Association of *Varroa destructor* females in multiply infested cells of the honeybee *Apis mellifera*

Alexis L. Beaurepaire[1,2] , James D. Ellis[3], Klemens J. Krieger[4] and Robin F. A. Moritz[1,5]

[1]Institut fűr Biologie, Martin-Luther-University Halle-Wittenberg, Halle a. d. Saale, Germany;

[2]UR 406 Abeilles et Environnement, INRA, Centre de Recherche Provence-Alpes-Côte d'Azur, Avignon, France;

[3]Entomology & Nematology Department, University Florida, Gainesville, Florida, USA;

[4]Bayer Animal Health GmbH, Leverkusen, Germany and

[5]Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

Abstract

The genetic diversity of *Varroa destructor* (Anderson & Trueman) is limited outside its natural range due to population bottlenecks and its propensity to inbreed. In light of the arms race between *V. destructor* and its honeybee (*Apis mellifera* L.) host, any mechanism enhancing population admixture of the mite may be favored. One way that admixture can occur is when two genetically dissimilar mites coinvade a brood cell, with the progeny of the foundresses admixing. We determined the relatedness of 393 pairs of *V. destructor* foundresses, each pair collected from a single bee brood cell (*n* = five colonies). We used six microsatellites to identify the genotypes of mites coinvading a cell and calculated the frequency of pairs with different or the same genotypes. We found no deviation from random coinvasion, but the frequency of cells infested by mites with different genotypes was high. This rate of recombination, coupled with a high transmission rate of mites, homogenized the allelic pool of mites within the apiary.

Key words*: Apis mellifera*; host–parasite coevolution; inbreeding avoidance; mating behavior; population genetics; *Varroa destructor*

Introduction

As a result of large-scale transhumance, the western honeybee (*Apis mellifera* L.) became host to multiple new parasites and pathogens since the early 1900s (Oldroyd, 1999; Higes *et al*., 2006; Genersch & Aubert, 2010), including *Varroa destructor* (Anderson & Trueman, 2000). *V. destructor* is a devastating mite endemic to Southeast Asia where its natural host is *A. cerana*, the eastern or Asian honeybee. *V. destructor* managed to switch hosts successfully after colonies of the western honeybees were introduced into parts of eastern Russia in the late 1800s (Danka *et al*., 1995). Since then, *V. destructor* has spread globally (Rosenkranz *et al*., 2010). Though the mite only moderately impacts on *A. cerana*, its natural host (Peng *et al*., 1987), it is considered the primary biotic driver of colony losses experienced by managed western honeybees (van Engelsdorp *et al*., 2008; Le Conte *et al*., 2010). *V. destructor* reproduces in worker cells of its new host, causing lethal exponential

population growth rates in *A*. *mellifera* colonies, but not in *A. cerana* where the mite only reproduces in drone cells (Vandame *et al*., 2000).

The life cycle of *V. destructor* is characterized by two phases: a phoretic phase, where the mite occurs on adult bees, and a reproductive phase, where the mite invades and reproduces in a brood cell. A female mite will normally perform two-to-three of these cycles during her lifetime (Fries & Rosenkranz, 1996). In the reproductive phase, a "foundress" mite (the mite invading the cell) will produce only one male and an average of 1.30–1.45 mature females when infesting worker cells, but close to double this amount when infesting drone cells (Martin, 1994, 1995). The reproduction of *V. destructor* is therefore highly dependent on brood availability in its host colony (Beaurepaire *et al*., 2017). When there are more brood cells than mites in the colony, the cells typically will be infested by a single foundress mite (single infestation). In that case, mating will occur between the offspring of this mite: a male and his sisters. As a result of incestuous mating, multiple, highly inbred mite lineages will coexist in the colony. However, as the infestation expands and the ratio of mites-to-brood cells increases, cell invasion by multiple foundresses ("multiple infestations") becomes more frequent and the progeny from different mites may admix. This differential mating dynamic essentially shapes the genetic structure of the mite population with a dramatic reduction of heterozygosity in the mite populations in the beginning of the season and an increase of recombination toward the end of the brood season (Beaurepaire *et al*., 2017).

