

Reproductive biology of *Varroa destructor* in Africanized honey bees (*Apis mellifera*)

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Abstract Since its first contact with *Apis mellifera*, the population dynamics of the parasitic mite *Varroa destructor* varies from one region to another. In many regions of the world, apiculture has come to depend on the use of acaricides, because of the extensive damage caused by varroa to bee colonies. At present, the mite is considered to contribute to the recent decline of honey bee colonies in North America and Europe. Because in tropical climates worker brood rearing and varroa reproduction occurs all year round, it could be expected that here the impact of the parasite will be even more devastating. Yet, this has not been the case in tropical areas of South America. In Brazil, varroa was introduced more than 30 years ago and got established at low levels of infestation, without causing apparent damage to apiculture with Africanized honey bees (AHB). The tolerance of AHB to varroa is apparently attributable, at least in part, to resistance in the bees. The low fertility of this parasite in Africanized worker brood and the grooming and hygienic behavior of the bees are referred as important factors in keeping mite infestation low in the colonies. It has also been suggested that the type of mite influences the level of tolerance in a honey bee population. The Korea haplotype is predominant in unbalanced host-parasite systems, as exist in Europe, whereas in stable systems, as in Brazil, the Japan haplotype used to predominate. However, the patterns of varroa genetic variation have changed in Brazil. All recently sampled mites were of the Korea haplotype, regardless whether the mites had reproduced or not. The fertile mites on AHB in Brazil significantly increased from 56% in the 1980s to 86% in recent years. Nevertheless, despite the increased fertility, no increase in mite infestation rates in the colonies has been detected so far. A comprehensive literature review of varroa reproduction data, focusing on fertility and production of viable female mites, was conducted to provide insight into the Africanized bee host-parasite relationship.

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Introduction

The mite *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae) (formerly named *Varroa jacobsoni*) is a worldwide ectoparasite of honey bees with serious economic importance for beekeeping (De Jong et al. 1982b). The problem is most acute in regions with a temperate climate, which have bees of European origin (De Jong 1997). Severe mortality due to this mite has been reported in colonies of European honey bees (EHB) in many countries of Europe, Asia and North America (Bailey and Ball 1991; Korpela et al. 1993; Mobus and Bruyn 1993). However, in tropical regions of Brazil, where honey bees are Africanized (AHB) the mite effect on the colonies appears to be reduced to the point where no control measures are necessary and no colony losses because of this mite seem to occur (Carneiro et al. 2007; De Jong 1997; Moretto 1997; Moretto and Mello 1999; Moretto and Leonidas 2003).

Different factors contribute to the population growth of mites, which eventually can lead to colony collapse (Boot et al. 1999). Although larval stages of the honey bee must be available for mite reproduction, this is only one factor that influences mite population growth (Garrido et al. 2003). The development time of worker brood, the hygienic behavior, the grooming behavior and the reproductive ability are characteristics long associated with varroa tolerance. Furthermore, regional differences in weather conditions and mite genotypes make it difficult to characterize the mite reproductive ability and the varroa population dynamics over a wide-spread area (De Guzman et al. 2007).

The serious impact of this mite on Africanized bees in other tropical American regions appears different from the situation in Brazil (Medina and Martin 1999). The geographical variation in fertility and growth rate of varroa populations between geographic regions may be a consequence of different conditions for the mite in these areas (De Guzman et al. 2007). According to Correa-Marquez et al. (2003) varroa reproductive success is considered a significant characteristic for determining the resistance of honey bees to this parasite. Nevertheless, objective comparisons between published studies in this topic are difficult, as each researcher has used different techniques. Frequently the methodology is not clearly explained (De Jong 1997; Correa-Marques et al. 2003).

A comprehensive review of the literature was conducted to collate the data of varroa mite reproduction to provide insight into this host-parasite relationship. Here we present an overview of the reproductive biology of varroa, focusing on some aspects of the mite reproduction rate (fertility and production of viable female mites) on Africanized bees.

Damage to honey bee colonies

Varroa mites live apparently in a longtime association with their natural host *A. cerana*, the Indian honey bee, in South East Asia. Serious damage to these bees has never been reported, and treatment against varroa is not needed in beekeeping with *A. cerana* (Rath and Drescher 1990; Rath 1993; Tewarson et al. 1992), meanwhile many reports exist of severe damage and loss of thousands of *A. mellifera* colonies caused by infestation with varroa mites, especially in temperate areas of Europe and Asia (De Jong et al. 1982a). Varroa is generally considered the most severe threat to world beekeeping (De Jong 1997; Oldroyd 1999; Sammataro et al. 2000).

