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**Review article**

# Breeding for resistance to *Varroa destructor* in North America*

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**Abstract** – Breeding for resistance to *Varroa destructor* in North America provides the long-term solution to the economic troubles the mite brings. This review reports the development of two breeding successes that have produced honey bees of commercial quality that do not require pesticide treatment to control *Varroa*, highlights other traits that could be combined to increase resistance and examines the potential uses of marker-assisted selection (MAS) for breeding for *Varroa* resistance. Breeding work continues with these stocks to enhance their commercial utility. This work requires knowledge of the mechanisms of resistance that can be further developed or improved in selected stocks and studied with molecular techniques as a prelude to MAS.

***Varroa* resistance / breeding program / Russian honey bees / *Varroa*-sensitive hygiene / marker-assisted selection**

## 1. INTRODUCTION

The introduction of *Varroa destructor* Anderson & Trueman (2000) into North America during the late 1980s caused dramatic changes to beekeeping practices and increased the costs of honey production and pollination. Increased costs stemmed primarily from the control measures necessary to prevent loss of colonies from varroosis. Most beekeepers relied on acaricides such as Apistan® (fluvalinate) or CheckMite™ (coumaphos) to control *Varroa* mites. Unfortunately, use of chemicals has led to the development of acaricide-resistant mites and to increased residues of chemicals in beeswax and honey.

A variety of non-chemical control methods were developed to circumvent or delay the problems of acaricide-resistant mites and chemical residues in beekeeping products. Non-chemical controls for *Varroa* mites included mite trapping by removal of capped drone brood, screened floors, sticky traps on the bottom board, and use of *Varroa*-resistant honey bees. Using *Varroa*-resistant honey bees is ideal since the need for acaricides is either reduced or eliminated without a need for additional *Varroa* control measures.

Breeding for *Varroa*-resistant honey bees became the primary goal for a number of research groups around the world. Within North America, *Varroa* resistance has been produced by at least three breeding programs. One program from the University of Minnesota produced measurable *Varroa* resistance as a consequence of selecting for improved general hygienic behavior (Boecking and Spivak, 1999; Spivak and Reuter, 2001a, b; Ibrahim et al., 2007). The "Minnesota Hygienic" stock (MNHYG) is sold commercially throughout the US (Spivak et al., 2009). Two other programs were initiated at the USDA-ARS Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, LA, and they are the

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primary focus of the current review. The Russian Honey Bee (RHB) Program and the *Varroa*-Sensitive Hygiene (VSH) Program were initiated specifically to produce *Varroa* resistant honey bees that would be suitable for commercial use.

Although they differ in general breeding approach, the two programs have produced and released *Varroa*-resistant honey bees that are sold commercially. These honey bees require substantially fewer acaricide treatments for controlling *Varroa* mites, and they retain the commercial qualities desired by beekeepers. Both programs have relied upon traditional breeding techniques and an understanding of the known mechanisms of *Varroa* resistance. One goal is that future selection will include the use of molecular genetics. Specifically, the development of marker-assisted selection (MAS) will likely accelerate breeding progress in these two programs and programs that are currently developing other *Varroa* resistance traits such as nestmate grooming.

## 2. MECHANISMS OF RESISTANCE

The Russian (or Korean) haplotype of *V. destructor* is the hypervirulent variant which threatens *Apis mellifera* beekeeping worldwide (de Guzman et al., 1997, 1999; Anderson and Trueman, 2000). Honey bee colonies that survive infestations of this *Varroa* haplotype have one or more behavioral or physiological traits which underlie their resistance to *Varroa*.

### 2.1. Behavioral mechanisms of resistance

#### *2.1.1. Hygienic behavior*

Hygienic bees are able to detect, uncap and remove diseased brood (Rothenbuhler, 1964; Gilliam et al., 1983; Spivak and Reuter, 2001b). A general test of hygiene, the removal of freeze-killed brood by colonies (Spivak and Reuter, 1998), correlates relatively well with removal of *Varroa*-infested brood (Boecking and Drescher, 1992; Spivak, 1996). Removal of mite-infested brood is well established in *A. cerana* (Peng et al., 1987).

The ability to remove brood infested with *Varroa* has been bred to high levels in *A. mellifera* colonies bred for VSH (Harbo and Harris, 2005; Harris, 2008). VSH is more pronounced in infested worker brood than in drone brood suggesting that increased mite infestation may occur in VSH colonies when drone brood is abundant (Harris, 2008). As VSH bees uncap and remove infested brood, freed adult female mites usually transfer onto the bees removing the brood (Aumeier and Rosenkranz, 2001) but may eventually become free on the combs and exposed to attack by bees. Thakur et al. (1997) documented that honey bees can detect, grab and bite free-moving mites. The mites may also become phoretic and exposed to grooming. Hence, VSH may be a basic mechanism which can enhance other traits such as nestmate grooming and an increased phoretic period (Ibrahim et al., 2007).

