

Original article

Resistance to the parasitic mite *Varroa destructor* in honey bees from far-eastern Russia

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(Received 15 February 2001; revised and accepted 18 May 2001)

Abstract – *Varroa destructor* is a parasitic mite of the Asian honey bee, *Apis cerana*. Owing to host range expansion, it now plagues *Apis mellifera*, the world's principal crop pollinator and honey producer. Evidence from *A. mellifera* in far-eastern Russia, (Primorsky) originating from honey bees imported in the mid 1800's, suggested that many colonies were resistant to *V. destructor*. A controlled field study of the development of populations of *V. destructor* shows that P colonies have a strong, genetically based resistance to the parasite. As control colonies (D) were dying with infestations of ca. 10000 mites, P colonies were surviving with infestations of ca. 4,000 mites. Several characteristics of the P bees contributed to suppressing the number of mites parasitizing their colonies.

Apis mellifera / mite resistance / *Varroa destructor* / Russia

1. INTRODUCTION

In Asia, *Varroa destructor* Anderson and Truman, 2000 is an innocuous external parasitic mite of *Apis cerana*, the Asian hive bee. However, *V. destructor* has made a host

shift to *Apis mellifera*, the western honey bee that is employed world-wide for pollinating crops and producing honey (Boot et al., 1997; Rath, 1999). The parasite spread rapidly and now infests most the world's *A. mellifera*. This host shift has been

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devastating to apiculture. Apiculturists use acaricides to maintain their colonies, but are stymied by mite populations that develop resistance to acaricides, and are burdened by growing costs of controlling mites and replacing lost colonies. Without effective acaricide treatments, repeated as often as three times a year, *A. mellifera* colonies die from mite infestations. Feral and wild populations of *A. mellifera*, and the pollination afforded by them, have been essentially eliminated in North America and Europe by the mite (Oldroyd, 1999). While some free-living colonies continue to be discovered (Kraus and Page, 1995), they are probably founded by recent swarms from kept colonies protected by acaracides.

Using *A. mellifera* that are resistant to *V. destructor* would greatly benefit agriculture and would help re-establish free-living populations of honey bees. However, producing or finding suitable resistant stocks has proved daunting since most of the variance in resistance in domestic European colonies originates from environmental or random sources (Kulinčević et al., 1997). Starting with colonies that survived an epizootic of *V. destructor*, Kulinčević et al. (1992) produced a stock of honey bees which was only slightly more resistant than other stocks (de Guzman et al., 1996). Harbo and Harris, (1999) have bred a line of bees which suppresses reproduction of *V. destructor* and may be a source of genetic resistance for breeding commercial stocks. However, numerous breeding programs have encountered insufficient genetic variance to produce resistant stock.

Africanized honey bees, hybrid descendants of African and European honey bees imported to South America, (Rinderer et al., 1991) have been reported to be resistant to *V. destructor* in Brazil based on various differences from European honey bees other than survival (Camazin,e 1986, 1988; Moretto et al., 1993; Moretto, 1997; Moretto and de Mello, 1999). However, considering survival, European honey bees kept in Brazil

after importation from the United States also appear to be resistant (De Jong and Soares, 1997) as do European honey bees in Uruguay (Rosenkranz, 1999). The origin of the *V. destructor* in Brazil is Japan via neighboring Paraguay (de Jong et al., 1982) rather than Russia, the origin of the mites in most of the world (de Guzman et al., 1997; de Guzman et al., 1998; de Guzman and Rinderer, 1999; de Guzman et al., 1999). Perhaps the *V. destructor* from Japan are hypovirulent. In any case, Africanized honey bees are undesirable for agriculture owing to their extreme defensive behavior (Collins et al., 1982), poor honey production (Rinderer et al., 1984, 1985), and management difficulties (Danka et al., 1987), as are the reportedly resistant honey bees of Tunisia (Boecking and Ritter, 1993). Occasionally, feral colonies in the USA have been reported to survive *V. destructor* infestations. However, when tested, none have been found to be resistant to *V. destructor* (Danka et al., 1997).

One possible source of commercially useful resistant European honey bees is far-eastern Russia (Primorsky) where European settlers took *A. mellifera* in the mid 1800s (Crane, 1978). The area has native *A. cerana* infested with *V. destructor* which most likely infested the arriving *A. mellifera*, resulting in the longest known association of *A. mellifera* and *V. destructor* (Danka et al., 1995). Preliminary examinations of *A. mellifera* in the Primorsky territory (P) suggested that they might have substantial levels of mite resistance (Danka et al., 1995). These observations inspired the importation and further testing of a sample of P honey bee queens (Rinderer et al., 1997). An initial evaluation indicated that their commercial traits such as honey production were similar to those of existing commercial stocks (Rinderer et al., 1999b). Most importantly, some of the imported P queens produced colonies which appeared to be resistant to *V. destructor* (Rinderer et al., 1999b).

An experiment was undertaken to compare the mite population growth (MPG) of

V. destructor in P colonies produced by daughter queens of resistant P queens with MPG in colonies that represented stocks used commercially in the United States (D). The goals of the experiment were to determine: (1) comparative resistance, (2) if the resistance was inherited, and (3) some possible mechanisms of resistance.

2. MATERIALS AND METHODS

Daughter queens were raised from promising P queens imported from Russia (Rinderer et al., 1999b). Queens were collected within a 200 km² north and west of Vladivostock (131°54'E 43°7'N). Mated to drones from similar queens on Gran Terre Island, Louisiana to assure controlled matings. Four stocks of commercial honey bees were represented in the (D) colonies. These stocks were derived from 10 or more years of selection by commercial breeders from apiaries debilitated by *V. destructor*. No further screening was done among the D colonies since numerous selection efforts within these stocks for vigor and production despite *V. destructor* had already occurred.

