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## Breeding for resistance to *Varroa destructor* in Europe\*

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**Abstract** – The rich variety of native honeybee subspecies and ecotypes in Europe offers a good genetic resource for selection towards *Varroa* resistance. There are some examples of mite resistance that have developed as a consequence of natural selection in wild and managed European populations. However, most colonies are influenced by selective breeding and are intensively managed, including the regular use of miticides. We describe all characters used in European breeding programs to test for *Varroa* resistance. Some of them (e.g., mite population growth, hygienic behavior) have been implemented in large-scale selection programs and significant selection effects have been achieved. Survival tests of pre-selected breeder colonies and drone selection under infestation pressure are new attempts to strengthen effects of natural selection within selective breeding programs. Some perspectives for future breeding activities are discussed.

**Varroa resistance / breeding program / tolerance character / vitality / natural selection**

### 1. INTRODUCTION

Europe has a high diversity of climatic regions and natural biotopes. As honeybees are endemic to most of regions in Europe, they have evolved into numerous natural subspecies and ecotypes (Ruttner, 1988) with different adaptive capacities. This diversity offers rich potential genetic resources for selection on mite resistance. Wild honeybee populations under natural selection are very rare, not only as a consequence of *Varroa* infestation but also due to a lack of natural habitats in densely populated areas with intensive use of farmland and forests. Today, nearly the entire honeybee population of Europe is managed by the beekeeping industry, which involves regular chemical treatments for diseases and mites that can con-

tribute to the propagation of susceptible bee populations.

Breeding and selection techniques have a long tradition in European countries and are widely used with varying degrees of rigor. Starting at the end of the 19th century, beekeepers transported queens and colonies across the natural ranges of different subspecies and ecotypes. This practice led to hybridisation followed by changes in the spread and frequency of certain genotypes. While the economically important subspecies *A. m. carnica* and *A. m. ligustica* are now widely spread throughout Europe, the native populations of *A. m. mellifera*, *A. m. siciliana*, *A. m. macedonica*, and other European subspecies have been diminished and partly extinguished from their natural ranges (Meixner et al., 2010).

Breeding programs are well established in many European countries, based on performance tests, statistical data analysis, and the mass propagation and controlled mating of

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selected breeder colonies (Lodesani and Costa, 2003). The strong impact of selective breeding on the population is evident due to the significant reduction of genetic variability in European compared to wild African colonies (Moritz et al., 2007).

In the majority of selection and breeding programs, economic traits (such as honey productivity and colony strength) together with traits desirable for modern beekeeping (such as gentle temper and low swarming tendency) have been of predominant importance. In contrast, disease resistance, viability, and adaptation to local conditions were considered less important, as deficiencies in these characters could often be compensated by pharmaceuticals, artificial feeding, and other management techniques.

Since *Varroa destructor* began spreading throughout Europe, the beekeeping industry has had to face a new situation. The regular use of chemical treatments has been accompanied by several disadvantages, such as high costs and labor, residues in bee products, and the selection of mites resistant to acaricides. However, repeated high colony losses due to varroosis could not be prevented. Consequently, research on mite resistance of honey bees started in the 1980s and continues to receive a large amount of scientific interest and practical attention in Europe.

We consider resistance in honey bees as the ability of a bee population to survive without therapeutic treatments in a given environment and management system. Resistance is therefore not an absolute trait, but rather has to be viewed as the result of successful interactions in a specific environment. High levels of resistance occur in some untreated European bee populations. However, as most colonies are under strong influence of modern beekeeping management, which includes regular use of acaricides and requeening with selected stock, *Varroa* resistance should be implemented on a broader scale in selective breeding activities. This implementation depends on having suitable test characters and an effective coordination of breeding programs. Comparative tests between European bee strains and Primorski lines from the US allow for an evaluation of the European breeding efforts.

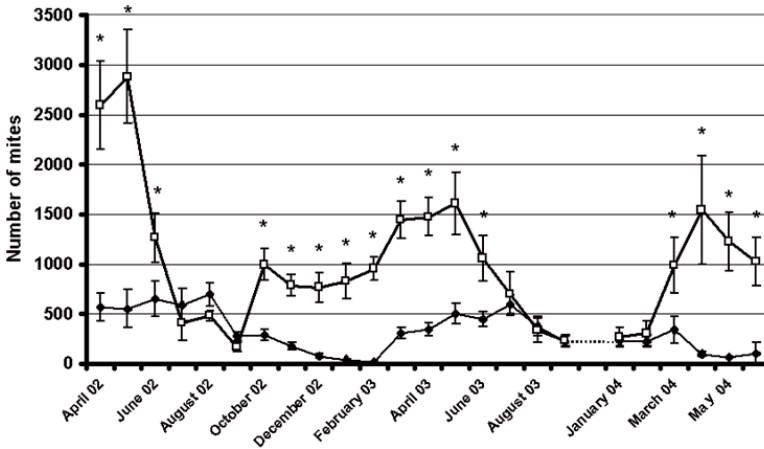
## 2. MITE RESISTANCE IN UNTREATED EUROPEAN BEE POPULATIONS

*Varroa* surviving bee (VSB) colonies were identified in untreated *Apis mellifera* populations from a few different locations in Europe. Similar observations were reported by Seeley (2007) from a small bee population in the Arnot Forest in the northeastern United States.