#### *2.1.2. Grooming behavior*

Honey bees clean themselves (autogrooming) and nestmates (allogrooming) (Haydak, 1945). Grooming may injure or kill *Varroa* mites (Ruttner and Hänel, 1992), or it may cause mites to either move to other parts of the autogroomer's body, transfer to a new host or be removed from the bee's body without causing visible injury (Büchler et al., 1992). Grooming is rarely observed directly. However, variation among honey bee stocks in grooming has been inferred from the proportion of mites that drop to hive floors that are damaged, apparently from bees' mandibles (Boecking and Spivak, 1999; Fries et al., 1996; Rinderer et al., 2001a; Arechavaleta-Velasco and Guzman-Novoa, 2001).

In a study in Mexico that compared mite population growth (MPG) in a genetically diverse set of colonies and beginning with equal mite infestations, the principal mechanism of resistance identified was grooming (Arechavaleta-Velasco and Guzman-Novoa, 2001). Colonies with the lowest MPG had fewer mites on adult bees, more mites falling to hive floors, and a higher proportion of chewed mites. A more recent study compared traits associated with MPG

and found that the two best predictors of reduced MPG were the average number of mated female mite offspring and the proportion of mutilated mites (Mondragon et al., 2005). This is one of only two studies in North America that have used diverse queen sources and investigated mechanisms of resistance. The other study identified VSH as the primary mechanism (see below).

Grooming is a heritable trait (Moretto et al., 1993). However, its usefulness in a breeding program is controversial (Rosenkranz et al., 1997; Bienefeld et al., 1999; Aumeier, 2001). Several measurement problems have been identified. Usually, the proportion of damaged mites, a difficult and time consuming measurement, is the only criterion considered. However, living apparently uninjured mites have been detected in high numbers on bottom board traps (Fries et al., 1996). They may also indicate grooming and actually be injured or debilitated (Thakur et al., 1997). Alternately, they may be healthy, fallen owing to hot weather (Webster et al., 2000).

Injuries to mites may result from: (a) grooming, (b) removal of dead mites (Rosenkranz et al., 1997; Bienefeld et al., 1999), or (c) predation by wax moth larvae and ants (Szabo and Walker, 1995). Davis (2009) asserted that indentation on the mites' idiosoma is not damage caused by bees but is acquired during mite development. However, pieces missing from the idiosoma and missing legs cannot be attributed to normal mite development. Laboratory assays of grooming using either individual bees or cages of bees have been developed and produce promising results that correlate with the proportion of damaged mites in source colonies, thus circumventing the difficulty of evaluating damaged mites (Arechavaleta-Velasco and Guzman-Novoa, 2001; Currie and Tahmasbi, 2008; A. Gandino and G.J. Hunt, unpubl. data). An efficient laboratory assay should improve measurement precision and accelerate selection.

#### *2.1.3. Removal of mites from the nest*

Morse et al. (1991) hypothesized that bees may carry and discard *Varroa* mites outside the nest. Lodesani et al. (1996) confirmed this hypothesis using external traps (Gary, 1960). Likewise, living *Varroa* mites can be lost during foraging flights (Kralj and Fuchs, 2006) and more frequently by RHB (Kralj, 2004). Kralj also observed that a higher proportion of the infested RHB did not return to the hive as compared to infested *A. m. carnica* and interpreted the behavior to be an adaptive contribution to resistance (Kralj and Fuchs, 2006).

### 2.2. Physiological mechanisms of resistance

#### *2.2.1. Brood characteristics*

Brood attractiveness – The use of this character in breeding programs appears questionable because several comparative studies exposing different *A. mellifera* brood yielded contradictory results (Büchler, 1990; de Guzman et al., 1995, 1996; Calis et al., 2006). Attractiveness is commonly measured as the percentage of cells infested, but measurements of the reproductive potential of infesting *Varroa* mites may be more useful. Higher rates of non-reproduction (NR) in VSH bees (Harbo and Hoopingarner, 1997) and RHB (de Guzman et al., 2007) by infesting mites may result from reduced brood attractiveness but may also be a direct result of VSH. However, less attractive hosts may also result in a reduced reproductive success among reproductive mites as found with RHB (de Guzman et al., 2008) and other stocks (Camazine, 1986; Harbo and Hoopingarner, 1997; Ibrahim and Spivak, 2006). This reduced reproduction might be a useful trait for breeding.

Brood attractiveness seems related to differential reproduction on worker and drone brood. *Varroa* mites prefer drone brood over worker brood in *A. mellifera* (Fuchs, 1990) and only reproduce in *A. cerana* drone brood. Identifying the chemistry underlying these species and caste differences may provide a superior trait for selecting for *Varroa* resistance. For example, worker larvae of *A. cerana* have higher concentrations of free amino acids and lower concentrations of copper and zinc than drone

larvae of *A. cerana* or both worker and drone larvae of *A. mellifera* (Xing et al., 2007). Copper and zinc are important for insect growth and fecundity (McFarlane, 1976).

Larval food and comb properties – Traits for selection may also include the chemistry of comb and larval food. Cocoons contain semiochemicals used for the deposition of *Varroa* feces (Donze'and Guerin, 1994). Larval food may also contain chemicals that attract or influence *Varroa* mite reproduction (Nazzi et al., 2001). De Guzman et al. (2008) showed that the comb built by RHB contributed to an increased rate of NR, and decreased numbers of progeny and viable female offspring.