Genetic diversity is a key component of the evolutionary arms race between *V. destructor* and its new host (Delaplane *et al*., 2015). Consequently, any inbreeding may severely limit the potential of mite populations to adapt to their host's defenses. Indeed, enhancing the limited opportunities for recombination and exogamy will be of high adaptive value for *V. destructor* and its coevolution with its host (Schmid-Hempel, 2011; Nazzi & Le Conte, 2016). As only multiple infestations can allow for restoring heterozygosity in the inbred mite lineages, it would be highly adaptive if foundresses could choose cells that are already infested by a foundress of another inbred line in order to avoid further inbreeding. Consequently, we determined if *V. destructor* females infested brood cells of related or unrelated mites by genotyping foundress pairs collected from freshly sealed worker brood cells with microsatellite DNA markers. Our findings shed light on the reproduction of *V. destructor* and help understanding how the mating behavior of this major honeybee parasite influences its gene flow within and between hives.

Materials and methods

Sampling

A total of 960 pairs of *V. destructor* female foundresses were collected in October 2015 from capped brood cells (Dietemann *et al*., 2013) in each of five highly infected European-derived honeybee colonies (192 reproductive *Varroa* pairs per colony) not treated in 2015 and managed in the research apiary of the University of Florida (Gainesville, FL, USA). The mites were collected only from cells containing two foundress mites rather than 1 or ≥3 mites. The capped brood cells contained honeybee prepupae or early-stage pupae to ensure that the mites being collected were foundress mites rather than offspring. All mite pairs were

separated, and placed in 96-well plates containing 100% ethanol. Each mite was placed into its own well to avoid contamination with DNA from other mites. All plates were marked such that the mite pairs could be distinguished from one another. The well plates were stored frozen (−20°C) until DNA extraction.

DNA isolation and genotyping

The mite samples were washed in ddH_2O, crushed, and their DNA extracted using a standard Chelex protocol (Walsh *et al*., 1991). A total of 24 previously published polymorphic microsatellites (Evans, 2000; Solignac *et al*., 2003; Cornman *et al*., 2010; Beaurepaire *et al*., 2017) were tested on a subsample of 72 mites from three colonies (24 mites per colony) in order to determine the degree of polymorphism in the focus population (Table 1). Out of the 24 markers tested, 6 were polymorphic in the subsample of 72 mites. Those six markers were therefore used to genotype the individuals in a MEGABACE DNA Analysis System (GE Healthcare Life Science, Buckinghamshire, England) using the Fragment Profiler software V. 1.2.

Table 1 Sample and marker statistics.

Microsatellite marker	N ➡	Colony 1 298	Colony 2 130	Colony 3 104	Colony 4 142	Colony 5 112	Overall 786
VD112	N_A	2	2	2	2	2	2
	N_P	0	0	0	0	0	0
	H_E	0.187	0.053	0.126	0.173	0.052	0.137
	H_O	0.040	0.023	0.038	0.077	0.018	0.041
Vj292	N_A	3	1	2	4	3	5
	N_P	1	0	0	0	0	1
	H_E	0.020	0.000	0.109	0.069	0.110	0.051
	H_O	0.013	0.000	0.038	0.014	0.027	0.017
Vj294	N_A	3	2	3	3	2	4
	N_P	1	0	0	0	0	1
	H_E	0.111	0.187	0.093	0.170	0.027	0.121
	H_O	0.017	0.023	0.038	0.028	0.009	0.022
Vdes-01	N_A	2	2	2	3	2	3
	N_P	0	0	0	1	0	1
	H_E	0.377	0.292	0.318	0.354	0.343	0.347
	H_O	0.060	0.108	0.106	0.141	0.063	0.089
Vdes-02	N_A	2	2	2	2	2	2
	N_P	0	0	0	0	0	0
	H_E	0.125	0.143	0.056	0.125	0.086	0.114
	H_O	0.054	0.046	0.019	0.021	0.036	0.039
Vdes-04	N_A	3	2	2	2	3	4
	N_P	1	0	0	0	1	2
	H_E	0.460	0.431	0.502	0.471	0.406	0.464
	H_O	0.067	0.038	0.067	0.077	0.098	0.069
Overall	H_E	0.213	0.184	0.201	0.227	0.171	0.206
	H_O	0.042	0.040	0.051	0.060	0.042	0.046

