Natural & Suppressed Reproduction of Varroa

Jeffrey W. Harris & John R. Harbo

In 1995 we began selective breeding of honey bees for resistance to *Varroa destructor* (the common *Varroa* mite that was formerly called *Varroa jacobsoni*). From a population of bees that we assembled from Michigan and Louisiana, we measured many characteristics that we thought could be associated with the growth of mite populations in bee colonies[4]. Based on that study and the reports of other researchers[11], we chose suppression of mite reproduction (SMR) as the trait of the honey bee that would be the basis of our selective breeding. Since that time we have intensified SMR from about 20% in an unselected colony to a recent group of queens that averaged 100% SMR in worker brood[5,6]. This trait is widespread in our bee population, so anyone can select for it as long as they know how to measure it. This article describes metamorphosis in worker bees, normal reproduction of varroa mites, and the abnormal reproduction of mites found in colonies of bees that have been selected for SMR.

Introduction

Our breeding objective is to control the growth of mite populations within bee colonies by the selective breeding of honey bees. At first, we settled for a slower growth of the mite populations, and then we wanted no growth. Now we want a decline in mite populations.

Our approach is to measure small changes in mite populations over relatively short periods (2 – 4 months). This requires accurate and sometimes tedious measurements of brood, bee populations, mite populations, and mite reproduction.

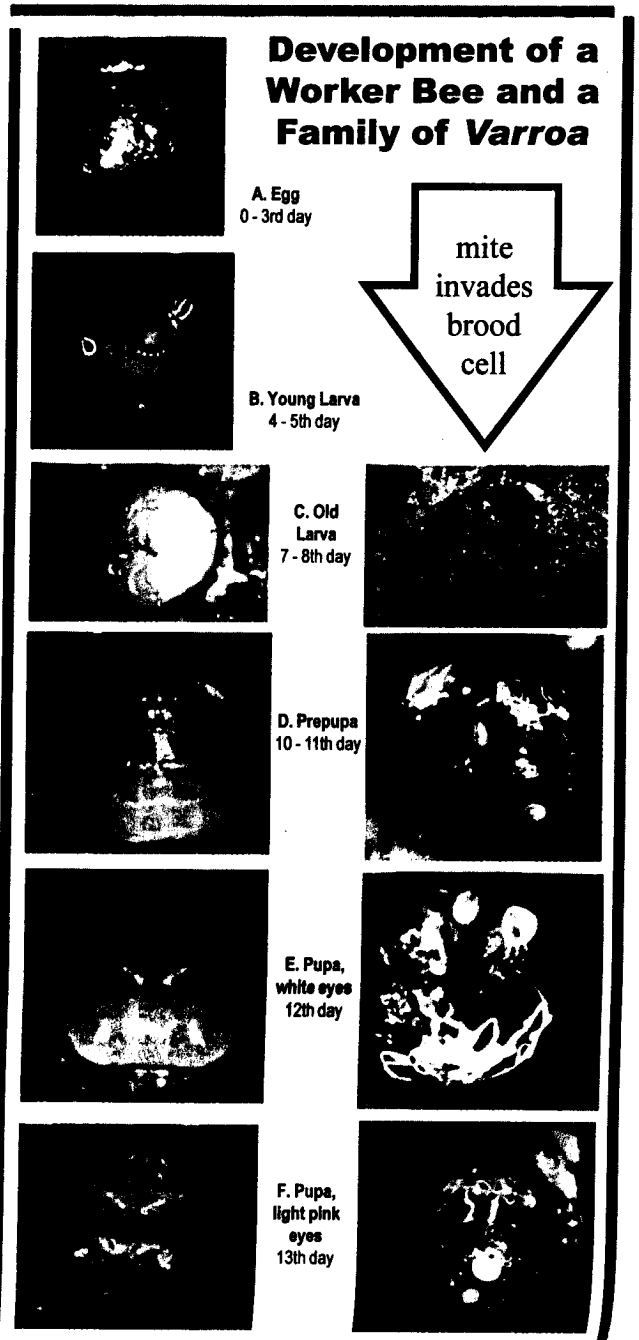
A colony does not need to show long-term tolerance of *Varroa* mites to be valuable in selective breeding for resistance. We selected queens for breeding by screening 25-30 colonies of bees for *Varroa*-resistance in 80-120 day field tests. Typically, each colony started with 2 pounds of worker bees and 400 mites. We measured populations of mites and bees from each colony at the beginning and end of a test, and we measured various traits during the experiment. Then we chose queens from the best colonies to become breeder queens. Later, we found that the percentage of nonreproducing mites (%NR) predicted the growth of a population of *Varroa* mites; mite growth was lowest in colonies with the highest %NR. We then began to select queens based on the %NR rather than on overall growth of the mite population.