In June 1998, 22 daughter queens were established in standard hives with about 1.35 kg of P worker bees. The worker bees came from a common pooling which was sampled (15 subsamples washed in alcohol) to estimate the number of adult female *V. destructor* in each colony. During the first week the bees were in the hives, dead mites that fell to the floor of the hive were collected on screen protected sticky boards, counted, and their numbers were subtracted from the initial mite infestation of each colony, resulting in an estimate of 305 ± 32 adult mites per colony. Similarly, 22 queens of D stocks were established with about 1.35 kg of D worker bees harboring about 223 ± 18 mites. All the *V. destructor* came from the current United States population of mites: at no time have we imported living mites from Russia.

The inequality in numbers of mites resulted from colonies being established from two different "bulk packages". We consider this technique to be the most certain way to establish precise numbers of mites in experimental colonies. However, because one bulk package was impossible because of size limits, either differential numbers of bees or differential numbers of mites at the beginning of the experiment had to be accepted. Since the P colonies would be disadvantaged by having more mites but would be similar to D colonies in respect to the number of bees, and since the experiment tested the null hypothesis that P colonies were not more resistant to *V. destructor*, we selected the option which challenged P colonies with more mites.

P and D colonies were divided equally into two apiaries near Baton Rouge, Louisiana with random colony placement within apiaries. All colonies were inspected weekly for maintenance purposes. The experimental apiaries lost 6 colonies due to non-mite related reasons during 1998 and one early in 1999 (Tab. I). These colonies were replaced with reserve colonies established for this purpose having similar stock, histories, and mite levels. Also, following our standard management practice, all colonies were requeened in Apr. When supersEDURE queens or queenless colonies were detected, the colonies were requeened with queens of the appropriate stock (Tab. I). SupersEDURE queens were unacceptable since they likely mated with mixtures of P and D drones and would produce hybrid colonies. When replacement queens were not available, the colonies were excluded from the remainder of the experiment (Tab. I). Many colonies "died" from varroosis (Tab. I). Some colonies were considered dead when they had lost half or more of their adult bee population, had numerous deformed bees, had scattered and dead brood, and had at least 35% worker brood infestations. The remainder of the dead colonies were approaching these conditions the previous month and had

Table I. Status of colonies through time showing requeening, colony replacement and loss.

dwindled to no or only a very few bees when they were declared dead.

V. destructor population dynamics were evaluated using a comprehensive suite of measurements. Each month (excepting Dec. and Jan. when cold would have damaged exposed brood), the number of adult female mites (including nearly adult female mites) in each colony was estimated. These estimates were derived from: (a) counts of mites in 200 worker brood cells (50 from each side of two combs), (b) counts of mites in 100 drone brood cells (50 from each side of one comb or from several nest areas when drone brood was scattered), (c) estimates of mites per 100 adult worker bees derived from counts of mites removed from 300 to 600 adult worker bees by the bees being washed in ethanol, (d) comb by comb estimates (to the nearest 5%) of the numbers of sealed worker and drone brood cells in the hive, and (e) comb by comb estimates of the number of bees (to the nearest 5%)

comprising the colonies. Brood sampling involved opening cells along a row of cells through the center of the brood pattern to avoid bias resulting from the patchy distribution of infested cells. Mother mites and their daughters were individually recognized according to the methods of Ifantidis (1983).

One week each month, the mites that fell to the floor of hives were collected on sticky boards. The boards were fitted with screens which prevented bees from biting mites after they were on the board. These mites were examined using a microscope for damage and mandible marks resulting from worker bees biting mites (grooming) (Ruttner and Hänel, 1992; Boeking and Ritter, 1993).

3. RESULTS

In 1998, estimated mite populations remained small in both groups of colonies (Fig. 1). However, the mite population

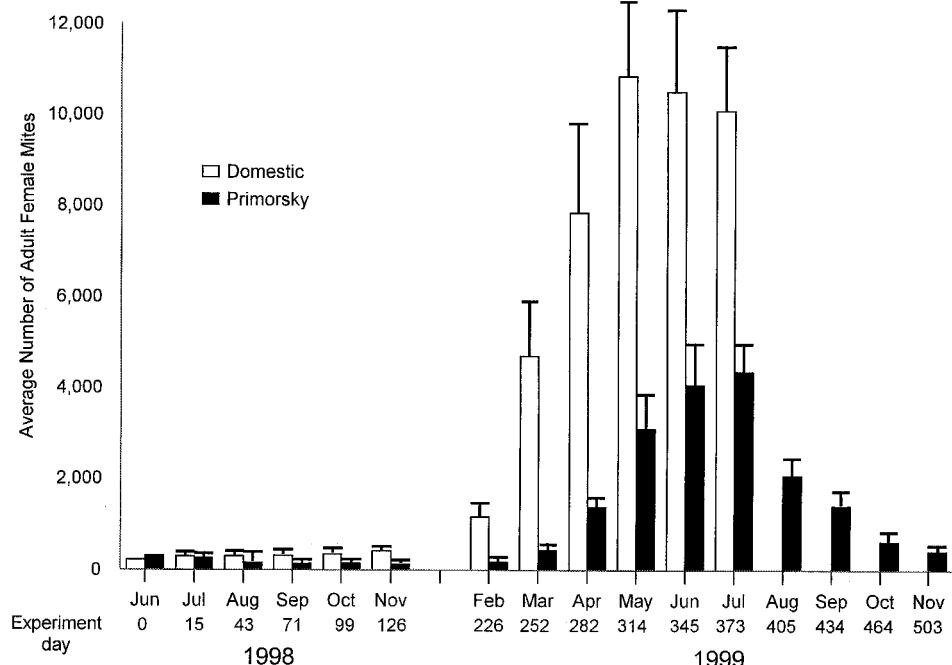


Figure 1. Average *V. destructor* infestations (numbers of adult female mites) in Primorsky (black bars) and domestic colonies (white bars) through time. Error bars = sem.

Table II. Month to month mite population growths. Numbers less than 1 indicate population declines.