Note: The table summarized diversity statistics for the five colonies and six microsatellite markers (N: sample size, N_A: number of alleles, N_P: number of private alleles, H_E: expected heterozygosity, H_O: observed heterozygosity). Other markers that were tested included VD125, VD126, VD152, and VD154 from Evans (2000); VjL3B2, Vj275, Vj295, and Vj296 from Solignac *et al.* (2003); VD305, VD306, and VD307 from Cornman *et al.* (2010); Vdes-03 and Vdes05–Vdes10 from Beaurepaire *et al.* (2017).

Microsatellite DNA analyses

In all, 393 pairs of mites were analyzed (149, 65, 52, 71, and 56 mite pairs from colonies 1–5 respectively, Table 1). Diversity estimates were calculated for the mites by colony and

marker with the Microsatellite Toolkit (Park, 2008). Hardy–Weinberg equilibrium within and among all loci and linkage disequilibrium between all pairs of loci were estimated using Fstat v 2.9.3 (Goudet, 1995). An Analysis of Molecular Variance (AMOVA) was performed to estimate how genetic variation in the mites was distributed between and within the sampled colonies using Arlequin 3.5.1.2 (Excoffier, 2010).

The mite genotypes were identified and analyzed manually to determine their occurrence, and distribution in the colonies. All following analyses were performed with R V. 3.1.2 (R Core Team, 2016). The genotypes were separated into two categories: inbred lineages (if all markers were homozygous) and recombinant genotypes (if at least one locus was heterozygous). Finally, the distributions of the different genotypes in the five colonies were compared using a Kruskall–Wallis test.

The frequencies of the mite genotypes were used to determine the probabilities for the pairing of mites among the various lineages assuming random infestation as follows:

$$P_E = \sum_{0}^{i} p_i^2, \quad (1)$$

where P_E is expected frequency of identical genotype association at random mating and p_i is observed frequency of the *i*th genotype in the colony.

The observed frequency of identical and nonidentical mite genotypes was compared to the random expectation calculated as described above and tested with a goodness of fit test. The power and the effect size of these tests were estimated using G-Power (Faul *et al*., 2007).

Results

A total of 393 *V. destructor* pairs (786 individuals) from the five honeybee colonies were analyzed (Table 1). None of the six markers were in Hardy–Weinberg equilibrium due to high levels of inbreeding (overall F_{IS} = 0.776). After Bonferroni corrections to adjust *P* values for multiple testing, none of the marker pairs showed significant linkage. The six markers had an average of 3.33 ± 1.21 alleles per marker (Table 1) and a low observed heterozygosity (H_O = 0.046 ± 0.003), as commonly reported in *V. destructor* (Solignac *et al*., 2003, 2005). A few alleles were found exclusively in one of the five colonies (Table 1), but these private alleles were very rare (<5%). Accordingly, the AMOVA showed that the genetic variation among colonies was low (<1%) but significant, and that almost all (>99%) observed variation resulted from differences among the individual mites within the colonies (Table 2).

Table 2 Analysis of molecular variance.

Level	Sum of squares	Variance	% Variation	*P* value
Among colonies	13.854	0.005	0.790	<0.05
Within colonies	1198.339	0.614	99.210	<0.001
Total	1212.193	0.619		

Note: The results of the AMOVA performed with the software Arlequin to detect the degree and significance level of genetic variance within and among the five *A. mellifera* colonies.

Altogether, 74 genotypes were detected within the mite samples across all five colonies. These genotypes included 27 inbred lineages (36.5%), which provide the vast majority of the mites in the total sample (81.1%, Fig. 1). The six most frequent mite genotypes were found in all five colonies, composing >72% of the total sample (Fig. S1) and the distribution of the genotypes did not differ significantly among the five colonies (Kruskall–Wallis test, P = 0.157).