Adult bees emerging from infested brood cells with *V. destructor* show a marked reduction in weight compared with their uninfested sister bees (Beetsma et al. 1989; De Jong et al. 1982a). In addition, the brood is neglected and parasitized bees may be deformed. Amongst the range of symptoms described in colonies infested with varroa mites are a reduced adult bee population, crawling bees in front of the hive and queen supersedure (Hung et al. 1995).

Shimanuki et al. (1994) have suggested the terminology “parasitic mite syndrome” for colonies showing the symptoms of varroa infestation (end stage of varroa infestation). Much damage is due to secondary infections. Bees from dying infested colonies have been found to be infected with a number of honey bee viruses (Ball 1993, 1996). It is known that acute paralysis and deformed wing viruses are transmitted by the mite (Ball 1985; Bowen-Walker et al. 1999). Furthermore, *V. destructor* can act as a vector for sacbrood and black queen cell viruses (Bailey and Ball 1991). According to Ball (1985) acute paralysis virus was the primary cause of both adult bee and brood mortality in German honey bee colonies severely infested with varroa.

According to Martin (2001b) deformed wing virus initially had little effect on the colony but during late summer, as the population of virus-transmitting mites increased, the virus caused a reduction in the number of healthy young bees entering the overwintering population. This imbalance in the age structure of the overwintering bees resulted in the eventual death of the colony.

Virus multiplication may be induced experimentally in adult bees by the injection of foreign proteins, and digestive enzymes or toxins secreted by the mite may have a similar effect in nature. Apparently the introduction of foreign proteins from varroa mites into the bee’s haemolymph can stimulate virus replication (Ball 1985).

Even though bees originating from African races appear more resistant to varroa mites (Moretto et al. 1991), newly emerged bees with damaged wings, bees crawling in front of the hive and unusually severe adult bee mortality were observed in AHB colonies in Costa Rica. A large proportion of the adult bee population in most of these colonies was infested with *V. destructor*. The analysis of adult bee samples showed the occurrence of deformed wing virus and Kashmir bee virus (Calderón et al. 2003a).

There is considerable evidence that colonies infested with varroa eventually collapse as a result of secondary infections, and of these, viruses activated and transmitted by the mite are most important (Ball 1996). This would imply that a factor for the development of mite tolerance in a honey bee population could be the relative absence of secondary pathogens in that population.

The effects of viral disease on bee colonies are complex, acting both at the level of the individual and the colony (Martin 2001a; Martin and Medina 2004; Sumpter and Martin 2004). The homogeneous genetic structure, close physical contact, and extensive social interactions among individuals make bee colonies especially vulnerable to the infection and transmission of pathogens (Chen et al. 2006; Chen and Siede 2007). However, the highly elaborate social organization of bee colonies poses a special advantage for bee immunity to defend against the infection of pathogens and to improve the survival of the colonies (Evans and Pettis 2005). The colony is only threatened if premature death or behavioural change affects many individuals (Martin 2001a).

Although the physical and chemical barriers usually keep pathogens from entering the bee body, pathogens occasionally break through these defenses and begin to multiply. Whenever physical and chemical barriers are breached, honey bees can actively protect themselves from infection by employing an innate immunity response which represents a second line of defense and occurs immediately on infection (Chen and Siede 2007; Evans et al. 2006).

Varroa mite reproduction

Reproductive cycle

Varroa depends on honey bee brood to reproduce (Oldroyd 1999; Webster 2001). Female mites initiate reproduction by entering the brood cells of last-stage worker or drone larvae, normally within about 20 or 40 h, respectively, before the cell is sealed (Bailey and Ball 1991), and hide from the nurse bees by submerging in the remaining liquid brood food, lying upside down. The mite's modified peritremes protrude snorkel-like out of the fluid surface, enabling them to respire (Donzé and Guerin 1997). Mites remain in this position for a short time, up to about 4 h after the cell is sealed in worker brood. After leaving the larval food on the bottom of the sealed cell, the mites begin feeding on the haemolymph of the prepupae. About 60–70 h after the brood cell is sealed, the first egg is laid (Bailey and Ball 1991). Eggs are laid one by one, usually on the cell wall, at intervals of about 30 h. The first egg is generally male, and the rest female (Martin 1994). Mites go through the following instars: eggs-larvae, ambulatory protonymph, immobile protonymph, ambulatory deutonymph, immobile deutonymph and adult (Martin 1994). Mobile nymphs actively feed (feeding phase) and grow while immobile instars are quiescent (non-feeding). The foundress mite keeps the feeding site on its juvenile bee host open to allow her offspring to eat (Donzé and Guerin 1994).

