INFECTION AND IMMUNITY IN THE HONEY BEE, *APIS MELLIFERA*

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Introduction

The honey bee is subject during its life to a nearly continual challenge by different saprophytic and pathogenic microorganisms (bacteria, viruses), protozoan and metazoan parasites (larvae of *Senotainia tricuspis, Mermis sp.*) and parasitic mites (*Acarapis woodi*, *Varroa jacobsoni*). In response to infection and injury a variety of immune processes have evolved to suppress invaders that have succeeded in reaching the haemocoel (DUNN, 1986). The anatomical and physiological barriers formed by the cuticle, midgut and tracheal system play a crucial role in protecting the insect against the penetration of microbial intruders into the haemolymph (GLIŃSKI and JAROSZ, 1995a). When the outer protective barriers are broken the invader encounters the internal immune responses active in the coelomic cavity of the bee (GLIŃSKI and JAROSZ, 1995b,c).

Haemocytes, the major cellular components in the immune system are involved in the cell-mediated immune reactions (SALT, 1970) while humoral defense is attributed to the activity of soluble protective factors, both innate and inducible of insect haemolymph (BOMAN and HULTMARK, 1987). The ability to discriminate between self and non-self is the first prerequisite that generate the immune defense phenomena (RATCLIFFE and GÖTZ, 1990). The recognition and attachment events that initiate cellular defense response in innate system are regulated by humoral and by membrane-bound molecules that discriminate self versus nonself. Bacterial LPS or peptidoglycan molecules and mannose, galactose or glucan residues in the case of fungi, hemolin and lectins are the pattern recognition molecules (SCHMIDT et al., 1993).

In addition, the honey bee as a social insect has developed mechanisms that efficiently protect both the individual and the whole colony against a great number of microbial pathogens (GLIŃSKI and JAROSZ, 1994). The environment polluting agents and pesticides can undoubtedly impair the non-self response system and protective defense of the bee against pathogens, parasites and predators.

Antiviral protection

Up to date more than 15 distinct viruses have been characterized from honey bees. Most of them persist as inapparent infections, however, the latent viruses can be induced to multiply to readily detectable levels under stress conditions. All known viral diseases of honey bees are specific for only one stage in the life cycle: acute paralysis virus (APV), chronic paralysis virus (CPV), cloudy wing virus (CWV) attack adults whereas sacbrood virus (SBV), black queen cell virus (BQCV) are restricted to larval stages (ALLEN and BALL, 1996).

The most common route of infection with bee viruses is the ingestion of intact virions by feeding insects. The ingested viral particles may infect the epithelial cells of the midgut or pass through the gut wall to enter susceptible target cells in the insect body. Bee viruses are polytrophic because they infect most, if not all, tissues of the host. The most important role in virus-host relation is played by a specificity of the virus to the insect, the portal of entry for virions to the host body and the efficacy of the bee defense mechanisms. The bee viruses like other insect viruses, have evolved mechanisms for avoidance or depression of the insect host responses. These mechanisms allow them to survive for a long periods in their hosts and cause latent infections (RATCLIFFE et al., 1985).

A number of the nonimmunological factors modify and control virus replication and the outcome of viral infections. Commonly known protecting the bee against viruses are the thresholds present in the midgut and formed by the biochemical environment of the gut juice, the peritrophic membrane and the midgut epithelium. In most cases, the peritrophic membrane limits the spread of infection because of its impermeable or hardly permeable barrier to viral particles (SMITH et al., 1993). Only viruses that passed the peritrophic membrane may infect the gut epithelium. They could trespass the epithelial lining of the midgut, infect coelomic cavity, cause a viraemia and then infect body tissues. A prerequisite for infection is adsorption of virions to the specific receptors on the surface of susceptible cell. One cause of inhibition of viral replication is the production of interferons released from virus infected cells within a few hours after viral invasion. Insect interferons are glycoproteins (20-34 kDa) stable to heat and to extremes of pH.