When *Varroa* mites first invaded France in the 1980s, feral and untreated colonies were destroyed by the mites. The first observations of natural VSB were made in 1994 in western France, near Le Mans, where feral and untreated colonies seemed to survive the mite infestation for a few years. In 1999, 10 out of 12 of such untreated colonies were still surviving (5 of them survived for more than 11 years). At that time, 82 colonies that were untreated for at least 2 years were collected to characterize their survival without *Varroa* control. The colonies were managed only to monitor their survival. They were allowed to swarm and replace their queens naturally. On average, the survival of those colonies was  $7.88 \pm 0.3$  years with a maximum of 15 years (Le Conte et al., 2007).

*Varroa* populations were estimated between VSB and *Varroa* susceptible (control) colonies by counting natural mite mortality using a screened bottom board to collect the mites (see Fries et al., 1991). Traps were left in place constantly, and the mites falling down on the bottom board were counted one to three times a week depending on the amount of brood in the colonies. In Avignon, from April 2002 to June 2004, natural mite mortality was observed in 12 VSB colonies and 16 control (*Varroa* susceptible) colonies.

The number of mites collected in VSB colonies was three times lower than in control colonies, which was statistically significant (Le Conte et al., 2007), and each year the highest differences were observed between March and June (Fig. 1). The infestation of the VSB colonies from July to September, as measured by mite fall, decreased in both groups. After that period, *Varroa* populations increased more rapidly in control colonies than in VSB colonies. The VSB maintained lower



**Figure 1.** Number of mites collected per month on the bottom board (mean  $\pm$  S.E.) from April 2002 to June 2004. Mites were not counted between October 2003 and December 2003.  $\diamond$  represent mean number of mites in VSB ( $n = 12$  colonies),  $\square$  represent control colonies ( $n = 16$ ). (\* Significant statistical differences, Mann-Whitney test,  $P < 0.05$ ).

*Varroa* populations year round, suggesting that VSB colonies had developed mechanisms to inhibit the growth of *Varroa* populations.

Various hypotheses could explain this phenomenon. First, the bees may have developed behavioral or physiological resistance against the mite. Martin et al. (2002) showed that the VSB have better ability to recognize the mite compared to control bees. Thus, VSB could be more efficient in their ability to detect and destroy the mite from workers through grooming behavior, similar to the behavior of *Apis cerana* (Peng et al., 1987). They may also be more efficient in removing mite-infested pupae from the cells as reported in the MN Hygienic stock (e.g., Boecking and Spivak, 1999; Spivak and Reuter, 1998a, 2001) and *Varroa* sensitive hygienic (VSH) bee strains from Louisiana (Harbo and Harris, 2005a). Indeed, preliminary observations show that VSB are able to detect and remove mite-infested pupae from cells (Anderson and Le Conte, pers. obs.). Interestingly, gene-expression analysis of the VSB shows over-expression of a set of genes related to responsiveness to olfactory stimuli compared with *Varroa* susceptible bee colonies (Navajas et al., 2008). Harbo and Harris (2005b) suggested only a few genes to be involved in VSH behavior, which

seems to be widespread in honeybee populations. The quantitative trait loci (QTLs) for hygienic behavior from the MN Hygienic stock (Lapidge et al., 2002) were recently confirmed, and some genes regulating this behavior are thought to be involved in olfactory sensitivity (Oxley et al., in press). The genes involved in olfaction in the VSB, VSH, and MN Hygienic stocks are potentially very interesting to characterize as they may be involved in the ability of these bees to detect and remove and thus resist *Varroa*.

Other characters – such as the inhibition of *Varroa* reproduction, the duration of the post-capping stage, and thermoregulatory abilities – may also contribute to the survival of the VSB. Moreover, an average of 41.5% of the VSB colonies swarmed each year. Fries et al. (2003) demonstrated that swarming affects *Varroa* populations in a colony for at least one year; thus, swarming could at least partly explain the survival of the VSB.

Differential virulence of the mite also can be hypothesized to explain the survival of VSB. After the first years of *Varroa* invasion in France, most of the untreated colonies were found dead with plenty of mites trapped in entire frames of dead sealed brood. Individual fitness of a mite in those cells was therefore

null. As a less-virulent parasite (such that it would not kill the host and would have an increased individual fitness), the hypothesis of sub-populations of mites with different levels of virulence was tested using mitochondrial and nuclear microsatellites markers (Navajas et al., 2002; Solignac et al., 2003). The structure of *Varroa* population in Europe was found to be an invasive clone (Solignac et al., 2005). Therefore, it is unlikely that sub-populations of less-virulent mites could explain VSB, or if they are, virulence would be due to a limited number of genes as it is the case with *Varroa* populations that are resistant to the acaricide flouvalinate-tau (Milani, 1999; Liu et al., 2006).