Period	Primorsky colonies	Domestic colonies
June 98 – July 98	0.89	1.31
July 98 – Aug. 98	0.57	1.02
Aug. 98 – Sept. 98	0.97	1.06
Sept. 98 – Oct. 98	0.94	1.08
Oct. 98 – Nov. 98	0.85	1.21
Nov. 98 – Feb. 99	1.38	2.82
Feb. 99 – Mar. 99	2.58	4.06
Mar. 99 – Apr. 99	3.39	1.75
Apr. 99 – May 99	2.36	1.32
May 99 – June 99	1.14	0.97
June 99 – July 99	0.91	0.96
July 99 – Aug. 99	0.50	–
Aug. 99 – Sept. 99	0.64	–
Sept. 99 – Oct. 99	0.40	–
Oct. 99 – Nov. 99	0.93	–

numbers showed no trend for P colonies ($P = 0.72$) and an increasing trend for D colonies ($P = 0.003$) (Gibbons, 1994). By Nov., the P colonies harbored fewer mites than D colonies ($0 \pm \text{sem}$ (mean \pm standard error of the mean)): $P = 108 \pm 31$, D = 395 ± 93 ; t -test with unequal variance, $P = 0.007$.

In 1999, mite populations in D colonies rose sharply (Fig. 1, Tab. II). The increase in numbers was greatest in Feb. to Mar. (4 fold) (Tab. II). The rate of increase then slowed from Mar. through May as numbers were reached that caused colony deaths. By May, mite populations averaged 10844 ± 1665 ($0 \pm \text{sem}$) in D colonies and 7 of the D colonies died from varroosis. After May, the average mite populations no longer increased in D colonies since the D colonies with the highest mite populations were dying. At the end of July, all of the D colonies were gone from the experiment. Eighteen died from varroosis and four were lost because of queen supersedure or loss.

In comparison, the MPG in P colonies was restricted (Tab. II, Fig. 1). The MPG was 2.6 fold in Feb. to Mar., grew to its

highest in Mar. to Apr. (3.4 fold) and then slowed. After July, mite populations decreased (Tab. II, Fig. 1). During each month of 1999 (Feb. to July) the number of mites in P colonies was substantially less than in D colonies (all t -tests; $P < 0.05$) with averages of about 6000 fewer mites in May, June and July. From July on, the average numbers of mites in P colonies showed a decreasing trend ($P = 0.002$), going from about 4 000 in July to about 400 in Nov. Near the end of July, three P colonies died of varroosis with an average of 7896 mites. No other P colonies were lost from varroosis (Tab. I).

Separate data sets for the amounts of worker brood, adult worker bees and worker brood/adult bee ratios were analyzed as a completely randomized design with a two-way structure (stock = P and D colonies) having 11 repeated measures over time. Although these measures changed seasonally in both P and D colonies ($P < 0.05$), neither the stock by time interactions nor the stock main effects were significant ($P > 0.35$) (Fig. 2). Numbers of drone cells were analyzed using a generalized linear model with a Poisson error structure. The stock by time interaction was not significant ($P = 0.18$). Both date ($P = 0.0001$) and stock ($P = 0.02$) effects were significant with P colonies having more drones (Fig. 2).

The population dynamics data from this experiment were further evaluated. P colonies consistently had a smaller percentage of their total mites infesting worker brood, and a larger percentage on adult workers than did D colonies (Fig. 2; Π^2 test of independence, $P = 0.0001$, P^2 test of heterogeneity, NS). Also, a greater proportion of mites in P colonies infested drone brood (Fig. 2; Π^2 test of independence, $P = 0.0001$).

A greater percentage of the dead mites collected from the P colonies had physical damage attributable to grooming ($P = 42\%$ (8265 of 19680 mites), D = 28% (15712 of 56116 mites), Π^2 test of independence, $P = 0.0001$, P^2 test of heterogeneity between

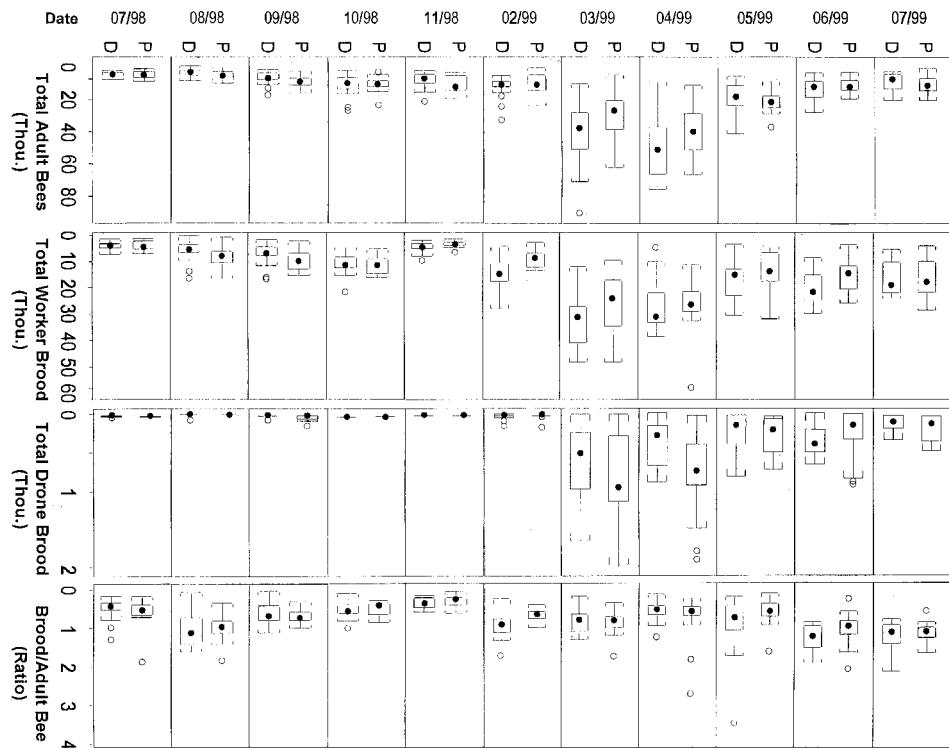


Figure 2. Box plots of total adult bees, total drone brood, total worker brood, and brood to adult bee ratios for Primorsky (P) and domestic (D) colonies for months from July 1998 through July 1999. ! = median observation, □ = range between 1st and 3rd quartile, [●] = range, ○ = outlying observation.

months, NS). No seasonal variation in these percentages was apparent, although the monthly total of dead mites increased as infestations grew.