It can be hypothesized that in bees, like in mammals, interferon could act by entering infected cells and depressing their DNA, so that produce translation inhibitory protein. This protein, can in turn, block the takeover of cell ribosomes by viral RNA, and hence inhibits virus replication.

The another factor that controls viral infections is lack of specific receptors at the plasma membrane to bind of ligands. The binding of ligands to receptors stimulates cells to change shape, endocytose, prolif-

eration or modulation of cell functions. Once a viral nucleoprotein penetrates the plasma or vesicular membrane it must make its way to the site in the cell where it will replicate. Within the infected cell, replication of bee viruses occurs entirely within the cytoplasm. Serologically related viruses, sometimes by unrelated or defective viruses may block the viral receptors on the susceptible cells. The blocking of viral receptors on sensitive cells is an effective mechanism of antiviral defense .

Viral infections induce unspecific cell defense reactions such as phagocytosis and nodule formation. Phagocytosis of virions develops in early and late stages of infection when viruses are liberated into the haemolymph from damaged cells. Phagocytosis and nodule formation is not always effective in prevention and control of viral diseases. The final result is both the recovery of the bee host due to the efficacy of the defense mechanisms or the viral invader prevails and the host is killed. A small number of viruses or viruses of a low virulence infecting the insect are killed by haemocytes whereas heavy infections or virulent strains can replicate and kill a specific types of haemocytes involved in antiviral defensive reactions.

Finally, many microbial parasites have evolved mechanisms for avoidance or depression of the insect host response. These mechanisms allow parasites to survive for long periods in the bee organism, which act as a source of new infection within a bee colony. Obviously, the neurons in the thoracic and abdominal ganglia protect replicating bee paralysis viruses from phagocytosis and other haemocytic defense reactions.

Bacterial infections and immunity

Bacteria associated with bees are widely distributed in soil, water, and air, stored bee food, surface of plants and skin of other living creatures. In most infections bacteria invade the bee body cavity through the intestines with ingested bacteria contaminated food. Bacteria may also infect the bee body via spiracles or with the aid of the piercing or chewing mouthparts of predators and external parasites, for example via cuticular abrasions caused by a stenophagous mite *Varroa jacobsoni* (GLIŃSKI and JAROSZ, 1992). Any break of the cuticle or the alimentary canal is a portal of entry for bacterial invaders.

The honey bee larvae can develop the American foulbrood (*Paenibacillus larvae larvae*), European foulbrood (*Mellissococcus pluton*) and the powdery scale disease (*Bacillus pulvifaciens*) whereas in adult bees septicaemias result mostly from *Pseudomonas aeruginosa*, *Hafnia alvei* and *Enterococcus faecalis* infections. Saprophytic and plant pathogenic bacteria may also accidentally induce the fatal septicaemia after invading the haemocoel.

Bee body coverings and the biochemical environment of the midgut juice by bacteriostatic or bactericidal action effectively restrict the development of most infections caused by bacterial saprophytes (JAROSZ, 1993; 1995). If the bacteria succeed in reaching the haemocoel through mechanically injured or enzymatically damaged anatomical protective barriers, they generate the internal immune responses in body cavity.

The bee has an open circulatory system and numerous haemocytes are contained in its haemolymph. Prohaemocytes, plasmatocytes, granular cells, cystocytes, sphaerula cells and enocytoids are the cells that comprise the bee haemocyte population (GUPTA, 1991). Commonly known haemocyte- mediated defense reactions of bees consist of phagocytosis, encapsulation, cytotoxicity, secretion of materials to damage foreign organisms (humoral encapsulation) or to modulate immunocyte functions. Haemocytes are able to recognize self and non-self. At least three possible factors may act as non self-recognition molecules in bees, that is the components of the prophenoloxidase cascade, lectins and chemokines. The prophenoloxidase activating system (proPO) consists of a cascade of enzymes and associated factors such as various plasma receptors, serine proteases and inhibitors (SÖDERHÄLL and SMITH, 1986). The multifunctional proPO cascade is comparable to the vertebrate complement system in a number of ways. Both systems generate opsonic intermediates that assist in the initial recognition and attachment phases of phagocytosis.