Acute paralysis virus (APV) and deformed wing virus (DWV) are resident in bee colonies and become more pathogenic when associated with *Varroa* (Sumpter and Martin, 2004). Therefore, survival of VSB could be due to a higher tolerance of the bees to those viruses. This hypothesis was tested, and data have shown that the VSB had less APV and CPV (chronic paralysis virus) compared to control bees. However, the VSB did not survive longer compared to control bees when injected with the two viruses (Le Conte, personal communication). This suggests that the VSB have fewer viruses because they have fewer mites to transmit virus in the bee population. Nevertheless, it is reasonable to suggest that honeybee resistance, *Varroa* virulence, and virus prevalence are constantly under selective pressure, and that natural selection favors a co-evolution that secures the survival of both the host and parasites.

The presence of a specific pathogen of the *Varroa* mite, such as fungus, cannot be ruled out and should be investigated as a factor contributing to the survival of VSB. Also, the effect of the environment and apicultural methods on the survival of VSB cannot be excluded. The areas where the experiments were done are outside France's major agricultural zone and are very favorable to the development of honeybee colonies. The colonies were manipulated only if necessary and were not moved or managed as professional beekeeping would recommend.

### 3. BREEDING FROM UNTREATED SURVIVOR COLONIES

The occurrence of VSB in natural European populations led to the study of survival in other infested honeybee colonies that were not treated for *Varroa* mites in order to select for *Varroa* resistance. This approach, called the Bond test ("Live and let die!"), has been used successfully in France (Kefuss et al., 2004) and Sweden (Fries et al., 2006).

Kefuss et al. (2004) initiated the first Bond test in 1993 on 12 *Apis mellifera intermissa* colonies imported from Tunisia in France, near Toulouse. The resistance of those bees was compared with 12 *A. m. carnica* *Varroa*-susceptible colonies after exposure to heavy *Varroa* infestations. Only *A. m. intermissa* colonies survived. They hybridized with local bee populations and most of the hybrids survived mite infestations indicating a genetic control of the resistance.

Since 1999, Kefuss et al. (2009) have not used any treatments against *Varroa* in their professional beekeeping enterprise. From this naturally surviving stock, they subsequently select their breeder colonies for economical traits. The best colonies are then tested for hygienic behavior (using a freeze-killed brood assay) and for *Varroa* infestation. Their colony losses are comparable to other beekeepers of the region that still treat their hives with acaricides. Adult bee infestation usually remains below 5% and, according to their report, does not economically justify the use of chemicals.

In Gotland, an island of the Baltic Sea, Fries et al. (2006) described the survival of 150 honey bee colonies that were subject to the Bond test. Five of the colonies survived over 5 years. Later, Fries and Bommarco (2007) compared the surviving colonies to *Varroa* susceptible bees parasitized by mites from a different source. The mite load in the Bond colonies was 82% lower compared to the control colonies. It was hypothesized that the differences in mite populations may have been due to the smaller brood areas in the Bond colonies and differences in mite distribution, with more mites present on adults rather than the brood in the Bond colonies.

Fries et al. (2006) pointed out that when beekeepers treat colonies to control the mite, they remove the selective pressure on both the host and the parasite, which could result in co-evolution with long term survival of both. The presence of wild colonies/swarms and untreated apiaries can promote co-evolution that could spread to larger areas. Even if those surviving colonies may not be interesting for professional beekeeping because of their lower honey production due to the cost of the tolerance (Le Conte et al., 2007), they can form the basis for integrated *Varroa* management and selection programs to increase honey production.

#### 4. RESISTANCE CHARACTERS FOR THE USE IN SELECTIVE BREEDING PROGRAMS

Because the effects of natural selection on increasing resistance to the mites have been widely eliminated by modern beekeeping practices (through the regular use of acaricides and requeening with productive and gentle breeding lines that have low swarming tendency), the development of genetic resistance depends on successful implementation of breeding programs that use suitable resistance characters in the selection process of honeybees.

The selection for mite resistance in treated populations has to rely on indirect selection characters, because the direct trait of survivability cannot be studied when the colonies are influenced by medical treatments. Starting about 25 years ago, much research in European institutes focused on the identification of suitable selection characters (Büchler, 1994b). In addition to the biological relevance (correlation with the direct selection goal), the heritability and the practicability of testing under field conditions were deemed to be of major importance in the implementation of such characters in breeding programs.

In the following, we briefly summarize the research on characters that have been used – at least temporarily – in European breeding projects for increased mite resistance.

#### 4.1. Mite population development

The effect of varoosis on the colony level depends on the infestation level (Garrido and Büchler, unpubl. data; Genersch et al., 2010). Therefore, slow and limited mite population growth is a fundamental criterion of resistant stock that can be used as a character for selective breeding on mite resistance, even if the underlying behavioral and physiological causes remain unknown. Different methods to accurately estimate the *Varroa* infestation level are well documented (Fuchs, 1985; Rademacher, 1985; Calatayud and Verdu, 1993; Garza and Wilson, 1994; Branco et al., 2006). However, methods used for the comparison of test colonies in large-scale selection programs need to be simple, reliable, and well standardized to optimize the heritability of the infestation data. Based on the number of mites killed by an annual Apistan® treatment (Pechhacker, 1992), Boigenzahn and Willam (1999) estimated the heritability of infestation to be  $h^2 = 0.13$ . The heritability can be increased if the test is started with a uniform artificial infestation of all colonies (Büchler, 1994a, 2000) but requires too much effort for routine field selection.