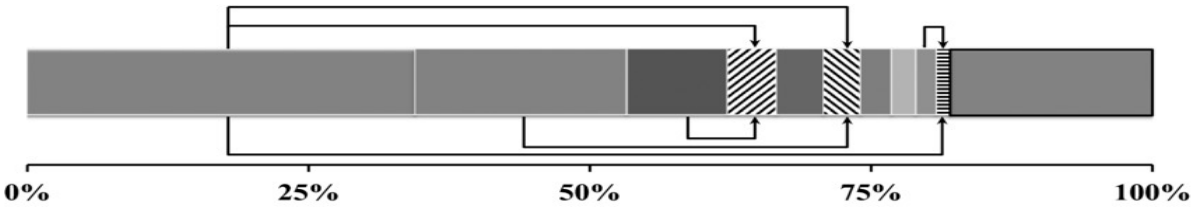

Figure 1 .Overall frequency of mite genotypes. The frequency of the ten most common genotypes from the five colonies. The black and grey boxes represent the common inbred lineages (N = 8), the striped ones the recombinants (N = 2), and the white box the frequency of the rare lineages (N = 64). The arrows indicate the inbred lineage crosses needed to produce each recombinant genotype.

Given the frequency of mite genotypes, the expected frequency of mites pairing with an identical genotype was low (16.13% ± 3.30%) and very similar to the frequency observed over all five colonies (16.79% ± 5.88%). Although the association of foundresses with different genotypes was slightly higher than expected, it was nowhere near to being significantly different from that of a random pairing of mites (χ^2, P > 0.05; Fig. 2). Hence, we have no evidence that foundress mites preferentially invade brood cells already invaded by mites with identical or different genotypes. This was also observed when analyzing inbred lineages and the recombinant genotypes separately (Table S1). The *post hoc* tests performed with G-Power (Faul *et al*., 2007) revealed that any potential effect size biasing the foundress choice in favor of an unrelated cofoundress was indeed minute (w = 0.016).

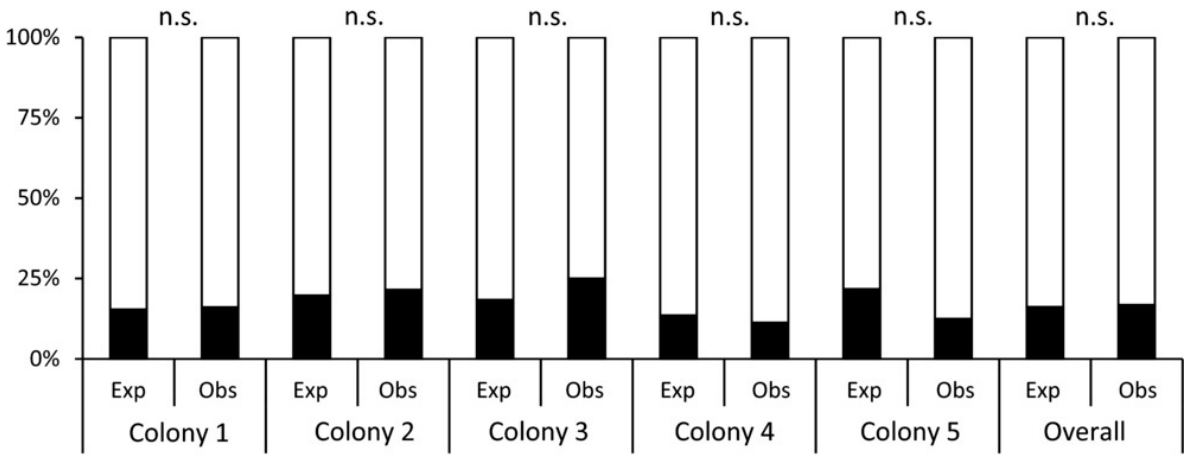

Figure 2. Comparison of genotypes association. The frequency of mites with the same (black) and different (white) genotypes coinfesting brood cells of five *A. mellifera* colonies. n.s. = not significant (χ^2 test, $P < 0.05$).