Male mites develop from egg to adult in about 6.5–6.9 days and females in 5.5–6.2 days (Martin 1994; De Jong 1997). Foundress mites can deposit a maximum of six eggs in worker cells and seven eggs in drone cells (Rehm and Ritter 1989). Guzman-Novoa et al. (1996) found no difference in the number of eggs or immature mites produced in AHB or EHB cells. Since males do not survive outside brood cells, fertilization of the adult daughters occurs within the cell. The deutonymphs molt directly to the adult phase, and mating takes place between the siblings (only when there is one foundress mite, otherwise cross breeding may occur) in the fecal accumulation site of the capped brood cell (female mites can only mate immediately after moulting) (Donzé and Guerin 1994).

Behavior of mites between reproduction periods (phoretic phase)

The bee hatches and leaves the cell along with the original female mite and the new adult females (Kraus 1994). Only the adult females survive after the bee emerges. Deutonymphs, protonymphs, eggs and males are left in the cell when the bee emerges. All of these apparently die, or are removed and killed by the nurse bees.

The females stay on the adult bees for a variable period lasting a few days to several weeks (Kuenen and Calderone 1997), possibly exhibiting a half-life function dependant on the ratio of bees to susceptible brood in a given colony (Boot et al. 1991). This is called the phoretic phase. After a variable period of time and a matter of chance (depending on the season and availability of brood) (Boot et al. 1993), the females begin the cycle again and invade new brood cells. They can do so immediately, though when they feed on an adult bee during this interval, their reproductive rate is increased (Beetsma and Zonneveld 1992).

Cyclic pattern of varroa reproduction

Varroa reproduction occurs in reproductive cycles, which start just after the host cell is sealed and end when the bee emerges. Female mites can go through two or three reproductive cycles in field populations (Martin and Kemp 1997) and as many as seven cycles

producing up to 30 eggs when artificially transferred between cells. Contact with adult bees is not indispensable for mite reproduction (de Ruijter 1987).

Preference of mites for drone brood versus worker brood

The reproductive ability of *V. destructor* varies according to the type of honey bee brood (worker or drone) being parasitized (Calderone and Kuenen 2001; Camazine 1988; Fuchs 1992; Ghandi and Hoopingarner 2003; Ifantidis 1988; Martin et al. 1997). Drone brood is more frequently infested and preferred by mites, and the average number of mites entering a cell with drone brood is higher than the number entering a cell with worker brood (Beetsma et al. 1999; Boot et al. 1991). In addition, mites prefer drone larvae to worker larvae when given the choice in laboratory tests (Le Conte et al. 1989; Trouiller et al. 1992).

Mite preference appears to be based, in part, on chemical attractants secreted by the larvae and present on the cuticle (Trouiller et al. 1991, 1994). Varroa mites are present on adult bees prior to invasion of brood cells. To decide to invade a brood cell or to stay on the bee, mites should obtain information about the content of the cell, e.g., a volatile chemical coming from the larvae (Boot 1994; Rickli et al. 1992, 1994). Such chemical information could be different for worker and drone brood, enabling discrimination by the mites (Boot et al. 1992; Calderone and Lin 2001). Le Conte et al. (1989) suggested that some aliphatic esters might be involved in mite invasion of cells.

In contrast to the chemical attractants related to cell invasion by varroa mites, there are a number of physical correlates to cell invasion (Calderone and Kuenen 2003; Kuenen and Calderone 2000). The shape of the brood cell appears to influence preference for drone cells (de Ruijter and Calis 1988). The surface of a drone cell is 1.7 times bigger than that of a worker cell, which increases the chance for a mite on a bee to come close enough to a suitable cell. Worker larvae in shallow cells are infested and capped sooner than larvae in control cells (Boot et al. 1995; Goetz and Koeninger 1992), whereas larvae reared in elongated cells are capped later than unmanipulated cells (Goetz and Koeninger 1993). Higher mite infestations occurred in worker cells that were individually raised above the surrounding cells (De Jong and Morse 1988).

To evaluate that it may be the brood type, which plays a role in mite reproduction success, we compared the reproductive rate of *V. destructor* in worker and drone cells of AHB colonies under the tropical conditions of Costa Rica. One of the most significant differences in mite reproduction was the higher percentage of mites producing viable offspring in drone cells (38.1%) compared to worker cells (13.8%). In worker brood fewer offspring are produced and higher rates of non-reproduction were found than in drone brood. A significant finding was that more than 74.0% of mites that did not reproduce in worker brood produced offspring when they were transferred to drone brood (Calderón et al. 2007). This indicates that brood type has an influence in the host-parasite relationship and plays a role in the ability of mite reproduction.