In the AGT breeding program (“Arbeitsgemeinschaft Toleranzzucht”, see below) mite population growth during the brood season is used instead of measures of absolute infestation level. For each colony, the infestation of a bee sample (at least 30 g per hive) collected in the first 10 days of July is compared to the natural mite fall during 3–4 weeks in spring (during *Salix* bloom as a phenological standardization for different climatic regions). The bee sample is taken from honey combs of the uppermost box, since they have a more uniform infestation (repeatability of infestation was 0.85 from honey combs versus 0.63 from samples taken from the hive entrance and 0.74, from the central brood nest; Garrido and Büchler, unpubl. data). Preliminary estimates on the basis of about 5000 colonies show median heritability values for this method of monitoring the mite population growth (Ehrhardt and Bienefeld, unpubl. data). The technique is easy and flexible in practice (independent of colony treatment before and



after the test period) and, in addition to its use in the AGT breeding program, is used routinely in several European countries.

#### 4.2. Hygienic behavior

Hygienic behavior towards infested worker brood cells has been recognized as a vital component of *Varroa* resistance in its original host *A. cerana* (reviewed by Rath, 1999) and as an antiseptic behavior against various brood diseases, including varroosis, in *A. mellifera* (reviewed by Boecking and Spivak, 1999; Wilson-Rich et al., 2009). The performance of colonies regarding this trait has been successfully improved by selective breeding, and it has been shown to be an effective measure to improve resistance of *A. mellifera* against *Varroa*, AFB, EFB, and chalkbrood (Spivak and Reuter, 1998a; Palacio et al., 2000; Harbo and Harris, 2005a; Ibrahim et al., 2007; Büchler et al., 2008; see also Rinderer et al., 2010, for further details and references).

Different methods have been developed to test brood-hygiene behavior under standardized conditions, including the assaying the bees response to *Varroa* infested brood cells (Boecking and Drescher, 1992; Hoffmann, 1996) as well as freeze-killed (Momot and Rothenbuhler, 1971; Spivak and Reuter, 1998b) and pin-killed brood (Newton and Ostasiewski, 1986; Hoffmann, 1996). These methods differ in the effort required and results, and their suitability for selective breeding programs is being discussed. While the freeze-killed brood assay is recommended as a more conservative test (Spivak and Downey, 1998), it shows higher variability between colonies (Espinosa-Montano et al., 2008). The pin-killed brood assay is preferred in most European breeding programs based on its higher repeatability, correlation with a removal of mite infested brood (Hoffmann, 1996), and lower costs (Espinosa-Montano et al., 2008).

For the pin-killed brood test, usually 50 cells containing young pupae are pierced with a fine insect pin. Since the removal of damaged or killed pupae in the course of time follows a sigmoid function, the highest discriminatory power of the test is reached when on

average 50% of the pupae are removed. Therefore, the time interval between piercing the cells and checking should be adapted to the average removal response of the test population. If the average removal rate is much lower than 50%, prolonging the time interval will result in higher differences among colonies with high and low hygienic behavior. If it is much higher than 50%, a shorter time interval should be realized in further test repetitions.

#### 4.3. Grooming behavior

Auto- and allo-grooming are resistance mechanisms against *Varroa* in its original host (reviewed by Boecking and Spivak, 1999; Rath, 1999), but its quantitative contribution to mite resistance in *A. cerana* is still not clear and seems to be very limited. Grooming behavior has also been observed in *A. mellifera* (Bozic and Valentincic, 1995; Thakur et al., 1997), but compared to *A. cerana*, grooming seems to be less effective in *A. mellifera* (Peng et al., 1987; Büchler et al., 1992; Fries et al., 1996).

Although it is affected by numerous biological and environmental factors, the proportion of chewed mites in the debris of a colony can be used as an indicator of grooming success under field conditions (Moosbeckhofer, 1992; Büchler, 1993, 2000; Rosenkranz et al., 1997; Bienefeld et al., 1999; see also Rinderer et al., 2010 for more details and references). After several generations of selection in a test population, colonies selected for this trait showed significantly more damaged mites and lower infestation rates compared to unselected colonies (Büchler, 2000). However, the estimated heritability was too low ( $h^2 < 0.15$ ; Ehrhardt et al., 2007) to justify the laborious sample collection and processing in a large-scale selection program.

#### 4.4. Postcapping stage duration

The postcapping stage duration (PSD) limits the reproduction of *Varroa* in sealed brood cells. There is a median heritability but low variability of the average PSD among European subspecies (Büchler and Drescher, 1990;

Le Conte et al., 1994; Schousboe, 1986). However, selection for faster development of worker brood could be quite effective if realized by direct selection on the reproductive individuals (queens and drones; Moritz and Jordan, 1992; Le Conte et al., 1994; Siuda and Wilde, 1996).