Much interest has recently been generated by the observation that the pPO is involved in immune recognition and cellular communication. This system itself can react to foreign materials and be converted into its active form by microbial products. The granular cell type may contain not only lectins but also components of the proPO system. This cell type rapidly degranulates in contact with non self materials to release the non self recognition molecules.

The occurrence of lectins in haemolymph of bees is scarcely documented. They have in insects a dual function, in defense and in development. The lectins enhance recognition and phagocytosis by insect haemocytes and they have ascribed roles in nutrition and development. They probably are involved in tissue reorganization and cell adhesion (OLAFSEN, 1986).

Hemokines, a group a small secreted proteins, function as important regulators of the immune system by inducing haemocytes to migration and by modulation haemocyte-antisome interactions (CHADWICK and ASTON, 1991).

The haemocytes can engulf and destroy smaller foreign objects such as bacteria or fungal spores, but larger parasites, bacterial clumps or fungal hyphae, are encapsulated by several haemocytes and then removed from circulation.

The cellular immune responses have been shown to be accompanied by changes both in the number of circulating haemocytes and the relative proportions of different haemocyte types in the blood. Generally, the infection mobilizes sessile haemocytes to migrate and increases a percentage of round plasmato-

cytes (BAHADUR, 1993). Predominant cells involved in phagocytosis are plasmatocytes, followed by granular cells (SHARMA et al., 1986). Although the cells involved in the process of phagocytosis in the bee are well recognized the mechanisms or the factors involved in this process and the role they play are not yet clearly understood. The engulfed bacteria are digested in phagolysosome by released hydrolytic enzymes, primarily lysozyme, alkaline phosphatase, ribonucleases and phospholipases.

Relatively little is known about oxygen-dependent microbial killing mechanism in insects. Superoxide anions have been detected in several lepidopterans and there is evidence that lipophorin is involved in production of these molecules. (ARAKAWA et al., 1996). Nitric oxide synthetase (NOS) has been detected in insect fat body and Malpighian tubules. The fat body NOS is induced by bacterial LPS. The generated nitric acid acts as signaling/messenger molecule or as a toxic antimicrobial agent (MIMS et al., 1995). The role of factors such as proPO, melanins and recruitment factors cannot be excluded in phagocytic process. They all may increase the number of collisions between phagocytes and foreign bodies and mobilize sessile haemocytes. In some instances, however, the captured bacteria may even multiply within the phagocytes, causing their death. Release bacteria then multiply in the insect blood and the bee perishes due to a fatal septicaemia.

Cellular encapsulation of large foreign invaders is a common phenomenon of cell-mediated immune reaction protecting the insect host. Components of the proPo system could function as signaling molecules to promote encapsulation. A 400 kDa complex consisting in part of of prophenoloxidase, phenoloxidase, and an interleukin 1 was isolated from *Manduca sexta* haemolymph (BECK et al., 1996). The invader is enclosed in several layers of cells and the capsule-like so formed melanizes and strictly isolates the parasite from circulation. The primary stimulus initiating the encapsulation may originate from either foreign substance present on the surface of an invader or metabolites and waste products released by the parasite. The granular cells that contact and recognize the foreign body as a non-self structure degenerate to release sticky proteins that attract the plasmatocytes. Melanization is not a regular process of cellular encapsulation. Only living organisms induce both encapsulation and melanization. The melanization of the capsule depends on the proPO activating system that kills the microorganisms and parasites, isolating them from the rest of the body in the hard and impermeable capsule. Death of larvae and embryos of parasites, adolescent protozoans and nematodes may result from asphyxiation or the accumulation of toxic wastes in the capsule. Encapsulated parasites may die through the toxic effects of quinones that contribute to melanization (VEY and GÖTZ, 1975).