Varroa mite reproduction in Africanized bees

Defense of Africanized bees to varroa mites in Brazil

Since its first contact with *A. mellifera*, it can be seen that the population dynamics of the parasite *V. destructor* varies from one region to another. In many regions of the world,

apiculture has become an activity depending on the use of acaricides, because of the extensive damage caused by varroa to bee colonies. It could be expected that in tropical climates, where worker brood rearing and varroa reproduction take place all year round, the impact of the parasite would be even more devastating. This has not been the case in tropical areas of South America (De Jong 1984). In Brazil varroa was introduced more than 30 years ago and established itself at low levels of infestation, without causing apparent damage to apiculture (Carneiro et al. 2007; De Jong 1997; Moretto and Leonidas 2001, 2003). Among the races of *A. mellifera*, bees originating from African races appear more resistant to this parasite than European races (Moretto et al. 1991).

In Brazil varroa was probably introduced in the state of Sao Paulo (Morse and Goncalves 1979). As varroa dispersed through regions of Brazil, infestation rates of more than 10% were first recorded and it was thought to be a serious threat to Brazilian apiculture. However, a strong reduction of the rate of infestation has been observed in AHB colonies in several regions of this country, since the introduction of the parasite (Moretto et al. 1995; Moretto and Mello 1999). For example, the mean infestation rate in Sao Joaquin in the spring of 1986 was about seven mites per 100 bees, whereas in 1993 it was 2.03 per 100 bees. The same tendency was found in other regions of Santa Catarina state. The decreasing infestation rates observed from 1986 to 1993 demonstrate that an adaptive process has occurred in this host-parasite relationship (Moretto et al. 1995). The natural resistance of AHB in Brazil is evident from the low levels of infestation through the years (De Jong 1997; Moretto and Mello 1999).

Even though reduced varroa infestation level has also been reported from Uruguay (Ruttner et al. 1984), where about 70.0% of the varroa mites found in worker brood cells are infertile mites, there are different levels of varroa tolerance compared to Brazil and Argentina. In Uruguay, long-term survival of untreated and presumably non-Africanized bee colonies was confirmed. Striking tolerance factors, however, were not evident in Uruguay and infestation levels are higher than in Brazil (Rosenkranz 1999). The mites found on Africanized bees in Brazil have the same origin and have populations contiguous with those found on European bees in Argentina, where annual treatments are essential to keep colonies alive (De Jong et al. 1984; Rosenkranz 1999).

Proposed tolerance mechanisms to varroa

In the next section, we discuss some resistance mechanisms of AHB to varroa, focusing on traits related with mite reproduction.

Grooming behavior

Grooming refers to the behavior in which the bees groom themselves and each other when they are irritated by the mites (Büchler 1994; Moretto and Mello 1999; Peng et al. 1987) and even kill them with their mandibles (Szabo et al. 1996).

Artificial infestation of bee colonies with adult varroa females in Sao Paulo, Brazil, has showed that Africanized bee workers were almost eight fold more efficient in getting rid of the mites on their bodies compared to pure Italian bee workers (Moretto et al. 1991). According to Moretto (1997), artificially infested Africanized bees reacted to the presence of varroa from the very beginning of infestation. Strong body movements involving the abdomen, legs and mandibles were performed by the infested workers. The movements executed by infested workers permitted nearby workers to identify the varroa on their body. When fellow workers identified the presence of the mite on the body of another

worker, they used their tongue and mandibles to attack it (strong interaction between adult bees). Of the total number of infested workers in the studied colonies, 31.0% removed the parasite due to their own action and the action of fellow bees (Moretto 1997). The active defense of the Africanized bees is recorded to be similar to that of the natural host *A. cerana* (Peng et al. 1987).

The results of Guzman-Novoa et al. (1999) and Arechavaleta-Velasco and Guzman-Novoa (2001) in Mexico are in agreement with those reported from Brazil. They suggested that grooming behavior may be an important mechanism conferring tolerance of honey bee colonies toward varroa mites in Mexico. Nevertheless, Vandame et al. (2002) found that grooming capacities were slightly higher in AHB than EHB. Only a maximum of 11.0% of the observed mites were definitely removed by AHB, versus a maximum of 8.0% in EHB. Thus, it seems very unlikely that in Mexico grooming behavior may explain the low compatibility between AHB and *V. destructor*.