Wilde and Koeniger (1992) selected a line of bees with a significantly shorter PSD compared to *A. m. carnica* and *A. m. caucasica* control colonies (276.4 hours versus 287.4 and 289.1 hours, respectively), but the observed effects on the reproductive success and the population increase of *Varroa* in test colonies were not significant (Siuda and Wilde, 1998). The selection program was stopped after seven generations, recognizing that the achieved breeding progress of 0.2–4.2 hours per backcrossed generation remained insignificant (Siuda et al., 1996).

#### 4.5. Mite reproduction

The failure to reproduce in worker brood is a basic aspect of the mite's biology in *A. cerana*, and thus it is a factor that enables co-existence between *Varroa* and its native host. Reproduction of *Varroa* in *A. mellifera* brood has been intensively studied by numerous authors (reviewed by Fries et al., 1994). However, the impact of bee genetics on the reproductive success of *Varroa* is still unclear. The significant lower fertility of *Varroa* in Africanized bees compared to European strains that was observed in previous studies (Rosenkranz and Engels, 1994; Rosenkranz, 1999) has not been observed subsequently (Garrido et al., 2003), although the Africanized bee population has managed to retain its high resistance level to the mites.

Reduced mite fertility was observed in an isolated apiary where the colonies were maintained without treatment and were selected for low mite fertility over 9 years at the institute in Lunz/Austria (Pechhacker et al., 1996). However, a comparison of bees and mites from this isolated population to bees and mites from Udine/Italy showed a significant effect on mite fertility due to the origin of the mites and not

due to the origin of the bees (Milani et al., 1999).

Recently, Rosenkranz et al. (2009) compared the infertility rates of *Varroa* in *A. m. carnica*, *A. m. ligustica*, *A. m. mellifera*, SMR (now called VSH; Rinderer et al., 2010), and Gotland bees, and they did not detect significant effects of bee type or sex of the bee. However, they found significant differences in the pattern of cuticular compounds on the bees, which should be further studied because certain compounds can reduce (Milani et al., 2004) or stimulate *Varroa* reproduction (Trouiller and Milani, 1999; Garrido and Rosenkranz, 2004). Thus, selection for specific bee cuticular patterns might have the potential to improve *Varroa* resistance in European bees.

## 5. BREEDING PROGRAMS

In most European countries, selection and breeding activities are mainly realized by numerous small-scale beekeepers with the support of several governmental institutions. In most countries, specialized bee-breeding associations have been formed, and selection guidelines have been compiled by beekeeper associations and governmental authorities to coordinate activities.

Most breeding programs use pure subspecies and are oriented towards preserving and improving local populations. Recently, significant progress has been achieved by establishing a genetic evaluation of performance test data, based on a BLUP animal model adapted to the peculiarities of honey bee genetics and reproduction (Bienefeld et al., 2007, 2008). This model estimates breeding values for queen and worker effects based on several colony traits, and it also considers environmental effects. A central online database with about 100 000 registered colonies of *A. m. carnica*, *A. m. ligustica*, *A. m. mellifera*, and *A. m. siciliana* comprising test data from Austria, Germany, Italy, Norway and Switzerland has been established at the Landerinstitut fur Bienenkunde in Hohen Neuendorf, Germany (<http://www.beebreed.eu>).



Within Europe, cross breeding of different subspecies or strains is of minor importance; it is used systematically in the selection of Buckfast bees and occasionally also by single breeders in search for new traits.

Meanwhile, resistance to *Varroa* has been recognized as a relevant selection criterion in most European bee breeding programs. In the following, we describe the AGT breeding program as an example of an ambitious selection strategy based on pure populations, and investigations of Russian Honey Bee (RHB) lines from the US that received special attention as promising candidates for crossbreeding efforts.

### 5.1. AGT selection strategy

The Arbeitsgemeinschaft Toleranzzucht (AGT, <http://www.toleranzzucht.de>) selection program was founded in 2003 to support the selection and propagation of productive, gentle queens with high resistance against *Varroa* and other diseases. Basically, the selection program consists of three elements: (a) pre-selection in a large population; (b) survival testing of pre-selected breeder colonies; and (c) drone selection under natural infestation pressure.

#### 5.1.1. Pre-selection in a large population

About 150 bee breeders are involved in testing about 2000 queens per year. As a sustainable selection progress directly depends on the size and the genetic variability of the population (Moritz, 1986), the limits to the structure of bee breeding in Europe – namely small numbers of colonies per apiary (uniform environmental conditions) and small numbers of colonies per breeder (usually less than 100 colonies) – have been overcome by organizing a systematic exchange of test queens among all breeders and apiaries within the AGT. This approach guarantees optimal evaluation of environmental and genetic effects in the central data processing. Using the estimated breeding values, all tested queens can be directly compared to each other, regardless of their individual test environment.

The uniform protocol for the annual testing is based on Apimondia recommendations (Ruttner, 1972) and includes monitoring of the *Varroa* population growth during the brood season and repeated pin-killed brood tests for brood hygiene behavior according to the methods described above. The central database at the institute in Hohen Neuendorf has been adapted accordingly, and breeding values for *Varroa* resistance are calculated on the basis of mite population growth and pin-killed brood test data in addition to breeding values for honey productivity, gentleness, and swarming behavior. Work is ongoing to combine the breeding values of all individual criteria into a single selection index.