Nodule formation is a phenomenon in response to both animate and inanimate substances that cannot be removed from circulation by phagocytosis. In this cellular reaction, the haemocytes loaded with bacteria are entrapped by a coagulum that is produced by the degranulating granular cells and then centrally melanized. A sheet of blood cells surrounds the entrapped invaders in that coagulum in the center. The fate of nodulated bacteria depends on their virulence. In general, bacterial saprophytes are quickly killed within melanized nodules while pathogenic bacteria may subsequently liberate from the nodules into the haemolymph (RATCLIFFE and ROWLEY, 1979).

Cell-free immune reactions involve synthesis and release of several antibacterial immune proteins, some capable of killing both Gram negative and Gram positive bacteria. The expression of this multicomponent humoral immune system requires the *de novo* synthesis of a specific immune mRNA in the fat body and response peptides and small protein synthesis with broad antibacterial activity (BOMAN and HULTMARK, 1987; JAROSZ, 1979).

The bee responds to fungal infections by altering immunocompetent cell motility. Haemocytes have been shown to migrate towards fungal spores and hyphae. Such migratory responses may help to explain the selective depletion of insect cells from circulation that is often evident during cell-mediated immune reactions directed against parasitic fungus.

Among the great majority of immune mediators in insects which have been infected with bacteria, lysozyme, apidaecins, abaecin and hymenoptaecin have been found to exist in the honey bee (GLIŃSKI and JAROSZ, 1995c). The bee lysozyme is a relatively small molecule (about 15 kDa), representing a group of true lysozymes shared characteristics with the chicken type lysozyme. The concentration of lysozyme in larval honey bees and in adults ranges from 5.0 μg to 25.0 μg/ml, and in pupae from 5.0-10.0 μg/ml of haemolymph. Bacterial infection or artificial inoculation of bacterial saprophytes increase the concentration of lysozyme in haemolymph of bee larvae even to more than 1300 μg /ml whereas in flying worker bees to not more than 40.0 μg/ml. It is hypothesized that in brood lacking of the inducible apidaecin-family antibacterial response peptides the enhanced potency of lysozyme reduced the risk of infection of preimaginal stages by saprophytic bacteria (GLIŃSKI and JAROSZ, 1993). The peptidoglycan fragments of Gram positive bacteria released by the lytic action of lysozyme act as a very potent elicitors of antibacterial peptides such as apidaecins. In several cases lysozyme acts as a synergistic with the smaller cationic peptides (BANG et al., 1997).

The apidaecins represent a family of inducible, non-helical, small (about 2 kDa) proline-rich antibacterial peptides of activity mostly against Gram- negative bacteria (CASTEELS et al., 1989). They are the most prominent components of the honey bee inducible cell-free defense against bacterial invaders. Apidaecins of the honey bee consist of four closely-related peptides (Ia, Ib, II and III) composed of 18 amino acid residues each (Fig. 1). Biologically active isoforms appear in haemolymph of the adult bee whereas the inactive precursor molecules – proapidaecins exist in the blood of the last instar larvae. Conversion of the apidaecin precursors into active peptides could occur through a stepwise cleavage of dipeptides ending ei-

ther a proline or an alanine. Structural analysis suggests that apidaecins contain both a constant region responsible for potency and a variable region which dictates the antibacterial spectrum (CASTEELS-JOSSON et. al., 1993).

Proapidaecin Ia/b EAKPEAKP ➔ **GNNRP**V**YI**P**QPRPPHPR** $^{I}/_{L}$
Apidaecin Ia **GNNRP**V**YI**P**QPRPPHPR**I
Apidaecin Ib **GNNRP**V**YI**P**QPRPPHPR**L
Apidaecin II **GNNRP**I**YI**P**QPRPPHPR**L
Apididaecin III **GNNRP**V**YI**S**QPRPPHPR**I

E Glutamic acid G Glycine I Isoleucine
A Alanine N Asparagine Q Glutamine
K Lysine R Arginine H Histidine
P Proline V Valine Y Tyrosine
L Leucine

Fig. 1 – Sequences for the precursor of apidaecins Ia and Ib and amino acid sequence of apidaecins Ia, Ib, III and III of the honey bee, Apis mellifera

A sharp increase of apidaecin transcript levels occurr 4-6 hours after infection, followed by a steady rise of several more hours. Peak concentration in the haemolymph is within 36 hour post-infection and then the concentration of the apidaecin compounds declines in bee blood gradually for the 3-4 next days.