The average number of mature female mites (mothers and daughters) in infested P worker cells was fewer than in infested D worker cells (D: 1.87 ± 0.20 , R: 1.53 ± 0.27 , *t*-test, $P = 0.0001$) Also, the average number of adult female mites per infested P drone cell was smaller (D: 3.14 ± 1.14 , P: 2.63 ± 1.20 , *t*-test, $P = 0.0001$).

4. DISCUSSION

The differences in changes in mite populations in P and D colonies and their

differential survival evidence genetic resistance to *V. destructor* by the P colonies. The resistance is inherited since the P queens were daughters of queens that showed a similar phenotype (Danka et al., 1995; Rinderer et al., 1997, 1999b). Further, the P and D colonies shared the same experimental environment including the same ecotype of mite. Sharing the same experimental apiaries in random placement may have muted the observed differences since large numbers of mites were produced in dying D colonies that had opportunity enter P colonies and mask their resistance phenotype.

The origin of the resistance appears to be numerous small differences which are both additive and interactive since no single

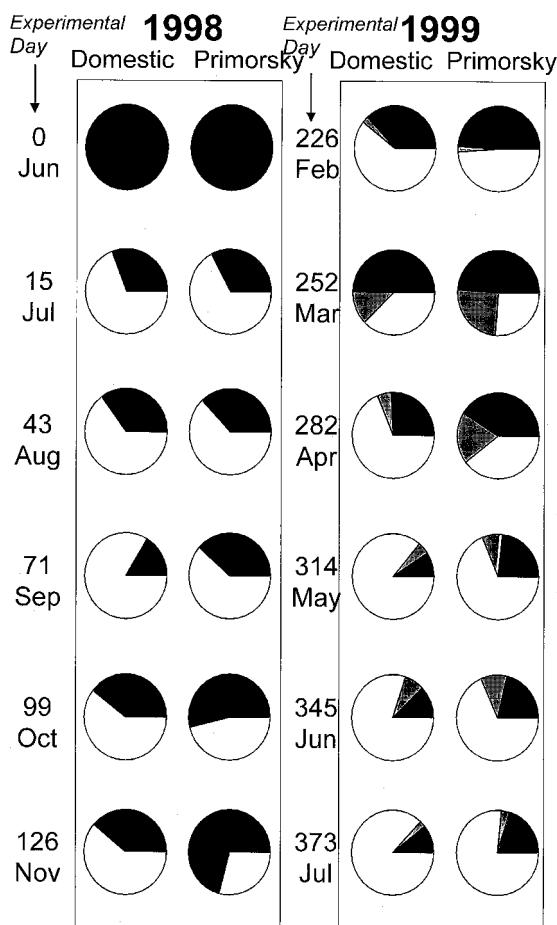


Figure 3. Pie charts showing the proportional distribution of adult female mites in Primorsky and domestic colonies through time. Black: phoretic mites on adult bees/total mites, White: mites infesting worker brood/total mites, Gray: mites infesting drone brood/total mites. Colony numbers are shown for each period and stock.

overwhelming resistance mechanism was identified. Although they had similar worker brood and more drone brood than D colonies, the P colonies somehow restricted the early season MPG (Jan. to Mar.; Fig. 1; Tab. II) which is normally exponential (Ifantidis, 1984; Schulz, 1984; Fries et al., 1994; Harbo and Harris, 1999). In other periods of the year, the P colonies had declining populations of *V. destructor* (Tab. II). After July 1999, when worker brood was still plentiful and drone brood was often present, the P colonies experienced a strong reduction in mite populations. This reduction also occurred in P but not D colonies in 1998,

although to a less pronounced degree in Sept. and Oct. These reductions led to much smaller mite populations by Nov. that were subject to further differential attrition during Dec. and Jan. Further differential attrition in P colonies could have led to still lower mite populations and contributed to the growing differences in population numbers observed in Mar. 1999. Similar "winter" attrition in colonies having brood has been observed in Africanized honey bees in Mexico (Vandame, 1996).

Several aspects of P honey bee biology may be involved in restricting MPG. First,

the greater proportions of phoretic mites would reduce opportunities to reproduce, assuming the mites have a similar life span in P colonies. Reproductive frequency is a key factor in MPG (de Ruijter, 1987; Otten, 1991; Fries and Rosenkranz, 1993; Fries et al. 1994; Martin and Kemp, 1997) and its reduction could be a key resistance mechanism.

Second, an increased phoretic period increases vulnerability to worker bee grooming (Büchler et al., 1992; Delfinado-Baker et al., 1992; Bienefeld et al., 1999). This vulnerability seems to be exploited since a disproportionately high number of dead mites collected from P colonies show physical damage attributable to grooming. Since the sticky boards were protected by screens, the biting occurred before the mites reached the sticky board. Our observed rate of bitten mites in D colonies were very similar to that observed in *A. mellifera* by Fries et al. (1996). Our rate of bitten mites in P colonies was 1/3 higher than that observed in *A. cerana* by Fries et al. (1966).

The smaller average number of mites in both worker and drone brood in P colonies might arise from differential reproductive rates or might be simply a result of P colonies having fewer mites and hence fewer instances of multiple infestations. However, the suppression of mite reproduction hypothesis gains support from the comparison of monthly MPG (Tab. II). In most months, mite populations were reduced in P colonies. Also, MPG in P colonies only exceeded that in D colonies when D colonies were highly infested, dying, and unable to support further increases in MPG. Additionally, field observations suggest the hypothesis that the late summer drop in mite populations is related to a very strong increase in non-reproduction by brood infesting mites.