The AGT publishes an annual breeding register with the breeding values of all tested colonies, providing a comprehensive overview over the whole population and stimulating the exchange and propagation of valuable stock.

#### 5.1.2. Survival test of pre-selected breeder colonies

Garrido and Büchler (unpubl. data) observed the wintering ability of untreated colonies with regard to bee population size and *Varroa* and virus infestation levels in different locations in Germany. Colonies with infestation levels below 10% of the adult bees and with adult bee populations of more than 10 000 bees in the fall had a high probability of surviving the winter. The size of the bee population in spring in relation to its size in October (wintering index) proved to be a useful indicator for the health status of the colony.

Within the AGT breeding program, bee breeders are encouraged to refrain from using acaricides on test colonies with an infestation of less than 1 mite/10 g bees in the samples routinely taken during the first decade of July. Subsequently, these pre-selected colonies are monitored for their mite infestation and bee population development on a monthly basis. As soon as colonies come close to 10% mite infestation or decrease to less than 10 000 bees, they are taken out of the test to prevent domino effects by an invasion of mites and secondary infections into the remaining

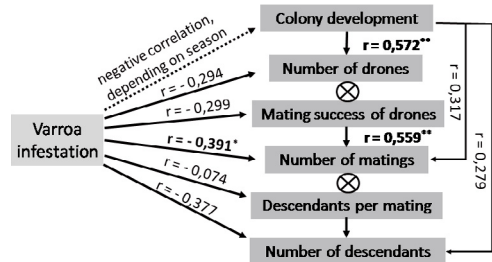
population of untreated colonies. After the spring evaluation, the wintering index will be regarded together with the breeding values estimated from the preceding performance test to finally select vital breeder colonies with a high level of *Varroa* resistance.

### 5.1.3. Drone selection under natural infestation pressure

The prime victim of parasitism by *Varroa* mites is the drone population (Fuchs, 1990). *Varroa* infestation during the pupal stage hampers the life expectancy and flight abilities of drones (Schneider et al., 1988; Duay et al., 2002; Bubalo et al., 2005), as well as the development of spermatozoa (Schneider and Drescher, 1987; Schneider et al., 1988; Del Cacho et al., 1996; Duay et al., 2002, 2003).

Thus, *Varroa* infestation obviously has very strong effects on the fitness of males in the colonies. Büchler et al. (2006) studied the effect of different levels of *Varroa* infestation on male fitness of 26 closely related drone colonies in an isolated mating station. From May to July 2005, brood activity, adult bee and drone populations, and *Varroa* infestation were estimated in regular intervals. At the same time, the mating success of the drone colonies was checked using molecular paternity tests. Using five closely linked microsatellite loci, the genotypes of 412 workers from 48 queens that were mated during this period could be assigned to one of the 27 haplotypes identified in the drone colonies. The *Varroa* infestation level (range: 0.2–8.8 mites/10 g bees) proved to be negatively correlated to the colony size (range: 20–31 comb streets of bees), the number of adult drones (range: 2275–8725), the mating success per drone (range: 2.7–18), and the number of descendants per mating (range: 4–39; Fig. 2).

Fostered by the long distance of mating flights, the strong and direct competition among drones from many different colonies within the range of each drone congregation area, and the multiple mating of each queen, differences in the mite infestation of colonies must result in strong selection effects under



**Figure 2.** Effects of *Varroa* infestation on components of drone fitness. Significant correlations are given in bold letters (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ).

natural mating conditions. This hypothesis is also supported by the observation of a remarkably fast development of significant mite resistance in some untreated *A. mellifera* populations.

Since 2004, the AGT breeding program is taking advantage of this element of natural selection by maintaining several isolated mating areas (so called “tolerance mating stations”) with untreated drone colonies (Büchler et al., 2009). These drone donors usually descend from several unrelated breeder queens in order to achieve high genetic variability within the selected drone population.

As long as the mite infestation of a given colony does not exceed the threshold level (10 mites/10 g bees), the colonies are maintained without any chemical treatments. The sole remedy permitted for use to reduce the mite load in these colonies at the end of the mating season is the complete removal of the sealed brood combined with introduction of a trapping comb (open brood comb into the broodless colony which is removed after sealing). The anticipated losses of infested drones are compensated for by a high number of drone colonies (usually 30–100 colonies per mating station).

## 5.2. Potential of crossbreeding local stocks with comparatively resistant stocks from far eastern Russia

To speed up selection efforts for *V. destructor* resistance, crossbreeding of comparatively resistant stocks was envisioned as an additional option and alternative to selection that

is solely based on local populations. Promising reports from the United States indicated a high level of resistance to *V. destructor* in honey bees from far eastern Russia (Rinderer et al., 2001, 2010). To test their potential for resistance breeding, comparative investigations on mite development and colony performance between Russian honey bee (RHB) from the US and local *A. m. carnica* (C) were carried out in Germany.

In a preliminary three-month study, a distinctly slower *Varroa* population increase in RHB colonies from the USDA selection program could be confirmed (Koeniger et al., 2000). Subsequent large-scale studies by several institutions using hybrids of RHB with other strains from the US also confirmed the slower increase of the *Varroa* population in the RHB, but the interpretation of the results was ambiguous. In some experiments, the colony performance of RHB was significantly worse compared to C, which was hypothesized as one main reason of slower mite growth (Rosenkranz and Liebig, 2003; Schuster, 2003). In other studies, the slower mite growth could not be explained by poor colony development (Berg et al., 2003).