Hygienic behavior

Hygienic behavior is related to bees opening up capped brood cells and removing the brood (Peng et al. 1987; Spivak 1996; Boecking and Spivak 1999). A specific response of bees to diseased and parasitized brood, is said to be the primary natural defense against diseases and pests (Peng et al. 1987). Bees that demonstrated hygienic behavior by fast removal of freeze killed pupae, also remove mites from infested cells (Boecking and Ritter 1993; Spivak 1996; Eischen 1997; Spivak and Reuter 1998). Spivak (1996) reported that colonies selected for hygienic behavior had lower mite levels than non-hygienic ones. Harbo and Harris (2005) suggest that bees with a suppressed mite reproduction trait (SMR) (colonies that have a low frequency of reproductive mites in worker brood) removed reproductive mites more often than they removed non-reproductive mites.

Colonies, strains, races and species of bees are highly variable as regards their hygienic behavior (Boecking and Spivak 1999). Workers of AHB colonies are more efficient at removing brood infested with varroa than are European bees, kept under the same conditions (Moretto et al. 1991; Spivak and Gilliam 1998). Vandame et al. (2000, 2002) found in Mexico that EHB were able to remove only 8.0% of infested brood while AHB removed up to 32.5% showing a possible mechanism that could contribute to the tolerance of AHB toward *V. destructor*. Thus, selective removal behavior as a type of hygienic behavior can be effective in the control of varroa (Ibrahim et al. 2003).

Varroa destructor haplotypes

Another consideration is the suggestion that it is the type of mite present that influences the level of tolerance in a honey bee population. The most common and important haplotypes are called Korean and Japanese. The Korean haplotype, which has also been referred to as the Russian genotype, was identified on *A. mellifera* in Europe, the Middle East, Africa, Asia and America. Less common is a Japanese/Thai haplotype. This was identified on *A. mellifera* in Japan, Thailand and South America. The Korean haplotype is considered to be more virulent than the Japanese haplotype (Anderson and Fuchs 1998; Anderson and Trueman 2000). These genotypes may represent mite populations with different virulence, which could explain why some populations of honey bees survive and maintain low levels of varroa infestations (Arechavaleta-Velasco and Guzman-Novoa 2001).

The degree of bee tolerance in Mexico and Costa Rica seems to be lower than that of AHB in Brazil. It is noteworthy that the populations of mites estimated in Mexico and

Costa Rica are far higher than those from Brazil. Arechavaleta-Velasco and Guzman-Novoa (2001) found that infestation levels in adult bees increased over 6-month period in some colonies up to 45.0% (reaching up to around 9,000 mites per colony). Furthermore, Mondragon et al. (2005) reported an increase in the amount of mites throughout the year on AHB in Mexico. A significant increase in varroa infestation levels was also observed in AHB colonies in Costa Rica. Infestation rates of up to 22.0% in adult bees, and a total of 8,000 mites found in some colonies during a 170-day study period, indicate that mite populations found in AHB colonies in Costa Rica are higher than those reported for AHB in Brazil (Calderón and Van Veen 2008).

As the degree of Africanization is supposed to be the same in Costa Rica, Mexico and Brazil, the mite haplotype has been considered as a cause for differences in mite fertility between Mexico and Brazil (Medina and Martin 1999), and Costa Rica. In Costa Rica it was confirmed that the mites belonged to the Korean haplotype (De Guzman, personal communication, 1999; Anderson and Trueman 2000). Mites reported from Mexico and recently from Brazil have also been reported as belonging to the Korean haplotype (though the Japanese haplotype was formerly thought to have been more abundant in Brazil) (Anderson and Trueman 2000). Thus, the fact that in Brazil the host-parasite situation is stable despite the presence of the Korean haplotype (Carneiro et al. 2007; Correa-Marquez et al. 2003; Garrido et al. 2003) shows that tolerance is not entirely dependant on the varroa haplotype.

Duration of the capped cell stage

The post-capping period in worker brood of European bees has been recorded as 12 days, generally sufficient for three female mites to reach adulthood (Martin 1994). In some African bee strains the duration of the sealed cell stage is 11 days. Africanized bees are reported to have a post-capping period in worker brood of 275–278 h (Rosenkranz 1990). Some studies suggest that the shorter post capping stage of Africanized bees (about 10–13 h less) is likely the cause of the low infestation levels of varroa (the more time a pupae is in the cell, the more time mites have to develop). The shorter development time exhibited by African races appears to result in a larger degree of infertility in adult mite females after invasion of worker brood (Camazine 1986; Ritter and De Jong 1984). According to Sammataro (1996), if the capped cell stage is reduced even by 6 h, this may be enough time to reduce mite levels.