#### *2.2.2. Phoresy*

If mites in some bee colonies are phoretic for longer periods, they may have fewer chances of reproducing during their life and an increased potential for being groomed (Ruttner and Hänel, 1992). Selection for increased phoresy would enhance mite resistance. However, phoresy may be influenced by other resistance traits. VSH reduces the number of mites in brood, and grooming reduces the number on adults. The mites released by VSH may either die or join the phoretic population, but in either case, the proportion of phoretic mites increases. In RHB, phoresy may be influenced by brood unattractiveness (de Guzman et al., 2007), winter- or nectar-dearth induced broodlessness (Tubbs et al., 2003), or be supplemented by increased and prolonged drone production (Rinderer et al., 2001a; de Guzman et al., 2007).

## 3. SELECTIVE BREEDING OF *VARROA*-SENSITIVE HYGIENE (VSH)

### 3.1. History of the suppression of mite reproduction (SMR)/VSH breeding program

This breeding program sought to identify and enhance traits of honey bees that limit growth of *Varroa* populations from bee stocks that were already in the U.S. The primary goal is to deliver useful *Varroa* resistance traits to the beekeeping industry by providing highly selected germplasm that can either be introgressed by selective breeding into existing commercial stocks or outcrossed to produce hybrid bees that retain significant *Varroa* resistance.

Early in the breeding program (1996–2001), selection for *Varroa* resistance focused on MPG among homogeneous infested colonies over about ten weeks (Harbo and Harris, 1999a). Resistance was defined as the ability of a colony to retard MPG. MPG was estimated by an exponential growth equation (Branco et al., 1999), and environmental variation (Harris et al., 2003) was minimized by forming colonies at the same time and within the same apiary. Success in finding genetic differences among colonies was enhanced by the use of single drone inseminations of queens. This mating technique produced workers of one patriline with reduced genetic variation within a colony, which allowed variation between colonies of diverse genetic backgrounds to be more apparent (Rothenbuhler, 1960). Of the four mechanisms of resistance [post-capping period, freeze- killed brood removal, grooming and NR] that were measured, only NR was found correlated with MPG (Harbo and Hoopingarner, 1997; Harbo and Harris, 1999a).

NR was caused by two heritable traits (Harbo and Harris, 1999b). Brood from queens with resistance genes caused increased NR, but the strongest effect came only after adult worker bees had been produced from the queens (Harris and Harbo, 2000). Breeding for the adult bee effect was favored because it had the strongest influence on *Varroa* resistance (Harbo and Harris, 1999b). This adult bee effect was called the SMR trait (Harbo and Harris, 2002). Beginning in 2001, SMR lines were selected for increased NR (Harris and Harbo, 1999). The brood effect still occurs in some VSH lines (Ibrahim and Spivak, 2006), and could probably be enhanced though selective breeding.

### 3.2. Mechanism of resistance in VSH bees

Selection for high percentages of NR mites continued until 2005 when it was discovered that infertility of mites was linked to hygienic removal of mite-infested pupae (Harbo and Harris, 2005; Ibrahim and Spivak, 2006). Because VSH is the primary mechanism of resistance, the name replaced SMR (Harris, 2007). The new understanding came when Ibrahim and Spivak (2006) observed that colonies of VSH bees removed freeze-killed more quickly than the MNHYG stock of bees, and so were more hygienic. Also, it was found that the infertility of mites in foreign brood increased after a 1-week exposure to VSH bees (Harbo and Harris, 2005). Increased mite infertility was correlated with a decrease in the brood infestation rate, which presumably resulted from VSH. This could be explained if VSH bees preferentially removed pupae that were infested by mites with offspring rather than pupae with infertile mites (Harbo and Harris, 2005, 2009).

However, recent experiments indicated that VSH bees remove mite-infested pupae whether mite offspring are present or not (Harris et al., 2009, 2010). Therefore, increased NR is likely caused by other aspects of hygiene. For example, uncapped pupae are sometimes recapped by non-hygienic bees within a hygienic colony (Arathi et al., 2006), and this frequently occurs in VSH colonies (Harris, 2008). Perhaps reproduction by *Varroa* is disrupted by the uncapping of the brood cells, and some uncapped pupae are recapped with NR mites inside them. Because VSH bees remove mite-infested pupae without regard to the presence of mite offspring, it seems unlikely that the stimulus triggering VSH is related to oviposition or to odors from mite offspring. Neither odors nor movements of adult *Varroa* mites elicit removal of mite-infested brood (Aumeier and Rosenkranz, 2001). Therefore, the stimulus for VSH probably originates from odors of infested hosts (Martin et al., 2002); however, there are other possible triggers for the removal of mite-infested brood, and the specific cues remain unknown (Vandame et al., 2002).