It is now known that the mode of action of the apidaecins is to attack bacterial cell membrane by the ionophoretic action, they form voltage-dependent ion channels causing leakage and cell death. This activity is non-specific and so apidaecins have a fairly broad range of activity. They are highly active against Gram negative bacteria, but show only a weak activity against Gram positive bacteria. The spectra of activity for all isoforms of apidaecins are very similar, and seems to be bacteriostatic rather than bactericidal.

The activity of apidaecins is directed against bacteria present commonly in the bee environment (Table 1).

Table I

Antibacterial activity of apidaecin Ia, Ib and II *Apis mellifera* (Casteels et al., 1989)

Bacterial strains	Minimum Inhibitory Concentration μg/ml		
	Ia	Ib	II
Agrobacterium tumefaciens DSM 3129	0,2	0,2	0,2
Erwinia salicis NCPPB 2530	0,02	0,02	0,02
Escherichia coli NCTC 9001	0,1	0,1	0,2
Pseudomonas syringae pv. tomato NCPPB 1106	0,2	0,1	0,1
Rhizobium meliloti ZB 314	0,1	0,02	0,02
Salmonella Newport CD 94	0,2	0,2	0,2
Salmonella typhimurium ATCC 23565	0,1	0,1	0,1
Serratia marcescens ATCC 17991	+200	+200	+200
Shigella flexneri CP 87	0,1	0,1	0,1
Corynebacterium insidiosum NCPPB 1109	50	50	100
Bacillus alvei LMG 6922	200	200	200
Bacillus megaterium QMB 1551	150	100	100
Bacillus subtilis NRRL-B-237	+200	+200	+200
Bacillus thuringiensis tenebrionis DSM 2803	+200	+200	+200

It can be suggested that the marked activity of apidaecins directed against enteric bacteria, plant-associated and phytopathogenic bacteria developed in the bee as a defense mechanism active against the bacterial species contaminating plant and water sources visited by flying worker bees. The presence of inactive precursor of apidaecins in the haemolymph of brood may reflect differences in the immune status of the larval and adult stages of the honey bee. The risk of brood infection by bacteria contaminating the worker

bees is reduced by factors of antibacterial activity of royal jelly, honey and pollen. Larva responds to bacterial infection by increasing the level of lysozyme in the haemolymph (GLIŃSKI and JAROSZ, 1995b).

A second antibacterial protein that was originally discovered from the honey bee is abaecin (CASTEELS et al., 1990). It is a large inducible, proline-rich, α-helical peptide (about 4.0 kDa) of antibacterial activity. Unlike the apidaecins, the range of activity of abaecin for bacteria is quite narrow and confined to a limited number of both Gram negative and Gram positive bacteria. The molecule of abaecin contains 10 proline residues but not cysteine (Fig. 2). Abaecin reveals striking similarities to apidaecins in *Apis mellifera* and diptericins in flies. Matching patterns of prolines and hydrophobic amino acids are present in the first 18 residues of each of the three immune peptides.

Apis YVPLPNVPQPGRRPFPTFPGQGPFNPKIKWPQGY

Fig.2 – Complete amino acid sequence of abaecin in the honey bee, Apis mellifera

Although the abaecin is induced upon microbial infection of the bee body cavity, its contribution to antibacterial defence of the bee remains still unclear. The mode of action of the abaecin is to alter the permeability of the outer membrane of the target bacterium. It is thought that the result of this action by abaecin allows improved access to the cell wall for lysozyme and to the cytoplasmic membrane for the apidaecins. Most probably abaecin, like apidaecins, represents an advanced level of internal cell-free antibacterial immunity of the bee. They both represent the highest level of adaptation of *Apis mellifera* response system to destroy plant pathogenic and enteric bacteria present ubiquitously in the bee living niche.