Discussion

In the animal kingdom, inbreeding avoidance can be achieved in many different ways, including long-range dispersal and kin avoidance (Blouin & Blouin, 1988; Pusey & Wolf, 1996). However, *V. destructor* has only a few options available to avoid inbreeding during its life cycle. In this parasite, dispersal after the reproduction phase is predominantly mediated by the emerging workers, which carry the mites on their body surface. Yet these bees hatch in a comb region that typically does not contain any brood stages that can be infested directly. Hence, the mites either need to switch host bees in the hive or wait until their newly emerged hosts move to other regions in the hive where the appropriate brood stages are available. Workers that are engaged in sealing brood cells can carry the mite to the right location in the hive and allow the mites to re-infest a brood cell. In addition, mites can disperse between colonies on foragers that drift into a foreign hive rather that their own. Mite movement to its new host (brood or another adult bee) may rely on the movement of its existing host. However, it can also decide its own fate by leaving an already infested cell and chose a neighboring one, either noninfested or infested with a related or unrelated mite.

Given recent findings showing low levels of genetic differentiation among *Varroa* populations over large scales (Beaurepaire *et al*., 2015; Roberts *et al*., 2015; Dynes *et al*., 2016), we chose to investigate a finite number of colonies from a single apiary. This way, we were able to analyze a large number of mite pairs per colony and managed to appreciate for the first time the true extent of intra-colonial genetic diversity in *V. destructor*. In spite of previous reports that included a few individuals sampled per colony and suggested a highly reduced genetic variance in *V. destructor* (Solignac *et al*., 2003, 2005), we found an average of 28.8 ± 11.7 genotypes/colony based on 157.2 ± 80.1 sampled mites for each of the five tested colonies. Additionally, our AMOVA showed that the genetic variation between individual mites within colonies was most important while only very few differences were found in genotypic composition of mites among the five colonies. As the colony level had only very little influence on the population structure of the parasite, our results suggest that *Varroa* moves readily among hives at the apiary level, in accordance with other studies

(Seeley & Smith, 2015; DeGrandi-Hoffman *et al*., 2016; Peck *et al*., 2016; Nolan & Delaplane, 2017).

The comparison of the genotypes of the foundress pairs suggested that the association of mites in a multiply infested cell is mainly driven by random choice rather than any specific adaptive behavior of the foundresses: mites neither with the same nor different genotypes preferentially coinvaded cells. Although we observed slightly more mites of the same genotype in brood cells (16.79%) compared to random expectations (16.13%), the pairing of *V. destructor* females in a brood cell did not significantly enhance the potential for inbreeding of mites beyond random choice in the colony. So despite their excellent chemical sensory system (Nazzi & Le Conte, 2016), *V. destructor* females do not discriminate between kin and nonkin when coinfesting a brood cell. Though the females do not appear to use such cues to avoid inbreeding, we do not know if the males are able to avoid sib-matings. Indeed, they would be in a very powerful position to reduce inbreeding by preferentially mating with the female offspring of a foundress mite that is not their mother. Yet, investigating this question would require more complex experimental genotyping studies.

Although our results suggest that *V. destructor* foundresses do not actively influence exogamy by coinvading a brood cell with a foundress mite of the same or different genotype, the vast majority of the cells (83.21%) contained foundress mites with different genotypes. Even if male mites would mate at random and not discriminate between sisters and unrelated females, the level of heterozygosity in the colonies would rapidly increase in the coinfestation phase when the mite-to-brood ratio is high (Calis *et al*., 1999; Beaurepaire *et al*., 2017).

To conclude, active inbreeding avoidance during coinfestation of multiple foundresses seems not to be a strong driver of *V. destructor* population structure in the colony. However, the gene flow among colonies is an important factor in the population dynamics of this parasite. The drift of mites among colonies ensures the presence of a high number of different lineages in all colonies on the apiary. This homogenous population structure greatly facilitates restoration of the levels of heterozygosity after phases of inbreeding in all colonies in the apiary. Despite that *V. destructor* is an invasive species that can reproduce incestuously, the high levels of genetic variability and potential for recombination we found must be considered carefully when designing studies with *Varroa* sp.