A key goal has been to develop more efficient methods for breeding the VSH trait. Selection for SMR involved field tests lasting 2–6 months. New understanding of VSH as a behavior of adult bees may allow accelerated selection based on direct measurements of behavior. However, current selection focuses on indirect measures of behavior such as a reduced *Varroa* infestation of brood after exposure to bees. The quickest bioassays for VSH involve the introduction of infested foreign brood into VSH colonies for either 40 h or one week (Harris, 2007; Villa et al., 2009a). The 40-h exposure showed a strong correlation between reduction in brood infestation and MPG, while strong correlations between reduced brood infestation, mite fertility, and MPG were apparent after the 1-week exposure. Currently, a 1-week exposure of brood to colonies is the primary method recommended for assessing VSH in breeding stock.

### 3.3. Performance of VSH bees in commercial beekeeping environments

The strongest VSH expression comes from purely mated queens, but early in the program some colonies of a VSH × VSH mating developed a poor brood pattern. The poor brood patterns were not related to a sex allele problem from inbreeding (Harbo and Harris, 2001). We know this because brood viabilities were often very high (>85%) for queens when they first begin egg-laying, and it is only after several months that poor brood patterns developed. The cause of poor brood production is not understood, but not all VSH lines developed the problem (Harbo, 2001). The problem is not inherent to queens or brood, and it can be selectively bred out of VSH lines while retaining *Varroa* resistance (Tom Glenn, unpubl. data). For example, pure naturally mated and hybrid VSH queens did not develop poor brood production over a 3-year field trial (Ward et al., 2008). Until there is a better understanding of the problem, commercial release of VSH through hybrids is recommended.

Hybrid VSH bees have provided substantial *Varroa* resistance and have retained good

brood production and colony size during routine maintenance of experimental lines. Hybrid VSH bees grew half of the mite populations of control colonies, and their adult bee populations and brood areas were larger (Harbo and Harris, 2001). Mite populations in hybrid VSH colonies were slower to reach an economic threshold in a 1-year study, but some of them developed poor brood production (Delaplane et al., 2005). Over a 2-year period the *Varroa* resistance of MNHYG was significantly increased in hybrids having less than the typical $F_2$ contribution from VSH parents (Ibrahim et al., 2007).

The performance of either hybrid VSH or pure VSH colonies was compared to RHB and a commercial control stock in beekeeping operations in Alabama over a 3-year period (Ward et al., 2008; Danka et al., 2008). Over the entire study, only 12% of VSH colonies reached a recommended treatment threshold (Delaplane and Hood, 1999), whereas 24% of RHB and 40% of the controls exceeded threshold, although treatment thresholds for resistant bees are not established and may be different. The stocks were similar in colony size, honey production and queen survival (Ward et al., 2008). Strong *Varroa* resistance can be obtained by using VSH honey bees without any significant loss of desired beekeeping characteristics. Beekeepers reported good beekeeping quality for all stocks, even pure VSH colonies.

### 3.4. Transfer of VSH germplasm to the beekeeping industry

High-VSH germplasm currently is released to the beekeeping industry through Glenn Apiaries (www.glenn-apiaries.com). Selected breeder queens containing the VSH trait are distributed to queen producers who raise daughters from the breeder queens and outcross them to unselected drones. In this way, significant *Varroa* resistance is delivered in the form of hybrid VSH colonies, while brood production and other desired beekeeping qualities are retained. VSH breeder queens have been sold to 50–80 queen producers in the US during each of the last few years, and about 12–15 of these queen producers sell a variety of outcrossed VSH queens to beekeepers (T. Glenn, unpubl. data).

Selection and breeding of VSH bees have been focused mainly on *Varroa* resistance, with some selection to avoid susceptibility to tracheal mites. The research lines maintained by us have been variable for other characteristics. Further breeding would be desirable in several areas. For example, recent surveys of beekeepers indicate a strong preference for Italian honey bees (T. Glenn, unpubl. data). Initial efforts have been made in the VSH breeding program to select for characteristics associated with Italian stock. Additionally, some VSH lines will be selected for improved performance in migratory pollination service.

Although high expression of the VSH trait can control growth of mite populations, reliance on a single resistance mechanism may be unwise. A goal of additive *Varroa* resistance produced by combining multiple mechanisms is highly desirable. Several of the other mechanisms of *Varroa* resistance are currently being selected by us. We are also trying to identify the semiochemicals that elicit removal of mite-infested brood and molecular markers associated with the VHS trait that could be used in future selective breeding. Until fully *Varroa* resistant bees have been developed, the VSH-trait should be part of a comprehensive integrated pest management scheme (Delaplane et al., 2005).

## 4. THE USDA-ARS RUSSIAN HONEY BEE (RHB) BREEDING PROGRAM

### 4.1. Early evaluations in Russia and the United States

The RHB breeding program has developed a novel stock, derived from the honey bees of far-eastern Russia, which is resistant to *V. destructor*. These honey bees were brought there from Western Russia in the mid-1800s by pioneers (Crane, 1978). The area is within the home range of *A. cerana*, the original host of *V. destructor*. Almost certainly, the imported *A. mellifera* became infested with *Varroa* rather

quickly, producing the historically longest association of *A. mellifera* and *Varroa*. It was hypothesized that this long association gave the best chance for natural selection to mold honey bees resistant to *Varroa* (Danka et al., 1995). Exploring this hypothesis led to the development of the RHB stock.