In the clarifying the honey bee haemolymph from bacteria participates hymenoptaecin (CASTEELS et al., 1993), apart from apidaecin-family peptides and abaecin. Hymenoptaecin is a glycine-rich peptide having 93 amino acid residues (about 10 kDa) with bactericidal activity for Gram negative and Gram positive bacteria. This inducible peptide requires higher doses of bacteria for induction and appears in the haemolymph at later time post-infection and at lower concentration than apidaecins.

Immune defenses against pathogenic fungi

The best known protective mechanisms active in the honey bee to fungal infection, notably in chalkbrood and stonebrood, are in the anatomical structure of the bee body (BARR and SHOPE, 1975; ORIHEL, 1975). The impermeable and hard cuticle, the biochemical environment of the midgut juice, peritrophic membrane of the midgut and tracheal system form together a mechanical and physiological barrier effectively protecting the bee` s body cavity against fungal invasion. A relatively low humidity in the tracheae is an important factor in restricting germination of spores and growth of fungus in the bee respiratory tract. Waxes and unsaturated fatty acids impregnating the cuticle or present on its surface have a potent antifungal action. Competition for food between gut bacteria and fungi could efficiently eliminate massive doses of fungal spores from the gut. Nevertheless, chitinase producing moulds and yeasts can actively penetrate the cuticular lining of the body and then infect the haemocoel. The cuticle damaged mechanically or enzymatically by growing hyphae also allow bacteria to enter body cavity and develop fatal septicaemias.

Non-degradable fungal materials are encapsulated by a large mass of haemocytes that serve as a barrier between the haemocoel and the object. Bee haemocytes may also directly kill fungal spores and other small foreign molecules in phagocytic process (GÖTZ, 1986). These cellular immune reactions have been shown to be accompanied by changes both in the number of circulating haemocytes and in the relative proportions of different haemocyte types in the blood (HINK, 1970). The infection of the haemocoel initiates a premature differentiation of haemocytes and their migration towards chemotactic stimulus. Phagocytosis predominates when the body cavity is exposed to small numbers of bacteria or fungal spores.

Encapsulation is the most effective haemocyte-mediated immune response in protection of insect haemocoel in fungal infections. This cell-mediated reaction consists of the formation of a capsule-like envelope around foreign objects with a diameter more than 10 μm that cannot be phagocytized by a single cell. The capsule is formed by attaching blood cells, mainly granular cells and plasmatocytes. The granulocytes release chaemotactic factors which attract the plasmatocytes to form the outer layer of the capsule around the encapsulated fungus. The role of the phenoloxidase activating system cannot be excluded in phagocytosis and melanization of encapsulated insect pathogenic fungi.

Neither lysozyme nor the haemolymph response proteins seem to inhibit or destroy spores or fungal mycelia in the invaded bee. The honey bee generate several groups of humoral immune factors to resist bacterial infections. The apidaecin-family peptides, abaecin and hymenoptaecin, the most prominent components of the honey bee inducible humoral defense, are inactive entirely to fungal invaders.

Protective mechanisms in protozoan invasions

The protozoans attack the epithelial cells of the midgut (*Nosema apis, Leidyana apis*) or Malpighian tubules (*Malpighamoeba mellificae*) of the adults only (DE GRAAF et al., 1993; 1994). Pathological changes

in epithelial cells and derangement of digestive processes by *N. apis*, both of which led to malnutrition and premature death of the invaded bees. In the parasitized individuals, desquamation of pathologically altered epithelial cells loaded with parasites, and their removal with feces, lowers the possibility of massive invasion of other parts of gut epithelium. Scars that develop at the site of removed dead epithelial cells, and newly developing epithelium, prevent the migration of the bee microflora from the gut into the body cavity. Variations in resistance could be assigned to the activity of chymozin in the honey bee ventriculus since this enzyme by improving the development of the peritrophic membrane prevents *N. apis* spores for coming into close contact with the epithelial gut cells.

