroa mites. Trends in Parasitology, 20: 112-114. doi: 10.1016/j.pt.2004.01.001.

Mendoza, Y., Gramajo, E., Invernizzi, C. & Tomasco, I. H. (2020). Mitochondrial haplotype analyses of the mite *Varroa destructor* (Acari: Varroidae) collected from honeybees *Apis mellifera* (Hymenoptera, Apidae) in Uruguay. Systematic and Applied Acarology, 25: 1526-1529.

Mondragon, L., Martin, S. & Vandame, R. (2006). Mortality of mite offspring: a major component of *Varroa destructor* resistance in a population of africanized bees. Apidologie, 37: 67-74. doi: 10.1051/apido:2005053

Moretto, G., Gonçalves, L.S., De Jong, D. & Bichuette M.Z. (1991). The effects of climate and bee race on *Varroa jacobsoni* Oud. infestations in Brazil. Apidologie, 22: 197-20

Moretto, G., Gonçalves, L.S. & De Jong, D. (1997). Relationship between food availability and the reproductive ability of the mite *Varroa jacobsoni* in Africanized bee colonies. American Bee Journal, 137: 67-69.

Mortensen, A.N., Schmehl, D.R., Allsopp, M., Bustamante, T.A., Kimmel, C.B., Dykes, M.E. & Ellis, J.D. (2016). Differences in *Varroa destructor* infestation rates of two indigenous subspecies of *Apis mellifera* in the republic of South Africa.

Experimental and Applied Acarology, 68: 509-515. doi: 10.1007/s10493-015-9999-8

Muntaabski, I., Russo, R. M., Liendo, M. C., Palacio, M. A., Cladera, J. L., Lanzavecchia, S. B., Scannapieco, A. C. (2020). Genetic variation and heteroplasmy of *Varroa destructor* inferred from ND4 mtDNA sequences. Parasitology Research, 119: 411-421. doi: 10.1007/s00436-019-06591-5.

Navajas, M., Solignac, M., Le Conte, Y., Cros-Arteil, S. & Cornuet, J.M. (2002). The complete sequence of the mitochondrial genome of the honey-bee ectoparasite *Varroa destructor* (Acari: Mesostigmata). Molecular Biology and Evolution, 19: 2313-2317.

Oddie, M.A.Y., Burke, A., Dahle, B., Le Conte, Y., Mondet, F., Locke, B. (2021). Reproductive success of the parasitic mite (*Varroa destructor*) is lower in honeybee colonies that target infested cells with recapping. Scientific Report, 11: 9133.

Peixoto, C.M., Correia-Oliveira, M.E., Silva, F.L., Ramos, C.E.C.O., Carvalho, C.A.L. (2021). *Varroa destructor* in *Apis mellifer* colonies in Brazil. Journal of Apicultural Research, doi: 10.1080/00218839.2021.1960746

Pinto, F.A., Puker, A., Message, D., Barreto, L.M.R.C. (2011) *Varroa destructor* in Juquitiba, Vale do Ribeira, southeastern Brazil: seasonal effects on the infestation rate of ectoparasitic mites in honeybees. Sociobiology, 57: 511-518.

Pinto, F.A., Puker, A., Barreto, L.M.R.C. & Message D. (2012). The ectoparasite mite *Varroa destructor* Anderson and Trueman in southeastern Brazil apiaries: effects of the hygienic behavior of Africanized honey bees on infestation rates. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 64: 1194-1199. doi: 10.1590/S0102-09352012000500017

Pinto, F.A., Puker, A., Barreto, L.M.R.C. & Message, D. (2015). Infestation rate of the mite *Varroa destructor* in commercial apiaries of the Vale do Paraiba and Serra da Mantiqueira, southeastern Brazil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 67: 631-635. doi: 10.1590/1678-7264

R Development Core Team (2012). R: A Language and Environment for Statistical Computing. Vienna: R foundation for Statistical Computing.

Rosenkranz, P., Aumeier, P. & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology, 103: S96-S119. doi: 10.1016/j.jip.2009.07.016

Sheppard, W.S., Rinderer, T.E., Garnery, L. & Shimanuki, H. (1999). Analysis of Africanized honey bee mitochondrial DNA reveals further diversity of origin. Genetics and Molecular Biology, 22: 73-75. doi: 10.1590/S1415-47571999000100015

Solignac, M., Cornuet, J.M., Vautrin, D., Le Conte, Y., Anderson, D., Evans, J., Cros Arteil, S. & Navajas, M. (2005). The invasive Korea and Japan types of *Varroa destructor*, ectoparasitic mites of the Western honey bee (*Apis mellifera*), are two partly isolated clones. Proceedings. Biological Sciences, 272: 411-419. doi: 10.1098/rspb.2004.2853

Spivak, M. (1996). Honey bee hygienic behavior and defense against *Varroa jacobsoni.* Apidologie, 27: 245-260. doi: 10.1051/apido:19960407

Strapazzon, R., Carneiro, F.E., Guerra, J.C.V. & Moretto, G. (2009). Genetic characterization of the mite *Varroa destructor* (Acari: Varroidae) collected from honey bees *Apis mellifera* (Hymenoptera, Apidae) in the state of Santa Catarina, Brazil. Genetics and Molecular Research, 8: 990-997. doi: 10.4238/vol8-3gmr567

Thoms C.A., Nelson K.C., Kubas A., Steinhauer N. & Wilson M.E. (2019). Beekeeper stewardship, colony loss, and *Varroa destructor* management. Ambio: 48, 1209-1218. doi: 10.1007/s13280-018-1130-z