Büchler and Drescher (1990) determined a positive correlation between the length of the capped stage and the infestation level, indicating that, on average, a reduction of the length of the capped stage by 1 h led to a 8.7% reduction in the final mite infestation level in European bees. However, although the sealed-cell stage in Africanized worker bees is significantly shorter than that of European workers, Rosenkranz (1990) found that the number of offspring per fertile female mite in EHB was only slightly different than that for varroa parasitizing AHB.

Cell diameter

In a comparative study, Piccirillo and De Jong (2003) found that European-sized worker brood cells were always more infested than the Africanized worker brood cells in the same colony. The small width of worker cells produced by AHB may have a role in the ability of these bees to tolerate infestations by varroa mites. In a similar way, worker larvae reared in European size brood cells (larger diameter) are infested at a greater rate than are larvae from the same queen reared in Africanized size brood comb (Message and Goncalves

1995). These authors found more than twice as many mites in Italian-sized compared to Africanized-sized brood cells. Africanized worker cells are approximately 4.7 mm in diameter, compared with 5.1 mm for European bees.

Tall worker brood cells, which protrude above the comb surface, are also significantly more infested than normal cells (De Jong and Morse 1988; de Ruijter and Calis 1988). This may be due to a shorter distance between the larvae and the cell rim at the time that the brood cell is sealed (Boot et al. 1995). Medina and Martin (1999) found an increased varroa offspring mortality in worker cells of Africanized bees, compared to European bees, and suggested this was because of the reduced cell size of Africanized bees.

Effect of juvenile hormone

The very close relationship between varroa and the host bees has led to the hypothesis that reproduction in the mite is affected by juvenile hormone (JH) levels found in the host (Hänel 1986; Hänel and Koeniger 1986). Maximum juvenile hormone levels in bee brood coincide with the initiation of egg laying by the mite (De Jong 1997). Rosenkranz et al. (1990), looked for hormonal differences in the haemolymph of three honey bee races: *A.m. carnica*, *A.m. lamarckii* and AHB. No differences were found in juvenile hormone titers between the three types of bees. These results do not support the hypothesis that a reduced reproduction of the mite in worker brood on AHB in Brazil is regulated by host derived juvenile hormone (JH III) titer in worker larvae (Rosenkranz et al. 1990, 1993).

Varroa mite reproduction rate as influenced by colony factors

The observation that large numbers of infertile mites were present in honey bee populations tolerant to varroa has led to mite infertility becoming perhaps the most widely accepted explanation for varroa tolerance. In fact, reduced reproduction of varroa is regarded as the most important factor in tolerance of honey bees toward this parasite. Minor variations in the reproductive rate of the parasitic mite may have an impact on its population dynamics (Calis et al. 1999) because of the large number of generations per year (Milani et al. 2004).

Mite reproductive rates would be reduced if adult workers detect infested brood cells and remove the pupae before the mites can reproduce (Spivak 1996) or remove mites from the bodies of nestmates and thus prevent the mites from entering cells and reproducing (Fries et al. 1996). Physiological characteristics of the honey bee larvae or pupae might also afford some resistance. For example, mite reproductive rates might be lowered if there was reduced feeding activity on the larvae or pupae by the foundress mites (Grandi-Hoffman et al. 2002). Larvae movements (longitudinal displacement) that reduced mite feeding were observed in transparent cells containing infested worker brood in Africanized bees (Calderón et al. 2009). Indeed, traits expressed by bee larvae and pupae might be responsible for the lower fertility rates of mites in the brood of Brazilian honey bees, which show an increased tolerance to *V. destructor* (Ritter and De Jong 1984). It has frequently been suggested that the mite infertility is particularly prominent in Africanized bees and that this is the reason for varroa tolerance in these bees (Camazine 1986). Of all the traits examined, only non-reproduction of mites was highly correlated with changes in the mite populations (Harbo and Hoopingarner 1997).

The best measure to quantify reproductive success in varroa mites is the number of fertile mites and the number of viable females produced in worker brood cells per foundress per reproductive cycle (Correa-Marquez et al. 2003; Medina and Martin 1999).

Fertility of female mites

During a normal infestation cycle, the female mite enters a brood cell, feeds on the haemolymph of the developing bee, and lay eggs that develop into nymphs and eventually new adult mites (De Jong 1997). However, many mites go through these cycles, feed on the pupae, but do not reproduce. It is not well known which components of the diet of the mites are responsible for inducing oogenesis (triggering the activation of oocyte maturation of the mite) (Garrido et al. 2000). Oocyte growth in reproductive varroa females depends on the consumption of haemolymph from freshly sealed larvae (Donzé and Guerin 1994). The juvenile hormone hypothesis, which has been discussed, is now refuted by different studies. The quantity of haemolymph available at a certain time may regulate mite reproduction, especially the initiation of vitellogenesis (egg protein production). The considerable weight increase observed in the mites shortly after cell capping is an indication for this (Büchler 1994).