To exclude possible hybrid effects, the following experiment compared 12 different RHB lines headed by purely mated queens ( $n = 113$ ; Rinderer et al., ?) with three local C lines ( $n = 36$ ; Berg et al., 2004). The colonies were maintained without interfering in colony performance and *V. destructor* population growth. After 1.5 years, the mite population (estimated from bee samples) of RHB was 50% of that in C, and the colony strength (estimated as number of occupied combs in regular intervals) did not differ between the two groups (Berg et al., 2005). The comparatively lower mite population increase in RHB colonies could have been due to their significantly stronger hygienic behavior (based on the pin-killed brood test) and a trend of having a higher proportion of infertile mites in their worker brood (estimated in at least 30 single infested brood cells/colony). However, differences in the distribution of the mites on adult bees and in worker brood as reported by Rinderer et al. (2001) were not observed in this experiment.

Although the mite population growth was slower in the RHB colonies, it did increase continuously and the colonies harbored an average of 10 000 mites/colony at the end of the experiment. In addition, RHB showed unfavorable attributes regarding their productivity and gentleness, barring further thoughts of including them into crossbreeding programs.

## 6. EFFICIENCY REVIEW OF EUROPEAN SELECTION ON MITE RESISTANCE

### 6.1. Survivability of selected and unselected European honeybee from different origins

To survey the success of selection programs for *Varroa* resistance, 14 European strains of bees were compared on an isolated Adriatic island (Unije, Croatia) over a period of 2.5 years (Berg et al., 2001; Büchler et al., 2002). Seven strains originated from selection programs for resistance to *V. destructor* (Carniolan  $n = 5$ ; Buckfast  $n = 1$ ; hybrid  $n = 1$ ), and the other seven were unselected strains (Carniolan  $n = 5$ ; Buckfast  $n = 1$ , Ligustica  $n = 1$ ). The 117 colonies were started as artificial swarms (1.6 kg) with 270 *Varroa* each and were maintained according to normal management practices but without any treatment against *Varroa*.

At the end of the experiment most of the colonies had died, with only 15 colonies still alive. While in the first year colony losses mainly were attributed to difficulties in adaptation to the specific local conditions on the island (hot and dry summer, strong winds), colony losses in 2001 and 2002 were predominantly caused by varroosis.

There were distinct differences in the survivability of the different strains of bees (ANOVA,  $P < 0.05$ ), with the colonies originating from selection programs showing a significantly higher proportion of surviving colonies (11 out of 63 colonies) compared to the unselected ones (4 out of 54 colonies; Wilcoxon,  $P < 0.01$ ). However, the higher survivability rate of colonies from selected strains could not be correlated to differences in the relative natural mite mortality (mite mortality/1000 bees), the number of damaged mites

in the natural mite fall, hygienic behavior (pin-killed brood test), or the infertility rate of *Varroa* in worker brood. Nevertheless, from the second year on, the selected strains had significantly stronger colonies compared to the unselected strains (all Wilcoxon,  $P < 0.05$ ). The higher colony strength and better survival rate convincingly demonstrate the advantages of the strains selected for *Varroa* resistance, compared to the unselected strains.

## 6.2. COLOSS genotype – environment interaction test

Recently, a European-wide experiment was initiated as part of the COLOSS project (<http://coloss.org>) to estimate the resistance and vitality of different European bee strains and the interactions of these factors with different environmental conditions. It comprises about 600 colonies of 17 different origins (*A. m. carnica*, *A. m. ligustica*, *A. m. macedonica*, *A. m. mellifera*, *A. m. sicula*) in 26 test apiaries throughout Europe. The survival, bee population and *Varroa* infestation development of the colonies will be tested without using any acaricides over several years.

## 7. PERSPECTIVES

High survival rates in some local populations and significant variability in the mite infestation levels between breeding lines convincingly demonstrate there is potential to establish *Varroa* resistance in European *Apis mellifera* populations. Several selection tools suitable for use by beekeepers have been developed and have been implemented in field selection programs. However, the resistance mechanisms are complex and are still only partially understood. Furthermore, resistance does not occur as an isolated interaction between a host colony and its parasite, but depends on hive management and environmental conditions, including other pathogens.

Based on this current status we envision the following perspectives for bee breeding in the future:

- So far, only minor attention has been paid to the importance of specific ecological

adaptations and the natural biodiversity of honeybees for disease resistance. Consequently, higher priority should be placed on the investigation of genotype-environment (climate, resources, management) interactions and the development of effective strategies to conserve and utilize natural honeybee gene resources. This effort may also include changes in currently used breeding techniques to boost natural selection mechanisms and high intra-colony diversity.