Collaborative research (Danka et al., 1995) with the Far-Eastern Branch of the Russian Academy of Sciences resulted in surveys and a natural history comparison of *Varroa* MPG in Russia and the US which suggested that RHBs perhaps were comparatively resistant to *Varroa*. Consequently, honey bee stock from Russia was imported through quarantine into the US. Confinement on the island quarantine lasted eight months where the imported RHB were subjected to rigorous regulatory inspection (Harris et al., 2002).

After quarantine, the RHB colonies were uniformly inoculated with *Varroa* and evaluated for MPG. Most colonies supported a MPG lower than expected for susceptible colonies. Many had a MPG that was half to a 10th of the standard, with one colony not showing any MPG. Forty of the queens were chosen to be further evaluated in a sib-test (Rinderer et al., 1999).

Although the tests of individual queens provided additional evidence that the RHBs were resistant to *Varroa*, a rigorous experiment to compare the RHBs with known susceptible honey bees in a side-by-side experiment was lacking. Consequently, a comparative experiment was begun (Rinderer et al., 2001a). Newly produced RHB and Italian queens selected for resistance to *Varroa* were established in colonies inoculated with *Varroa* mites. The colonies were evaluated for numbers of adult female *Varroa* and the presence of varroosis from June, 1998 to November, 1999. The average numbers of adult female *Varroa* in Italian colonies continually grew to about 10 000 in the summer of 1999 (Fig. 1). The average number of mites in RHB colonies also grew, but only to about 4000 during this time. By July of 1999, all of the Italian colonies had died, most of them exhibiting varroosis, and all of them having high numbers of mites while only three RHB colonies died, apparently because of varroosis. The comparative survival

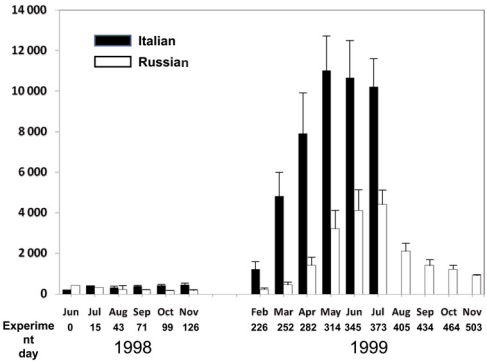


**Figure 1.** Average *Varroa* infestations (estimates of the total number of adult female mites) in RHB (white bars) and Italian colonies (black bars) through time. Error bars = sem (From: Rinderer et al., 2001a).

of the RHB colonies, the comparatively fewer mites infesting the RHB colonies and the decline in mite numbers in late-summer and autumn all supported the conclusion that RHBs were resistant to *Varroa.*

### 4.2. Mechanisms of RHB resistance to mites

Comparative studies of RHB and Italian honey bees found several mechanisms underlying RHB's resistance to *Varroa* mites. RHB consistently had low proportions of brood infested (Rinderer et al., 2001b; de Guzman et al., 2007; de Guzman et al., 2008) and fewer multiply infested cells in both worker and drone brood (de Guzman et al., 2007). A reduced attractiveness of RHB brood and a strong expression of hygiene (de Guzman et al., 2002) may have contributed to the increased rate of non-reproductive mites and decreased number of progeny and number of viable female offspring in RHB (de Guzman et al., 2008). Decreased reproductive success also may have been increased by the combs built by RHB (de Guzman et al., 2008). Reduced brood attractiveness and a higher rate of brood removal may have contributed to the extended phoretic period of *Varroa* mites in colonies of RHB (Rinderer et al., 2001a; de Guzman et al., 2007) increasing the

vulnerability of *Varroa* mites to be groomed in RHB colonies. RHB colonies had a higher proportion of damaged mites (42% vs. 28%) on bottom board traps than did Italian bees, which suggests that they have a strong *Varroa* grooming trait (Rinderer et al., 2001a).

In addition to having resistance to *Varroa*, RHBs were found to have other valuable traits which have been maintained or improved through selection. First, they are highly resistant to *Acarapis woodi* (de Guzman et al., 2001). Resistance to *A. woodi* was a contributing factor to comparatively very high winter survival of RHBs (de Guzman et al., 2005; Villa et al., 2009b). Resistance to *A. woodi* in RHBs is attributed to autogrooming (Villa, 2006), which might also contribute to resistance to *Varroa*. The genetic control of autogrooming is polygenic with some of the genes having a strong dominance effect (Villa and Rinderer, 2008). Also, RHBs are very hygienic (de Guzman et al., 2002) according to a standardized test (Spivak and Reuter, 1998).

### 4.3. Selection procedures

Testing and stock selection began with cooperating beekeepers who provided apiaries in northeastern IA, known for having both harsh winters and perennial problems with tracheal mites, apiaries in central MS that experienced a mid-summer soybean (*Glycine max*) nectar and pollen flow and apiaries in southern LA that experienced a late spring Chinese tallow *(Triadica sebifera*) nectar and pollen flow. These test apiaries provided a diversity of conditions that allowed the selection program to produce a stock adapted to a wide range of beekeeping. On occasion, a line would prove exceptional in one area but poor in a different area and was discarded.