The differences in resistance to *V. destructor* did not originate from differences in worker brood, adult worker bees or worker brood/adult bee ratios (Fries et al., 1994; Calis et al., 1999) but occurred despite

P colonies having more drone brood. The additional drone brood in P colonies may lead to a tolerance effect. It may account for the greater proportion of mites being found in drone brood and reduce damage to springtime colonies without unduly enhancing MPG since reproduction appears to be comparatively less in P drone brood. When drone brood becomes less available in late summer, the penetrance of the resistance phenotype is sufficient to reduce mite populations. Since this process would lead to better survival with more mites, it would be tolerance rather than resistance.

Natural selection is the most likely cause of the heightened resistance. We are not aware of any efforts to select for resistance in the area. Also, the mid-1800 importations were prior to general use of movable frame hives and about 60 years before the naming of the genus *Varroa* (Oudemans, 1904; Crane, 1978). Lacking movable frame hives, beekeepers could only divide surviving colonies or catch swarms. Founder effects are unlikely to have produced resistance since the originating Ukrainian population (or any other European honey bees) has not been reported to have any notable resistance.

The Primorsky territory has a specific, and perhaps unique condition which may have fostered this selection. Although *A. cerana* and *V. destructor* range throughout much of Primorsky, their occurrence is patchy (De Jong et al., 1982), perhaps because the area is at the edge of the *A. cerana* range (Ruttner, 1988). Hence, at the colony level, where infestation of worker bees indirectly reduces reproductive fitness, the selection by *V. destructor* on the imported *A. mellifera* may have been weak and intermittent, permitting marginally resistant colonies to express enhanced fitness via comparatively enhanced reproduction. *V. destructor* preferentially infests drone brood (Fuchs, 1990), overcoming the protection from environment afforded by the honey bee nest. Thus, weak or moderate selection at the colony

level may have been comparatively strong on drones. If so, intense selection on drones would accelerate selection even though infestation of worker bee may be insufficient to kill the colony. *V. destructor* kills many infested drones and many adult drones surviving infestation suffer severe reproductive impairment (Weinberg and Madel, 1985; Del Cacho et al., 1996; Rinderer et al., 1999a). Also, since drones are haploid, (Woyke, 1986) selection among drones is effectively at the gametic level, unencumbered by genetic conditions arising from heterozygosity.

Overall, Phoney bees appear to have several mechanisms which act in concert to provide them with substantial resistance to *V. destructor*. It is unlikely that we have yet identified all of the factors that may contribute to this resistance. Indeed, a substantial number of hypotheses remain wholly or partially untested. However, the diversity of traits identified in this study that may contribute to the resistance suggests that a constellation of traits and genes underlie the overall resistance and provide opportunities for further development of the resistance through selective breeding.

Résumé – Résistance des abeilles domestiques de Russie extrême orientale à l'acarien *Varroa destructor*. En Asie *V. destructor* est un parasite anodin d'*Apis cerana*. Cet acarien est passé de l'abeille asiatique à l'abeille européenne *A. mellifera*; il s'est répandu rapidement et infeste maintenant la majeure partie des colonies d'*A. mellifera*, causant un énorme préjudice à l'apiculture.

La Russie extrême orientale (Primorsky), où les colons ont apporté *Apis mellifera* au milieu du 19^e siècle, constitue une source éventuelle de résistance à *V. destructor*. Nous avons importé certaines de ces abeilles aux États-Unis, où les tests ont montré qu'elles convenaient pour le commerce. Au cours d'une série d'expériences nous avons comparé des colonies de Primorsky (P) et

des colonies locales (D) et déterminé (i) la résistance comparative, (ii) que la résistance avait une base génétique et (iii) certains mécanismes possibles de résistance.

Des reines sœurs issues de colonies P ont été élevées, fécondées sur une île et enruées avec environ 305 acariens *V. destructor* adultes. De la même façon des reines sœurs issues de colonies D ont été enruées avec environ 233 acariens. Nous avons évalué les populations d'acariens en comptant les acariens dans le couvain d'ouvrière, le couvain de mâle et sur les adultes et en estimant les surfaces de couvain operculé et le nombre d'abeilles. Nous avons récolté périodiquement les acariens tombés sur le fond de la ruche et cherché des indices de toilettage.

En 1998 les populations d'acariens sont restées généralement faibles. Pourtant, en novembre, les colonies P avaient moins d'acariens (Fig. 1). En 1999 les populations d'acariens dans les colonies D ont augmenté de façon exponentielle (Fig. 1). En mai, elles atteignaient environ 10000 et sept des colonies D sont mortes de varroose. À la fin de juillet 18 colonies D sont mortes de varroose et quatre autres ont été perdues pour d'autres raisons. La croissance des populations d'acariens dans les colonies P a été fortement restreinte (Fig. 1). Au cours de chaque mois de 1999 le nombre d'acariens dans les colonies P était nettement inférieur à celui des colonies D, où il atteignait environ 4000. À partir de juillet, le nombre moyen d'acariens a montré une tendance à la décroissance pour atteindre 400 en novembre. À la fin de juillet, trois colonies P étaient mortes de varroose. Les différences dans les populations d'acariens et la longévité des colonies sont des preuves de l'existence de différences génétiques dans la résistance à *V. destructor*. La résistance est héritée puisque (i) les reines P étaient des sœurs de reines qui présentaient un phénotype semblable, (ii) le test comparatif a été fait dans le même environnement.