Acknowledgments

We thank Bayer Animal Health for providing funding for this project. We graciously acknowledge the field assistance of Logan Cutts, Cameron Jack, Brandi Simmons, Mary Bammer, Ashley Mortensen, Siria Gamez, Liana Teigen and Brandon Stanford (all with the University of Florida). We also want to thank Petra Leibe and Denise Kleber for their help in the laboratory, together with Christin Hesse, David Matuschek and Tom Wippold for their help in the lab during the summer courses in 2016 (all with Martin Luther University).

Disclosure

The authors of this work declare no conflict of interest.

References

Anderson, D.L. and Trueman, J.W.H. (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology*, 24, 165– 189.

Beaurepaire, A.L., Krieger, K.J. and Moritz, R.F.A. (2017) Seasonal cycle of inbreeding and recombination of the parasitic mite *Varroa destructor* in honeybee colonies and its implications for the selection of acaricide resistance. *Infection, Genetics and Evolution*, 50, 49– 54.

Beaurepaire, A.L., Truong, T.A., Fajardo, A.C., Dinh, T.Q., Cervancia, C. and Moritz, R.F.A. (2015) Host specificity in the honeybee parasitic mite, *Varroa* spp. in *Apis mellifera* and *Apis cerana*. *PLoS ONE*, 10. https://doi-org.uplib.idm.oclc.org/10.1371/journal.pone.0135103.

Blouin, S.F. and Blouin, M. (1988) Inbreeding avoidance behaviours, *Trends in Ecology and Evolution*, 3(9), 230– 233.

Calis, J.N.M., Fries, I. and Ryrie, S.C. (1999) Population modelling of *Varroa jacobsoni* Oud. *Apidologie*, 30(2–3), 111– 124.

Le Conte, Y., Ellis, M. and Ritter, W. (2010) Varroa mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie*, 41, 353– 363.

Cornman, S.R., Schatz, M.C., Johnston, S.J., Chen, Y.P., Pettis, J., Hunt, G. *et al*. (2010) Genomic survey of the ectoparasitic mite *Varroa destructor*, a major pest of the honey bee *Apis mellifera*. *BMC genomics*, 11, https://doi-org.uplib.idm.oclc.org/10.1186/1471-2164-11-602.

Danka, R.G., Rinderer, T.E., Kuznetsov, V.N. and Delatte, G.T. (1995) A USDA-ARS project to evaluate resistance to *Varroa jacobsoni* by honey bees of far-eastern Russia. *American Bee Journal*, 135, 746– 748.

DeGrandi-Hoffman, G., Ahumada, F., Zazueta, V., Chambers, M., Hidalgo, G. and deJong, E.W. (2016) Population growth of *Varroa destructor* (Acari: Varroidae) in honey bee colonies is affected by the number of foragers with mites. *Experimental and Applied Acarology*, 69, 21– 34.

Delaplane, K.S., Pietravalle, S., Brown, M.A. and Budge, G.E. (2015) Honey bee colonies headed by hyperpolyandrous queens have improved brood rearing efficiency and lower infestation rates of parasitic *Varroa* mites, *PLoS ONE*, 10(12), https://doi-org.uplib.idm.oclc.org/10.1371/journal.pone.0142985.

Dietemann, V., Nazzi, F., Martin, S.J., Anderson, D.L., Locke, B. and Delaplane, K.S. (2013) Standard methods for *Varroa* research. The Coloss Beebook, Volume II: Standard Methods for Apis Mellifera Pest and Pathogen Research (eds. V. Dietemann, J.D. Ellis & P. Neumann), *Journal of Apicultural Research*, 52, 1– 54.

Dynes, T.L., De Roode, J.C., Lyons, J.I., Berry, J.A., Delaplane, K.S. and Brosi, B.J. (2016) Fine scale population genetic structure of *Varroa destructor*, an ectoparasitic mite of the honey bee (*Apis mellifera*). *Apidologie*, 48, https://doi-org.uplib.idm.oclc.org/10.1007/s13592-016-0453-7.

van Engelsdorp, D., Hayes, J., Underwood, R.M. and Pettis, J. (2008) A survey of honey bee colony losses in the U.S., fall 2007 to Spring 2008. *PLoS ONE*, 3, https://doi-org.uplib.idm.oclc.org/10.1371/journal.pone.0004071.