Inclusion of stock into a closed breeding population based on selection began in 1999 and continued to 2007. Daughters of the best of the queens imported from Russia were subjected to an intensive sib-test in the nine apiaries supplied by the cooperating beekeepers. Overall, daughters of 42 queens identified by individual tests of the 362 queens imported from Russia were evaluated in sib-tests, and 18 queen lines (5%) were included in the closed breeding population. Sib-tests evaluated MPG in the colonies and their honey production. Data for each colony were converted to within apiary Z-scores, permitting the comparison of lines and colonies among all apiaries. Using these comparisons, some lines were chosen for potential inclusion into the closed population of breeder lines using an un-weighted selection index score which combined each colony's Z-score times –1 for MPG and the Z-score for honey production to produce a single number for comparisons between colonies (Rinderer et al., 2001b). These lines were further tested to assure *A. woodi* resistance using a standardized test (Gary and Page, 1987). Lines not highly resistant to *A. woodi* were culled regardless of their selection index score.

Selection for mite resistance was based solely on colonies having low MPG. Any mechanism that promotes reduced MPG would be selected for using this criterion. Some mechanisms of resistance to *Varroa* might be associated with colonies being too small to be commercially useful (Büchler, 1997). However, lines were also concurrently selected for increased honey production which acts as a reasonable counter measure to prevent colonies from simply getting smaller as a response to selection for reduced MPG. Both selection criteria are broad. Any specific trait that contributes to a reduction in MPG would potentially be enhanced by selection. Likewise, any trait that generally enhances fitness would potentially be enhanced by selection for honey production.

### 4.4. Development of a closed breeding population of RHB

Groups of sister queens were produced from individual imported queens and "sib-tested" in multi-state trials. The 18 best sibling groups were used to found breeder lines with the best two or three siblings serving as mothers of the next generation. The 18 breeder lines were organized into three groups of six breeder lines for conducting matings within a closed population. Queens of each group of lines are

mated to drones of the other two groups of lines. This plan is designed to reduce inbreeding while also providing a practical method to arrange the open mating of 18 separate lines on an isolated island. Queens of one group can be produced and mated simultaneously. This mating scheme has resulted in a stock that retains good genetic diversity among groups and lines (Bourgeois et al., 2008). Also, allelic frequency differences at molecular loci enable RHBs to be distinguished from other commercial stocks with very high accuracy (Bourgeois and Rinderer, 2009). Using this suite of loci, the diversity among RHBs compares favorably to the diversity of non-RHB stocks in the US.

Between 1999 and 2007, the program emphasized sib-testing of lines that could potentially be added to the closed population. However, each year those lines that had been added to the program were tested and propagated. Owing to limited resources only between 8 and 12 colonies were used in tests of each line. However, in 2001 one line was included in the trials for the next three years as a test of the success of selection. The line had a comparatively high average Z-score for honey production and a moderately negative z-score for MPG (negative being desirable). When the scores were combined in an unweighted selection index, the line ranked highest of the lines tested that year. Each year the MPGs for the line were lower than the previous year (Fig. 2) suggesting that selection improved resistance to *Varroa*. The line continued to be the best honey producer in subsequent years, but honey production was not substantially improved. A separate experiment (de Guzman et al., 2007) compared RHB colonies from lines in the closed population to Italian colonies from 2001 to 2003 in the same apiary. Each year new queens were used. MPGs for Italian colonies were always larger and varied among the years without having a year to year trend. MPGs for RHB colonies trended lower through the years (Fig. 3). Hence, selection within the closed population increased the stock's resistance to *Varroa*.

Improvements in honey production are less well documented. However, honey production by RHBs has equaled or surpassed the honey

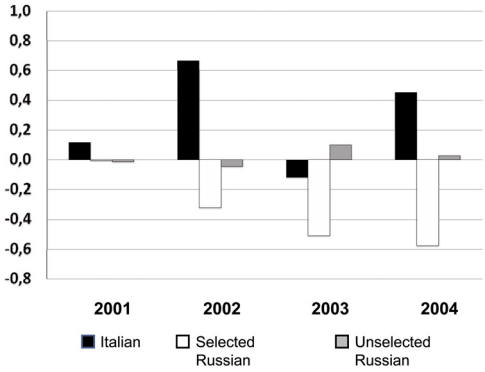


**Figure 2.** Changes in Z-scores of mite population growth estimated from a sib-test in four years for Italian colonies, a RHB line (selected Russian) that underwent selection for reduced mite population growth and a group of RHB lines (unselected Russian) being evaluated for inclusion into the closed breeding population. The selected line became comparatively more resistant each year.