When spores invade the bee body cavity, cell-mediated defense reactions seem to play a crucial role in the control of *N. apis* invasion. Very often, haemocytes aggregate around the parasite and are active in phagocytosis and nodule formation. Cysts and spores of parasites, like vegetative forms, can be effectively encapsulated. The effectiveness of haemocytic reactions in protozoan invasions is high but the parasites become increasingly difficult to eliminate as invasion progresses. Bacterial infections associated with the mechanical destruction of host cells by the developing protozoan accelerate the death of bees.

The protozoan *Malpighamoeba mellificae* attacks the epithelium of the Malpighian tubules, which usually become swollen and degenerated. The greatly distended tubules occasionally rupture and elicit a massive inflammatory response. Since the number of parasitic cysts produced in the body of the bee is low, massive lethal invasions of bees are not frequent. The anatomical protective barriers of the gut limit the destructive action of the parasite.

Mechanisms of resistance to the mite *Varroa jacobsoni*

Of about one hundred species of mites which live in or around honey bee colonies in various parts of the world, three mite species are dangerous to the honey bee: *Varroa jacobsoni, Acarapis woodi* and *Tropilaelaps clareae*.

The pathogenic effects of *V. jacobsoni* invasion closely related to the number of parasites and their developmental stage are attributable to the mechanical injuries, depletion of haemolymph proteins and to toxic effects of the parasite. Furthermore, the parasitic mite, induces latent viral infections (ALLEN and BALL, 1996), and transmits bacterial and fungal infections to the recipient bee host (GLIŃSKI and JAROSZ, 1992). It is possible that the parasite impairs the internal defense reactions of the bee and brood (GLIŃSKI and JAROSZ, 1984; 1988a).

At least, the five mechanisms that minimize the impact of *V. jacobsoni* on honey bees have so far been discovered: grooming behaviour, removal of mite infested brood, rapid development time, unattractive brood for the mite and infertility of mites on some bees brood (BOECKING, 1994).

In the Asian bee *Apis cerana* exists behavioural tolerance to *V. jacobsoni*. *A. cerana* grooms herself, removing the mite, while the European bee does not or at most grooms very little. After *A. cerana* caught a mite, it would kill it by biting and crushing it, then taking it out of the hive. Moreover, successful reproduction of *V. jacobsoni* on *Apis cerana* is limited by seasonal occurring drone brood and is lacking in worker brood (RATH, 1991; ROSENKRANZ et al., 1993). The infested drones become weakened by the parasitic mites, are not able to uncapping their cells, and in consequence they die together with mites inside uncapped cells. The bees which show the hygienic behaviour uncap and remove bee pupae that contain a *Varroa* mite.

Behavioural tolerance of *Apis mellifera* to *V. jacobsoni* was found in Africanized and European honey bees from Brasil, Tunisia and Uruguay (RITTER et al., 1990). In *Varroa*-tolerant *A. mellifera* colonies there is a reduced fertility of *V. jacobsoni* in worker brood compared to the successful reproduction of the mite in drone brood.

One of the reasons why *V. jacobsoni* is not a problem with the Asian bee is the short period of time the pupae is capped which does not permit a long enough time for the mite to develop. Also differences in the susceptibility to *V. jacobsoni* in *A. mellifera carnica* compared to *A. mellifera capensis* were found and *A. mellifera ligustica* compared to *A. mellifera monticola* hybrids. A shorter development time in bees is a time factor limiting the reproduction rate of *V. jacobsoni* because it would prevent the mite from completing its development (WILDE and KOENIGER, 1992). Selection for a post-capping period, that is even a few hours shorter, may decrease the development of *V. jacobsoni* infestations.