Les différences dans la résistance ne proviennent pas de différences dans la quantité de

couvain d'ouvrière, d'abeilles adultes ni dans les rapports couvain/adultes et existent bien que les colonies P aient eu beaucoup plus de couvain de mâles (Fig. 2). Les colonies P ont eu moins d'acariens qui ont infesté le couvain d'ouvrières et plus d'acariens sur les abeilles adultes. Ceci peut réduire la reproduction de la durée de vie et accroître la vulnérabilité au toilettage. Dans les colonies P un plus grand nombre d'acariens tombés présentaient des lésions dues au toilettage ($P = 42\%$, $D = 28\%$). Le nombre moyen d'acariens femelles matures dans les cellules d'ouvrières infestées des colonies P était plus faible ($P : 1,53$; $D : 1,87$) ; ce qui suggère que le taux de reproduction de *V. destructor* peut être inférieur dans le couvain d'ouvrières des colonies P. De même, on a trouvé un plus grand pourcentage d'acariens dans les colonies P infestant le couvain de mâles (Fig. 2). Mais le nombre d'acariens par cellule de mâles infestée dans les colonies P était plus petit ($P : 1,53$; $D : 1,87$), suggérant que la reproduction de l'acarien peut aussi être plus faible sur le couvain de mâle des colonies P. Au total les abeilles P semblent avoir plusieurs mécanismes de résistance qui agissent de concert. Elles offrent donc des possibilités de produire des lignées utilisables commercialement qui présentent une résistance à *V. destructor*.

*Apis mellifera / résistance au parasite / *Varroa destructor* / Russie*

Zusammenfassung – Resistenz gegen die parasitische Milbe *Varroa destructor* in Honigbienen aus dem fernöstlichen Russland. Eine mögliche Fundstelle für Resistenz gegen *V. destructor* ist das fernöstliche Russland (Primorski), wohin um die Mitte des 19. Jahrhunderts *Apis mellifera* von Siedlern verbracht wurde. Wir importierten einige dieser Honigbienen in die Vereinigten Staaten, wo ihre Überprüfung zeigte dass sie für die kommerzielle Imkerei annehmbare Eigenschaften hatten. In einem

Experiment wurden Primorsky (P) und einheimische (D) Völker untersucht. Hierbei zeigte sich, dass Primorskybienen (1) vergleichsweise resistent sind, (2) die Resistenz eine genetische Grundlage hat und (3) wurden einige mögliche Mechanismen der Resistenz bestimmt.

Tochterköniginnen der P Linie wurden nachgezogen, auf einer Insel verpaart 22 dieser Königinnen zusammen mit etwa 305 *V. destructor* Milben in Völker eingesetzt. D Königinnen wurden in vergleichbarer Weise zusammen mit 223 V Milben eingeweiselt. Die Populationsgrösse von *V. destructor* wurde durch Zählungen von Milben in Arbeiterinnenbrut, in Drohnenbrut und auf adulten Milben sowie durch Schätzungen der Bienenanzahl sowie der Anzahl verdeckelter Brutzellen bestimmt. In regelmäßigen Abständen wurden abgefallene Milben gesammelt und auf Anzeichen von Putzverhalten bei den Bienen hin untersucht.

1998 blieben die Milbenpopulationen im allgemeinen niedrig. Allerdings enthielten die P Völker im November weniger Milben (Abb. 1). 1999 wuchsen die Populationen in den D Völkern exponentiell an (Abb. 1). Im Mai waren die Populationen im Mittel auf ungefähr 10000 Milben angewachsen und 7 der D Völker gingen an der Varroose zu Grunde. Ende Juli waren 18 der D Völker an der Varroose gestorben, 4 waren aus anderen Gründen umgekommen. Das Wachstum der Milbenpopulationen in den P Völkern war stark begrenzt (Abb. 1). In allen Monaten 1999 war die Anzahl von Varroamilben in den P Völkern deutlich geringer als in den D Völkern und erreichte im Mittel etwa 4000 Milben. Von Juli an zeigte die mittlere Anzahl der Milben in P Völkern eine abnehmende Tendenz und ging auf etwa 400 im November zurück. Gegen Ende Juli gingen 3 der P Völkern an der Varroose ein.

Die Unterschiede in den Milbenpopulationen und in der Lebensdauer zeigten, dass genetische Effekte der Resistenz gegen *V. destructor* zu Grunde liegen. Die Resistenz wird vererbt: a.) die P Königinnen waren Töchter von Königinnen eines

ähnlichen Phänotyps und b.) fand der Vergleichstest in der gleichen Umgebung statt. Die unterschiedliche Resistenz war nicht auf Unterschiede in der Erzeugung von Arbeiterinnenbrut, in den Anzahlen von Arbeiterinnen oder das Verhältnis von Brut zu Arbeiterinnen zurückzuführen, sondern trat auf obwohl die P Völker mehr Dronenbrut erzeugt hatten (Abb. 2.). In den P Völkern befanden sich mehr Milben auf den Arbeiterinnen und weniger in der Arbeiterinnenbrut. Dies könnte die Lebensreproduktionsleistung vermindern und die Verwundbarkeit durch das Putzverhalten der Bienen erhöhen. In den P Völkern zeigten mehr der abgefallenen Milben Verletzungen durch Putzverhalten (P = 42 %, D = 28 %). Die mittlere Anzahl ausgewachsener weiblicher Milben in den befallenen P Arbeiterinnenzellen war geringer (P: 1,53, D: 1,87) was auf eine geringere Reproduktionsrate von *V. destructor* in P Arbeiterinnenzellen hinweist. Ebenso war der Anteil von Milben in Dronenzellen in P den Völkern höher (Abb. 2). Allerdings war die Anzahl von Milben pro befallener P Dronenzelle geringer (P: 1,53, D: 1,87). Dies könnte auf eine geringere Reproduktion in den Dronenzellen hinweisen. Insgesamt scheinen die P Honigbienen mehrere zusammenwirkende Resistenzmechanismen aufzuweisen. Daher bieten P Honigbienen Möglichkeiten kommerziell verwendbare Zuchtlinien mit Resistenz gegenüber *V. destructor* zu erstellen.