Varroa fertility can be defined on the basis of whether a female lays an egg or not (Rosenkranz 1999). A significant lower percentage of mites reproduce on Africanized worker brood than on brood of European bees, kept in the same regions of Brazil (Camazine 1986; Moretto et al. 1995). Rosenkranz (1999) reported that 43.0% of the mites do not reproduce at all in Africanized colonies compared with only 19.0% in European colonies, at least in the tropics. However, recent studies on AHB colonies from Brazil indicated that mite fertility has increased and is currently at European levels. Garrido et al. (2003) revealed that mite fertility in singly infested worker brood cells increased to 82.0%, whereas previous studies had demonstrated a 50.0% average mite fertility (Table 1). Furthermore, Correa-Marquez et al. (2003) reported a mite fertility of 72.0% in Riberao Preto and Carneiro et al. (2007) indicated that the percentage of fertile mites in worker brood of AHB in Brazil increased from 56.0% in the 1980's to 86.0% in 2005–2006 (Table 1).

A link between the temporal dynamics of fertility and haplotype change is suggested. Recently the Korean haplotype was reported from Brazil, whereas formerly the Japanese-haplotype was considered to have been more abundant (Garrido et al. 2003). Taking into account that in Costa Rica we found the Korean haplotype since varroa detection in 1997 (Anderson and Trueman 2000), this suggests that AHBs in Costa Rica are less tolerant to *V. destructor* than AHB in Brazil, due to the higher mite fertility in worker brood cells.

Table 1 Relative fertility of varroa mites in worker brood cells of Africanized honey bees (AHB) reported from different countries

Country of study	Fertility in worker brood (%)	Reference
Uruguay	50.0	Ruttner (1977)
Brazil	49.0	Camazine (1986)
Brazil	50.0	Rosenkranz (1999)
Brazil	78.0	Correa-Marques et al. (2003)
Brazil	82.0	Garrido et al. (2003)
Brazil	86.0	Carneiro et al. (2007)
Mexico	88.0	Medina and Martin (1999)
Mexico	82.0	Vandame et al. (1999)
Mexico	85.5	Mondragon et al. (2005)
Costa Rica	69.8	Calderón et al. (2003b)
Costa Rica	76.5	Calderón et al. (2007)

Each female mite laying at least one egg was considered fertile

In Costa Rica, mite fertility for varroa infesting worker brood cells in AHB ranged between 69.0% and 76.0% (Calderón et al. 2003b, 2007) (Table 1). In addition, we observed a mite population increase in experimental costarican AHB colonies through the year to significant levels (Calderón and Van Veen 2008).

Independent studies carried out in different tropical regions of Mexico showed that AHB colonies were able to survive without the application of any mite control measures (Guzman-Novoa et al. 1996), while the mite population cycled between 1,000 and 8,000 mites (Mondragon et al. 2006). According to Medina and Martin (1999) the percentage of fertile mites in AHB in Mexico was about 88.0% (Table 1). In addition, Mondragon et al. (2005) reported that the mean annual percentage of mites that reproduced in AHB in Mexico was 85.5%, with a minimum level observed in June (Table 1). In AHB from Mexico, mite fertility does not seem to be the main reason for bee resistance because its fertility level (Medina et al. 2002) is similar to that reported for EHB from Europe (Martin et al. 1997).

Viable offspring

Viable female offspring is the progeny that includes one live adult male mite and at least one live adult female mite (female mites that can reach the adult stage and have a mate available) (Medina and Martin 1999). The percentage of mites with viable offspring reported from worker brood cells was similar in Africanized bees in Mexico and Brazil, and was slightly lower in Costa Rica (Table 2). This is unexpected when we consider that varroa population increase in Africanized colonies in Mexico and Costa Rica, and not so in Brazil.

A direct consequence of offspring mortality in worker cells results in only 0.73 viable female offspring produced per mother mite, which rises to 0.85 if both mated (viable) and non-mated females are considered from AHB in Mexico (Medina and Martin 1999). For AHB in Costa Rica the number of viable females produced per reproductive female mite in worker cells was 0.30. If only the foundress producing viable offspring is considered this rises to 1.44 (Calderón et al. 2007).