The grooming behaviour towards phoretic *V. jacobsoni* mites seems to exist to a lower degree in *A. mellifera* compared to *A. cerana*. It is evident that *A. mellifera* is able to kill *V. jacobsoni* mites (RUTTNER and HÄNEL, 1992). The effect of this defence behaviour on the development of the colony infestation is till unknown. *A. mellifera* from Europe and North America stocks also remove *V. jacobsoni* from capped brood cells. However, in some cases the caps of mite infested brood are opened and then closed again without eliminating of parasitized brood. Mite removal may limit the *Varroa* population because immature mites are killed, female mites are killed and those that survive removal process cannot reproduce. *A. mellifera* removes not only mite infested drone brood but also worker brood which is in contrast to the behaviour of *A. cerana* (BOECKING, 1994).

Resistance mechanisms and factors of the honey bee as a social insect

Analysis of the immune responses in the bees revealed the well developed system through which the individual bee acquires a special type of immunity. This system is based on cell-mediated reactions and a number of innate and inducible immune proteins, some of them potent antibacterial proteins like lysozyme and apidaecin-family peptides. Apart from internal immune defenses, other mechanisms of the colony resistance to invaders are known. They include the antibacterial activity of honey, nectar and pollen, secretions of the honey bee exocrine glands, antimicrobial activity of propolis. A specific behaviour resistance protects the be colony from bacterial and fungal infections.

The antimicrobial activity of honey, nectar and pollen is an important factor in the colony that inhibits the development of many saprophytic bacteria and fungi in stored food, and that could destroy some pathogenic microorganisms (BURGETT, 1978). The acidity, osmotic pressure and production and accumulation of hydrogen peroxide are responsible for this effect in honey and nectar (WHITE and SUBERS, 1963). Honey as a hyperosmotic medium may kill many living cells, except those of osmophilic fungi and bacteria.

Secretions from honey bee exocrine glands contain biologically significant components. The hypopharyngeal gland secretions of young workers contain proteins to be bacteriostatic and bactericidal to a wide range of bacterial species (ROSE and BRIGGS, 1969). At least two bacterial inhibitors are identified in royal jelly: 10-hydroxy-2-decenoic acid and glucose oxidase. It can also inhibit or delay the growth of many fungi, for example *A. apis*.

Propolis, that is a highly complex mixture of waxes, resins, balsams, oils and a small amount of pollen forms a part of antimicrobial defense of the bee colony. Flavanones together with flavones, caffeic acid and its esters are considered to be responsible for antibacterial action of propolis (GREENEWAY et al., 1990). It is quite possible that fungi of plant origin and from animal sources, polluting environment and contaminating pollen sources and water gathered by bees are inhibited by biologically active compounds of propolis.

Hygienic behaviour can be characterized by the rapid detection of sick and dead brood by worker bees, removal of dead insects from the colony, and the thorough cleaning of the cell of honey comb. Worker bees groom their own bodies and those of other bees, maintain the hygiene of the nest and remove debris from the hive. This hygienic activity is important in the resistance to chalkbrood and stone brood. The adults remove the mummified larvae using their mandibles and carry the larvae away from the nest. Bees that have no means of removing the pathogenic fungi from the gut and the body hair subsequently reinfect susceptible larvae when feeding them or pass on infectious fungal spores to other adults of the colony (SOUTHWICK, 1994). Resistance is supported by an ability of some worker bees to filter ingested spores and mycelial fragments from the proventriculus. Inhibitors in the glandular-produced brood food are strong antibacterial and antifungal agents.

There are at least two mechanisms of behavioural resistance, both are genetic in nature. Hygienic behaviour is believed to be controlled by two recessive genes, one for uncapping diseased brood, and one for the removal of mummy (TABER, 1992). The expression of hygienic behaviour depends on the strength of the bee colony. When colony size is reduced by removing frames of brood and associated bees, hygienic activity is depressed in hygienic colonies but there is no effect in non-hygienic colonies. The expression of hygienic behaviour is also altered by adding hygienic or non-hygienic bees to colony, and by the colony composition. TABER (1992) has stated that all bees with hygienic behaviour tested to chalkbrood were resistant. SOUTHWICK (1994), however, has suggested that there is not straightforward correlation between hygienic behaviour and resistance to chalkbrood. The chalkbrood infected colonies showed a weak correlation with hygienic behaviour.

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