***Apis mellifera* / Resistenz / *Varroa destructor* / Russland**

REFERENCES

- Anderson D.L., Trueman J.W.H. (2000) *Varroa jacobsoni* (Acaria: Varroidae) is more than one species, *Exp. & Appl. Acarol.* 24: 165–189.
- Bienefeld K., Zautke F., Pronin D., Mazed A. (1999) Recording the proportion of damaged *Varroa jacobsoni* Oud. in the debris of honey bee colonies (*Apis mellifera*). *Apidologie* 30, 249–56.
- Boecking O., Ritter W. (1993) Grooming and removal behaviour of *Apis mellifera intermissa* in Tunisia against *Varroa jacobsoni*, *J. Apic. Res.* 32, 127–134.
- Boot W.J., Nguyen Q.T., Pham C.D., Luong V.H., Nguyen V.D., Le T.L., Beetsma J. (1997) Reproductive success of *Varroa jacobsoni* in brood of its original host, *Apis cerana*, in comparison to that of its new host, *A. mellifera* (Hymenoptera: Apidae), *Bull. Entomol. Res.* 87, 119–126.
- Büchler R., Drescher W., Tornier I. (1992) Grooming behaviour of *Apis cerana*, *Apis mellifera* and *Apis dorsata* and its effects on the parasitic mites *Varroa jacobsoni* and *Tropilaelaps clareae*, *Exp. Appl. Acarol.* 16, 313–319.
- Calis J.N.M., Fries I., Ryrie S.C. (1999) Population modeling of *Varroa jacobsoni*, *Apidologie* 30, 111–124.
- Camazine S. (1986) Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae) on Africanized and European honey bees (Hymenoptera: Apidae), *Ann. Entomol. Soc. Am.* 79, 801–803.
- Camazine S. (1988) Factors affecting the severity of *Varroa jacobsoni* infestations on European and Africanized honey bees, in: Needham G.R., Page R.E. Jr., Delfinado-Baker M., Bowman C.E. (Eds.), *Africanized honey bees and bee mites*, E. Horwood Ltd./Halsted Press, New York, pp. 444–451.
- Collins A.M., Rinderer T.E., Harbo J.R., Bolten A.B. (1982) Colony defense by Africanized and European honey bees, *Science* 218, 72–74.
- Collins A.M., Rinderer T.E., Tucker K.W., Pesante D.G. (1987) Response to alarm pheromone by European and Africanized honeybees, *J. Apic. Res.* 26, 217–223.
- Crane E. (1978) The Varroa mite. *Bee World* 59, 164–167.
- Danka R.G., Rinderer T.E., Collins A.M., Hellmich R.L. III (1987) Responses of Africanized honey bees (Hymenoptera: Apidae) to pollination-management stress, *J. Econ. Entomol.* 80, 621–624.
- Danka R.G., Rinderer T.E., Kuznetsov V.N., Delatte G.T. (1995) A USDA-ARS project to evaluate resistance to *Varroa jacobsoni* by honey bees of far-eastern Russia, *Am. Bee J.* 135, 746–748.
- Danka R.G., Villa J.D., Harbo J.R., Rinderer T.E. (1997) Initial evaluation of industry-contributed honey bees for resistance to *Varroa jacobsoni*, *Proc. Am. Bee Res. Conf. Am. Bee J.* 137, 221–222.
- de Guzman L.I., Rinderer T.E. (1999) Identification and comparison of *Varroa* species infesting honey bees, *Apidologie* 30, 85–95.
- de Guzman L.I., Rinderer T.E., Delatte G.T., Macchiavelli R.E. (1996) *Varroa jacobsoni* Oudemans tolerance in selected stocks of *Apis mellifera* L., *Apidologie* 27, 193–210.
- de Guzman L.I., Rinderer T.E., Stelzer J.A. (1997) DNA evidence of the origin of *Varroa jacobsoni* Oudemans in the Americas, *Biochem. Genet.* 35, 327–335.
- de Guzman L.I., Rinderer T.E., Stelzer J.A., Anderson D. (1998) Congruence of RAPD and mitochondrial DNA markers in assessing *Varroa jacobsoni* genotypes, *J. Apic. Res.* 37, 49–51.