- More research is needed to elucidate the relevance and causality as well as the molecular processes and the genetics of resistance characters. Numerous questions still need to be answered. For example: How do bees influence the reproductive success of *Varroa* using chemical stimuli for example? What role does the entombing behavior of infested pupae, known as an important mechanism of resistance in *A. cerana*, play in *A. mellifera*? Is there variability in susceptibility to virus infections that influence susceptibility to *Varroa*?
- Based on a better understanding of resistance mechanisms, suitable selection methods need to be developed and improved. Identifying the genes involved in *Varroa* resistance and establishing genetic markers for resistance traits are very stimulating challenges to incorporate characters such as grooming behavior that are difficult to estimate under field conditions and to speed up the selection progress. The honeybee genome sequence as molecular and genomic tool may enable such projects, as *Varroa* resistant bee populations become available.
- Accustomed management techniques have to be revised. Regular and uniform treatments of bee populations with highly effective acaricides are in opposition to field selection for resistance. To support the spread of more resistant stock, beekeepers need to identify (through monitoring infestation level) and exclude highly susceptible colonies from further propagation. As soon as the individual infestation of a colony exceeds certain threshold levels colonies should either be destroyed, or

treated and requeened to prevent domino effects. Preference of shorter brood rearing periods, acceptance of temporary breaks in brood rearing and complete brood removal once a season are some tools beekeepers can use to lower the population growth of *Varroa* and thus to reduce their dependence on the use of miticide treatments which mask the advantages of mite resistant stock.

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### Sélection d'abeilles résistantes à *Varroa destructor* en Europe.

#### résistance au *Varroa* / programme d'élevage / caractère tolérant / vitalité / sélection naturelle

**Zusammenfassung – Auslese auf Widerstandsfähigkeit gegen *Varroa destructor* in Europa.** Seit Einzug der *Varroa* Milbe in Europa sind in einigen Regionen Völker aufgetreten, die langfristig ohne Behandlungsmaßnahmen überleben. Eine weitergehende Untersuchung von 82 derartiger Völker (VSB) in Frankreich zeigte eine mittlere Überlebensdauer von 7,8 Jahren. Der *Varroa*-Befallsanstieg während der Brutsaison verlief signifikant schwächer als bei normal anfälligen Kontrollvölkern (Abb. 1). Dabei spielen offenbar ein intensives Putzabwehr- und Bruthygieneverhalten eine Rolle, möglicherweise aber auch häufige Schwarmereignisse, spezifische Umweltbedingungen, Veränderungen der Milbenpopulation und die Virusprävalenz.

Durch einen völligen Verzicht auf Bekämpfungsmaßnahmen ist es innerhalb weniger Generationen sowohl in einem kommerziellen Zuchtbetrieb in Frankreich als auch in einer isolierten Versuchspopulation in Schweden zur Auslese resistenter Bienen gekommen. Als mögliche Ursachen der abgeschwächten Befallsentwicklung wurden u.a. eine geringere Brutdynamik und ein geringer Brutbefall bezogen auf die Gesamtmilbenzahl beobachtet.

Aufgrund umfangreicher Untersuchungen liegen heute standardisierte Methoden zur Beurteilung der Befallsentwicklung und der Bruthygiene vor, die dank guter Heritabilitäten und effizienter Zuchtwertschätzverfahren in vielen europäischen Ländern Eingang in die Zuchtpraxis gefunden haben. Zuchtprogramme zur Selektion auf Putzabwehrverhalten, kürzere Verdecklungsdauer oder beein-

trächtige *Varroa*-Reproduktion scheiterten hingegen an ineffizienten Messverfahren, geringer genetischer Variabilität oder unzureichend geklärter biologischer Zusammenhänge.

Beispielhaft wird die Organisation eines umfangreichen Zuchtprogramms zur Steigerung der Resistenz leistungsfähiger Zuchtlinien vorgestellt. Die Kooperation zahlreicher Zuchtbetriebe, einheitliche Prüfstandards und eine gemeinsame Zuchtwertschätzung ermöglichen eine Auslese in großer Population. Potenzielle Zuchtvölker werden auf ihre Überwinterungsfähigkeit ohne vorangehende Behandlung gegen *Varroa* geprüft und durch den Betrieb von Belegstellen mit unbehandelten Drohnenvölkern werden Effekte unterschiedlicher *Varroa*-Anfälligkeit auf den Paarungserfolg von Drohnen (Abb. 2) in die Selektion einbezogen.

Aufgrund schlechterer Honigleistung und Sanftmut konnten US-Linien Russischer Bienen (RHB) trotz relativ hoher Widerstandsfähigkeit in Europa keine Bedeutung erlangen. Die meisten europäischen Zuchtprogramme zielen auf einer stetige Verbesserung einheimischer Populationen. Inwieweit es auf diesem Weg möglich ist, hochgradig resistente und zugleich leistungsfähige Zuchtlinien zu entwickeln, bleibt offen. Immerhin zeigte sich in einem mehrjährigen Vergleichsversuch eine höhere Überlebensfähigkeit der bereits auf Resistenz gegen *Varroa* ausgelesenen Linien.

Wichtige Zukunftsperspektiven ergeben sich aus der Weiterentwicklung der Selektionsmethoden, der Berücksichtigung spezifischer Umweltpassungen und verbesserter Haltungstechniken.

### *Varroa* Resistenz / Zuchtprogramm / Toleranzmerkmal / Vitalität / natürliche Selektion

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