The death of a male plays a central role in explaining a decrease in the production of viable daughters. When mites produce daughters but no living son, the daughters mites will remain unmated in single infested cells, because varroa mites mate just after their final molt inside the cell (Boot et al. 1997). The considerable higher rate of mortality suffered by the first (specifically the male protonymphs) and second (female) mite offspring found in AHB, is thought to contribute in part to the tolerance of the bees (Martin and Medina 2004; Medina and Martin 1999).

Table 2 Reproduction parameters display by *Varroa destructor* in worker brood cells of Africanized honey bees (AHB)

	Africanized bees in Brazil (Correa-Marques et al. 2003)	Africanized bees in Mexico (Medina and Martin 1999)	Africanized bees in Costa Rica (Calderón et al. 2003b)
Viable offspring	40.0	40.0	28.0
Nonviable offspring	38.0	48.0	42.0
Non-reproductive	22.0	12.0	30.0

This table was adapted from Correa-Marques et al. (2003)

Mite fertility in worker brood cells of AHB in Costa Rica was about 70.0%. Nevertheless, the combined effects of different factors results in less than 30.0% of the foundress mites producing viable female offspring (Calderón et al. 2003b). The absence of the male in a considerable number of worker cells naturally infested with varroa is the major factor in our study which reduces the production of viable daughters in AHB colonies in Costa Rica (Calderon et al. unpublished data). Harris and Harbo (2000) indicated that reduce numbers of progeny from mites may result from factors associated with immature bees. We observed in transparent cells containing infested worker brood in Africanized bees that larvae movements (longitudinal displacement) reduced feeding activity on the larva by the foundress mites (Calderón et al. 2009). Furthermore, in a considerable number of cases the first egg was disturbed and damaged when the prepupa molts into a pupa. The first egg is generally male and will not have any chance to get the adult stage. We consider that the absence of male in an important number of worker cells could be explained by this effect. This may affect mite population growth in a negative way. However, since varroa completes between two and three reproductive cycles (Martin and Kemp 1997), positive population growth is possible (infestation rates of up to 22.0% in adult bees were observed in AHB colonies in Costa Rica) although at a reduced rate in AHB compared to EHB.

Furthermore, we hypothesized that, due to the preference of mites to invade drone brood to reproduce, a high proportion of the mite reproduction should occur in drone cells. To test this hypothesis, we compared the reproductive rate of *V. destructor* in worker and drone cells of AHB colonies. We found that mite reproduction was greater in drone brood because more foundress mites produced viable female offspring (Calderón et al. 2007). The number of eggs and viable females produced per foundress mites was higher in drone brood compared to worker brood. Furthermore, a high percentage of the non-reproducing mites in worker brood produced offspring when they were transferred to drone brood.

So far, it is not well-known whether the severity of the effects caused by varroa depends on the genotype of the bees, on the genotype of the mite or on the interaction of both. The tolerance of AHB to varroa in Brazil is apparently attributable, at least in part, to resistance mechanisms. The low fertility of this parasite in Africanized worker brood has been considered an important factor for maintaining the host-parasite equilibrium. Grooming behavior and hygienic behavior are also referred as important mechanism used for the bees to keep low infestations in the colonies. Another consideration is the suggestion that it is the type of mite present that influences the level of tolerance in a honey bee population. The Korea haplotype is predominant in unbalanced host-parasite systems, as exist in Europe, while in stable systems, as in Brazil, the Japan haplotype formerly predominated (Anderson and Trueman 2000). However, the patterns of varroa genetic variation have changed in Brazil. All recent mites corresponded to the Korea haplotype, independent of whether the mites had reproduced or not.

The fertile mites of *V. destructor* on AHB in Brazil significantly increased from 56.0% in the 1980s (De Jong 1997; Rosenkranz 1999) to 86.0% in recent years (Correa-Marques et al. 2003; Garrido et al. 2003; Carneiro et al. 2007). Nevertheless, despite the increased fertility, no increase in mite infestation rates in the colonies has been detected so far. Currently, the rate of infestation on adult bees in Africanized bees in Blumenau has remained low (Carneiro et al. 2007). As the low fertility of mites in AHB worker brood has been discussed as one of the most significant factors contributing to varroa tolerance, the new status of mite fertility in Brazil demands for further attention. In addition, the number of viable females per mite that invade the worker brood in singly infested cells should be revised.

We conclude that several of the factors related to why AHB in tropical regions are able to tolerate *V. destructor* infestations were found to involve the reproductive ability of the mites. However, a detailed study into the mites reproduction biology in AHB colonies has not yet been undertaken. Further studies are needed to analyze in more detail the reproduction ability and the actual dynamic population, especially in Brazil where low mite infestation levels in Africanized colonies without causing measurable damage and the recent increase in mite fertility and the presence of the Korean haplotype are not fully congruent.

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