- de Guzman L.I., Rinderer T.E., Stelzer J.A. (1999) Occurrence of two genotypes of *Varroa jacobsoni* Oud., in North America, *Apidologie* 30, 31–36.
- 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, *Am. Bee. J.* 137, 742–745.
- De Jong D., Morse R.A., Eickwort G.C. (1982) Mite pests of honey bees, *Annu. Rev. Entomol.* 27, 229–252.
- Del Cacho E., Martí J., Josa A., Quilez J., Sanchez-Acedo C. (1996) Effect of *Varroa jacobsoni* parasitization in the glycoprotein expression on *Apis mellifera* spermatozoa, *Apidologie* 27, 87–92.
- Delfinado-Baker M., Rath W., Boecking O. (1992) Phoretic bee mites and honeybee grooming behavior, *Int. J. Acarol.* 18, 315–322.
- de Ruijter A. (1987) Reproduction of *Varroa jacobsoni* during successive brood cycles of the honeybee, *Apidologie* 18, 321–326.
- Eguaras M., Marcangeli J., Fernandez N.A. (1994) Influence of ‘parasitic intensity’ on *Varroa jacobsoni* Oud. reproduction, *J. Apic. Res.* 33, 155–159.
- Fries I., Rosenkranz P. (1993) Number of reproductive cycles in the *Varroa* mite, *Apidologie* 24, 485–486.
- Fries I., Camazine S., Sneyd J. (1994) Population dynamics of *Varroa jacobsoni*: a model and a review, *Bee World* 75, 5–28.
- Fuchs S. (1990) Preference for drone brood cells by *Varroa jacobsoni* Oud. in colonies of *Apis mellifera carnica*, *Apidologie* 21, 193–199.
- Fuchs S. (1992) Choice of *Varroa jacobsoni* Oud. between honey bee drone or worker brood cells for reproduction, *Behav. Ecol. Sociobiol.* 31, 429–435.
- Fuchs S., Langenbach K. (1989) Multiple infestation of *Apis mellifera* L. brood cells and reproduction in *Varroa jacobsoni* Oud., *Apidologie* 20, 257–266.
- Gibbons R.D. (1994) Mann-Kendall non-parametric test for trends, in: Statistical methods for groundwater monitoring, John Wiley, New York, pp. 178–181.
- Harbo J.R., Harris J.W. (1999) Selecting honey bees for resistance to *Varroa jacobsoni*, *Apidologie* 30, 183–196.
- Ifantidis M.D. (1983) Ontogenesis of the mite *Varroa jacobsoni* Oud. in the worker and drone brood cells of the honey bee, *J. Apic. Res.* 22, 200–206.
- Ifantidis M.D. (1984) Parameters of the population dynamics of the Varroa mite on honeybees, *J. Apic. Res.* 23, 227–233.
- Kraus B., Page R.E. Jr. (1995) Effect of *Varroa jacobsoni* (Mesostigmata: Varoidae) on feral *Apis mellifera* (Hymenoptera: Apidae) in California, *Environ. Entomol.* 24, 1473–1480.
- Kulinčević J.M., Rinderer T.E., Mladjan V.J., Buco S.M. (1992) Five years of bi-directional genetic selection for honey bees resistant and susceptible to *Varroa jacobsoni*, *Apidologie* 23, 443–452.
- Kulinčević J.M., de Guzman L.I., Rinderer T.E. (1997) Selection of honey bees tolerant or resistant to *Varroa jacobsoni*. Cahiers Options Méditerranéennes, Volume 21, Varroosis in the Mediterranean region; Proceedings of the seminar on the Varroosis in the Mediterranean region, Granada, Spain, 22–23 September 1996, Centre International de Hautes Études Agronomiques Méditerranéennes, pp. 59–75.
- 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, *J. Apic. Res.* 34, 187–196.
- Martin S., Cook C. (1996) Effect of host brood type on the number of offspring laid by the honeybee parasite *Varroa jacobsoni*, *Exp. Appl. Acarol.* 20, 387–390.
- Martin S.J., Kemp D. (1997) Average number of reproductive cycles performed by *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies, *J. Apic. Res.* 36, 113–123.
- Moretto G. (1997) Defense of Africanized bee workers against the mite *Varroa jacobsoni* in southern Brazil, *Am. Bee. J.* 137, 746–747.
- Moretto G., de Mello L.J. Jr. (1999) *Varroa jacobsoni* infestation of adult Africanized and Italian honey bees (*Apis mellifera*) in mixed colonies in Brazil, *Genet. Mol. Biol.* 22, 321–323.
- Moretto G., Gonçalves L.S., DeJong D. (1993) Heritability of Africanized and European honey bee defensive behavior against the mite *Varroa jacobsoni*, *Rev. Brasil Genet.* 16, 71–77.
- Oldroyd B.P. (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees, *Trends Ecol. Evol.* 14, 312–315.
- Otten C. (1991) Factors and effects of a different distribution of *Varroa jacobsoni* between adult bees and bee brood, *Apidologie* 22, 466.
- Oudemans A.C. (1904) Note VIII. On a new genus and species of parasitic Acari (*Varroa*), Notes from the Leyden Museum XXIV, pp. 216–222.
- Rath W. (1999) Co-adaption of *Apis cerana* Fabr. and *Varroa jacobsoni* Oud., *Apidologie* 30, 97–110.
- Rosenkranz, P. (1999) Honey bee (*Apis mellifera* L.) Tolerance to *Varroa jacobsoni* Oud. in South America, *Apidologie*, 30, 159–172.
- Rinderer T.E., Bolten A.B., Collins A.M., Harbo J.R. (1984) Nectar-foraging characteristics of Africanized and European honeybees in the Neotropics, *J. Apic. Res.* 23, 70–79.
- Rinderer T.E., Collins A.M., Tucker K.W. (1985) Honey production and underlying nectar harvesting activities of Africanized and European honeybees, *J. Apic. Res.* 24, 161–167.
- Rinderer T.E., Stelzer J.A., Oldroyd B.P., Buco S.M., Rubink W.L. (1991) Hybridization between European and Africanized honey bees in the neotropical Yucatan Peninsula. *Science* 253, 309–311.
- Rinderer T.E., Kuznetsov V.N., Danka R.G., Delatte G.T. (1997) An importation of potentially Varroa-resistant honey bees from far-eastern Russia, *Am. Bee J.* 137, 787–789.

- Rinderer T.E., de Guzman L.I., Lancaster V.A., Delatte G.T., Stelzer J.A. (1999a) *Varroa* in the mating yard: I. The effects of *Varroa jacobsoni* and *Apis-tan* on drones, Am. Bee J. 139, 134–139.
- Rinderer T.E., Delatte G.T., de Guzman L.I., Williams J., Stelzer J.A., Kuznetsov V.N. (1999b) Evaluations of the varroa-resistance of honey bees imported from far-eastern Russia, Am. Bee J. 139, 287–290.
- Ruttner F. (1988) Biogeography and taxonomy of honey bees, Springer-Verlag, Berlin.
- Ruttner F., Hänel H. (1992) Active defense against *Varroa* mites in a Carniolan strain of honeybee (*Apis mellifera carnica* Pollmann), Apidologie 23, 173–187.
- Schulz A.E. (1984) Reproduktion und Populationsentwicklung der parasitischen Milbe *Varroa jacobsoni* Oud. in: Abhangigkeit vom Brutzyklus ihres Wirtes, *Apis mellifera*. Apidologie 15, 401–420.
- Weinberg K.P., Madel G. (1985) The influence of the mite *Varroa jacobsoni* Oud. on the protein concentration and the haemolymph of the brood of worker bees and drones of the honey bee *Apis mellifera* L., Apidologie 16, 421–436.
- Woyke J. (1986) Sex determination, in: Rinderer T.E. (Ed.), Bee genetics and breeding, Academic Press, New York, pp. 91–119.