Evans, J.D. (2000) Microsatellite loci in the honey bee parasitic mite *Varroa jacobsoni*. *Molecular Ecology*, 9, 1436– 1438.

Excoffier, L. (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564– 567.

Faul, F., Erfefelder, E., Lang, A. and Buchner, A. (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39, 175– 191.

Fries, I. and Rosenkranz, P. (1996) Number of reproductive cycles of *Varroa jacobsoni* in honey-bee (*Apis mellifera*) colonies. *Experimental and Applied Acarology*, 20, 103– 112.

Genersch, E. and Aubert, M. (2010) Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). *Veterinary Research*, 41, 6, https://doi-org.uplib.idm.oclc.org/10.1051/vetres/2010027.

Goudet, J. (1995) FSTAT (Version 1.2): a computer program to calculate *F*-statistics', *Heredity*, 96, 485– 486.

Higes, M., Martin, R. and Meana, A. (2006) *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *Journal of Invertebrate Pathology*, 92, 93– 95.

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.

Martin, S.J. (1995) Ontogenesis of the mite *Varroa jacobsoni* Oud. in drone brood of the honeybee *Apis mellifera* L. under natural conditions. *Experimental and Applied Acarology*, 19, 199– 210.

Nazzi, F. and Le Conte, Y. (2016) Ecology of *Varroa destructor*, the major ectoparasite of the Western honey bee, Apis mellifera. *Annual Review of Entomology*, 61, 417– 432.

Nolan, M.P. and Delaplane, K.S. (2017) Distance between honey bee *Apis mellifera* colonies regulates populations of *Varroa destructor* at a landscape scale. *Apidologie*, 48, 8– 16.

Oldroyd, B.P. (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. *Trends in Ecology and Evolution*, 312– 315. https://doi-org.uplib.idm.oclc.org/10.1016/S0169-5347(99)01613-4.

Park, S.D.E. (2008) Excel Microsatellite Toolkit.

Peck, D.T., Smith, M.L. and Seeley, T.D. (2016) *Varroa destructor* mites can nimbly climb from flowers onto foraging honey bees. *PLoS ONE*, 11(12). https://doi-org.uplib.idm.oclc.org/10.1371/journal.pone.0167798.

Peng, Y.S., Fang, Y., Xu, S. and Ge, L. (1987) The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. *Journal of Invertebrate Pathology*, 49, 54– 60.

Pusey, A. and Wolf, M. (1996) Inbreeding avoidance in animals. *Trends in Ecology and Evolution*, 11, 201– 206.

R Core Team (2016) R: A Language and Environment for Statistical Computing. Vienna, Austria. Available at: http://www.r-project.org/.

Roberts, J.M.K., Anderson, D.L. and Tay, W.T. (2015) Multiple host shifts by the emerging honeybee parasite, *Varroa jacobsoni*. *Molecular Ecology*, 24, 2379– 2391.

Rosenkranz, P., Aumeier, P. and Ziegelmann, B. (2010) Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103, S96– S119.

Schmid-Hempel, P. (2011) Evolutionary Parasitology. Oxford University Press, Oxford, UK.

Seeley, T.D. and Smith, M.L. (2015) Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. *Apidologie*, 46, 716– 727.

Solignac, M., Cornuet, J.M., Vautrin, D., Le Conte, Y., Anderson, D., Evans, J. *et al*. (2005) The invasive Korea and Japan types of *Varroa destructor*, ectoparasitic mites of the Western honeybee (*Apis mellifera*), are two partly isolated clones. *Proceedings. Biological Sciences / The Royal Society*, 272, 411– 419.

Solignac, M., Vautrin, D., Pizzo, A., Navajas, M., Le Conte, Y. and Cornuet, J.M. (2003) Characterization of microsatellite markers for the apicultural pest *Varroa destructor* (Acari: Varroidae) and its relatives. *Molecular Ecology Notes*, 3, 556– 559.

Vandame, R., Colin, M.E., Morand, S. and Otero-Colina, G. (2000) Levels of compatibility in a new host-parasite association: *Apis mellifera/Varroa jacobsoni*. *Canadian Journal of Zoology*, 78, 2037– 2044.

Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechnique*, 10, 506– 513.