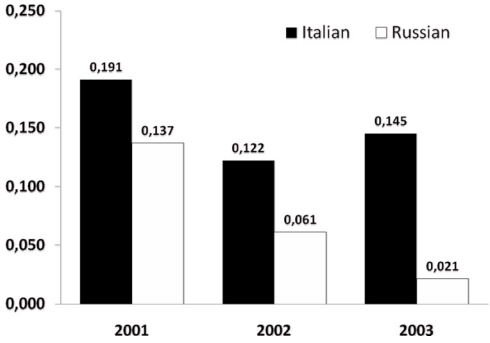


**Figure 3.** Instantaneous mite population growths (IMPG) across three years for an Italian stock not selected for resistance to *Varroa* and lines of RHBs in a closed breeding population that were selected for reduced *Varroa* population growth (from data presented in: de Guzman et al., 2007). IMPG showed annual decreases for the selected RHB but not for the unselected Italian stock.

production of a well respected Italian stock of honey bees in several experiments (Rinderer et al., 2001c, 2004). These results contrast with European studies that found "Primorski" honey bees were resistant to *Varroa* but produced less honey than locally selected *A. m. carnica* (Berg et al., 2004, 2005). These studies also reported "Primorski" honey bees to

be less gentle, although these studies included most lines of RHB and their hybrids rather than only lines released for general distribution. Some RHB hybrids are not gentle. However, purebred lines released for general distribution overall have acceptable traits including honey production and gentleness.

### 4.5. Transfer of RHB to the beekeeping industry

The breeding and selection program continues as a commercial activity. A group of United States queen breeders have formed the Russian Honeybee Breeder's Association and are continuing the selective breeding of the stock (Brachman, 2009). Members of this group and their customers use no other method to control *Varroa* beyond using the stock and have done so for many years. This management is consistent with studies of the stocks resistance (Rinderer et al., 2003, 2004). Additionally, RHB's outcrossed to susceptible stock express enough resistance to permit reduced schedules of *Varroa* control treatments (Harris and Rinderer, 2004).

## 5. MOLECULAR GENETIC APPROACHES TO RESISTANCE BREEDING

The publication of the sequence of the honey bee genome provided a means to study all of the genes in the bee (Honey Bee Genome Sequencing Consortium, 2006). How can molecular genetics help us to breed bees that resist mites? If we could identify the genes that influence resistance, we could select alleles directly by looking at the bee's DNA (marker-assisted selection: MAS). One approach is to use microarrays to study gene expression in resistant and susceptible lines to identify resistance genes, and one study using *Varroa*-surviving bees in France has been conducted (Navajas et al., 2008). Microarrays have been very useful for characterizing gene expression patterns for behavioral and physiological states, suggesting genes that may influence them (e.g., Whitfield et al., 2003; Grozinger et al., 2007). But microarray studies would not necessarily identify the genes that need to be selected for resistance. It is quite possible that the gene(s) responsible for resistance is not among them since it may control the expression of other genes, or is only differentially expressed at certain times, or in a specific tissue. Another difficulty for microarrays comes from effects of genetic backgrounds on gene expression, since inbred lines are difficult to develop for honey bees and resistant and susceptible strains would have many genetic differences unrelated to resistance. Probably the greatest benefit of differential gene expression studies is to identify genes involved in physiological processes that occur during mite infestation and the development of parasitic mite syndrome (Navajas et al., 2008).

Another possibility is to identify chromosomal regions that contain genes influencing a trait. The technique of quantitative trait locus (QTL) mapping is applicable to any heritable trait. The number of QTLs affecting the trait, their relative effects and their locations on chromosomes can be estimated. Basically, this involves studying the quantitative trait's association with DNA markers in a family of individuals descended from a hybrid individual derived from a cross between parents having low and high phenotypes of the trait (e.g. resistant and susceptible). The assumption is that there are multiple genes influencing the trait and that certain DNA markers that are close to genes influencing the trait will be nonrandomly associated with the trait values, despite crossing over during meiosis (recombination). Recombination is the basis for genetic mapping because the number of crossovers between two points on a chromosome correlates with their physical distance. A honey bee genetic map revealed a higher rate of meiotic recombination than any reported for a higher eukaryote (Hunt and Page, 1995; Solignac et al., 2004). This high rate of recombination is very useful for QTL mapping because it results in higher resolution of physical chromosome distance which reduces the effort required to find which gene(s) is influencing the trait.

In the honey bee, QTL mapping has been used to identify genes that influence stinging, foraging and guarding behaviors, foraging

age, response to sucrose and ethanol, worker egg-laying, and even ability to learn (Hunt et al., 1995, 1998; Page et al., 2000; Chandra et al., 2001; Arechavaleta-Velasco and Hunt, 2004; Rueppell et al., 2004, 2006; Ammons and Hunt, 2008; Oxley et al., 2008). If the sequences of DNA fragments used as markers are known, it is possible to identify the trait's candidate genes. For pollen-foraging and stinging, the QTL regions each contained about 40 genes (reviewed by Hunt et al., 2007). Regarding traits that influence resistance to *Varroa*, one study identified seven putative QTLs influencing general hygiene, but the markers were of unknown sequence so it was not possible to align the genetic map with genome sequence or to identify candidate genes (Lapidge et al., 2002).

What are the future prospects for using MAS? MAS, is most valuable when the cost of determining the phenotype (resistance) is high, and the time between generations is long (Hospital, 2009). Traits such as mite-grooming and VSH appear to be the most desirable *Varroa* resistance traits but are difficult to measure. Also, measurement of trait expression requires full colonies. MAS may permit bypassing the production of colonies and thereby speed selection while insuring the presence of the right alleles of specific genes. On the other hand, because of the high recombination rate of the honey bee we may require markers that are within the actual gene sequence, and the gene would need to be identified. Single-nucleotide polymorphisms (SNPs) often are found within honey bee genes (Whitfield et al., 2006). Genotyping arrays can be used to analyze thousands of SNPs in a set of several hundred individuals to make a high-density QTL map. SNPs in candidate genes identified by QTL mapping could then be tested for association with the trait in populations (Blangero, 2004; Anholt and MacKay, 2004).

We believe that MAS will not be a 'silver bullet' for making the super-resistant bee. The level of resistance found in *A. cerana* most surely is regulated by several genes and markers must be developed for several favorable alleles. As genotyping costs continue to fall, MAS may become a useful tool for combining several resistance traits in the same stock.

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**Sélection d'abeilles résistantes à *Varroa destructor* en Amérique du Nord.**

**résistance au *varroa* / programme de sélection / abeilles russes / comportement hygiénique sensible à varroa / sélection assistée par marqueur**

**Zusammenfassung – Zucht auf Resistenz gegen *Varroa destructor* in Nordamerika.** Die Zucht auf Resistenz gegen *Varroa destructor* in Nordamerika bietet die langfristige Lösung für die von der Milbe verursachten wirtschaftlichen Schwierigkeiten. Dieses Review untersucht mehrere potenzielle Mechanismen der Resistenz gegen *Varroa* und berichtet über die Entwicklung von zwei Zuchterfolgen, aus denen Bienen von wirtschaftlicher Qualität hervorgegangen sind, die weniger Pestizidbehandlungen gegen *Varroa* benötigen als unselektierte Bienen.

Das VSH Zuchtprogramm konzentriert sich auf die Selektion eines spezifischen Resistenzmechanismus, der Varroasensitive Hygiene genannt wird. Das Merkmal VSH wird über den Verkauf von VSH Königinnen, die mit Drohnen bereits vorhandener kommerzieller Linien gepaart wurden, für die Imker verfügbar gemacht. Die größte Resistenz kommt zwar in reinen VSH-Linien vor, die nachhaltigste Verbreitung wird jedoch durch VSH Hybridvölker erzielt. Durch das Auskreuzen reiner VSH Linien mit einer Vielzahl anderer kommerzieller Linien kann die genetische Diversität der Bienenpopulation in den USA auf relativ hohem Niveau gehalten werden. Reine VSH Zuchtköniginnen werden von Glenn Apiaries produziert und an kommerzielle Produzenten von Königinnen verkauft, die ihrerseits ausgekreuzte VSH Königinnen an Imker verkaufen.

Das Programm zur Russischen Biene nutzt ein Zuchtschema, das auf einer geschlossenen Population basiert, um gegen *Varroa* resistente Linien zu verbreiten, die ursprünglich aus dem fernöstlichen Russland stammten. Die Russischen Honigbienen (RHB) des ARS wurden aus 18 importierten Linien durch Geschwistertests über mehrere Jahre hinweg entwickelt. Ihre Varroaresistenz geht auf mehrere Mechanismen zurück, zu denen gegenseitiges

Putzen, varroasensitive Hygiene und für die Milbe geringe Attraktivität der Brut gehören.
RHB Linien wurden gleichzeitig für Varroaresistenz, gute Honigproduktion und Resistenz gegen Tracheenmilben, *Acarapis woodi*, selektiert. Die Resistenz gegen Tracheenmilben trägt zu ihrer ausgezeichneten Überwinterungsfähigkeit bei. Der Erfolg der experimentellen RHB Selektion regte eine große kommerzielle Nachfrage an, und RHB werden zurzeit von einer als Russian Queen Breeder's Association bekannten Züchterkooperative gezüchtet, vermehrt und an die Imker in den USA verbreitet.
Die Zucht auf Varroaresistenz wird in der Zukunft wahrscheinlich auch markergestützte Selektion (MAS) mit einbeziehen, in welcher entweder die Expression von mit Resistenz verbundenen Genen (RNA) oder molekulare Marker, die mit Resistenzgenen in Verbindung stehen (DNA), benutzt werden um die Zuchteltern auszuwählen. Das endgültige Ziel ist, die arbeits- und zeitaufwändige Selektion im Feld durch eine Labordiagnose zu ersetzen. Es wird erwartet, dass MAS den Selektionsfortschritt sowohl für Resistenzmerkmale, die schon entwickelt wurden, als auch für Merkmale, für die diese Entwicklung hin zu nutzbaren kommerziellen genetischen Linien noch aussteht, wie z.B. gegenseitiges Putzen und Entfernen von Milben, beschleunigen wird.

**Varroaresistenz / Zuchtprogramm / Russische Honigbienen / Varroasensitive Hygiene / markergestützte Selektion**

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