Varroa mites reproduce within the capped brood cells of the honey bee. Nonreproducing mites are those that enter brood cells and do not lay eggs, or if they do lay eggs, none of the daughters can mature before the adult bee leaves the brood cell. Experiments showed that a genetic characteristic of bees caused mites to become nonreproductive. We call this trait the "suppression of mite reproduction" (SMR). Before describing the abnormal reproduction of *Varroa* mites from colonies having the SMR trait, we will describe the normal life histories of worker bees and *Varroa* mites.

Metamorphosis of Worker Bees

Honey bees develop within cells on a brood comb. The total development time from egg to adult varies among and within the three castes: about 15 days for queens, 23 days for drones, and 20 days for workers. The following description focuses on worker metamorphosis. Although *Varroa* mites prefer drone brood cells to worker cells by about 8:1, we did not provide drone cells in our field trials, so the mites invaded only worker cells.

Like beetles and butterflies, honey bees undergo complete metamorphosis. The immature



insect looks dramatically different from the adult insect in this type of development. Honey bees advance through four morphologically distinct life stages: egg → larva → pupa → adult (Fig 1).

The rate of metamorphosis for most insects depends on temperature. Generally, development slows at cooler temperatures. This is also true for honey bees. However, healthy colonies of bees hold their brood nest temperature constant (about 95°F), and this constant temperature makes a predictable rate of development for the bee. Therefore, the size of a larva or the external coloration of a pupa can be used to estimate age (age is measured from the moment the queen bee lays the egg). A pupa with purple eyes and a white body is probably 15 days old (Fig 1H) give or take a few hours. The duration of each stage of development is constant. For a worker bee, an egg is 3 days, a larva is 8 days, and a pupa is 9 days. The egg and young larva live in uncapped brood cells, and the duration of the uncapped period is 8 days. The oldest larval stages, pupal stages and first half-day of the adult stage occur in capped brood cells. The capped period, or postcapping time, spans 12 days.

The life of a worker bee begins when a queen lays a single fertilized egg onto the base of a worker-sized brood cell (Fig 1A). An egg stands erect from its point of attachment. The external appearance of an egg does not change before hatching; however, the embryo within the egg dramatically changes from a tiny blob of cells into a segmented larva (Fig 1B). Just before hatching, the larva begins to flex within the egg while dissolving the eggshell. This occurs at the end of the third day.

Nurse bees feed a larva within minutes of hatching and continue to provide food (Fig 1B) while the cell is uncapped. By the end of the 7-8th day, a full-grown larva weighs about 1,500x its original weight at hatching. Older larvae completely fill the bases of their brood cells (Fig 1C). Attendant bees construct a wax cap by the end of the 8th day, and the larva eats the remaining brood food during the first several hours postcapping. Afterwards, the larva defecates or clears her gut and spins a cocoon around herself. Glands within the mouthparts of the larva produce the silk used to weave the cocoon. The cocoon usually separates the larva from her feces. Immature bees molt and shed their skins as they grow; 5 molts occur in the larval stages, and 1 at the end of the pupal stage. The final larval molt occurs soon after the cocoon is spun. This molt differs from the previous 4 molts because the newly formed prepupa (Fig 1D) remains within the old larval skin for a couple of days (rather than shedding it immediately after molting). The prepupal stage spans about 2 days.

The pupal stage begins after the prepupa sheds the fifth larval skin at the end of the 11th day (Fig 1E). The external body of a 12-day-old is all white. Subsequent external changes involve darkening of the eyes and the cuticle. Although the external shape of a pupa does not change dramatically, the internal structures undergo major rearrangements. The organs and tissues of the larva degrade, and new organs and tissues of the adult bee gradually replace them. The most obvious external changes in pupae involve pigmentation of the compound eyes (large eyes), the ocelli (three small eyes at the top of the head), and the cuticle or outer skin. A young pupa (Fig 1E) lacks pigment in the eyes or the body. The eyes and ocelli gradually change color from white to pink (Figs 1 F—G) to purple (Fig 1H) by the end of the 15th day. The cuticle of the bee slowly darkens from white (Figs 1 E—H) to tan (Figs 1 I—J) to gray or black by the end of the 18th day (Fig 1K). The wings expand fully as the bee sheds the pupal skin on the 19th day. This completes the pupa-to-adult molt, which is the sixth and final molt of the worker

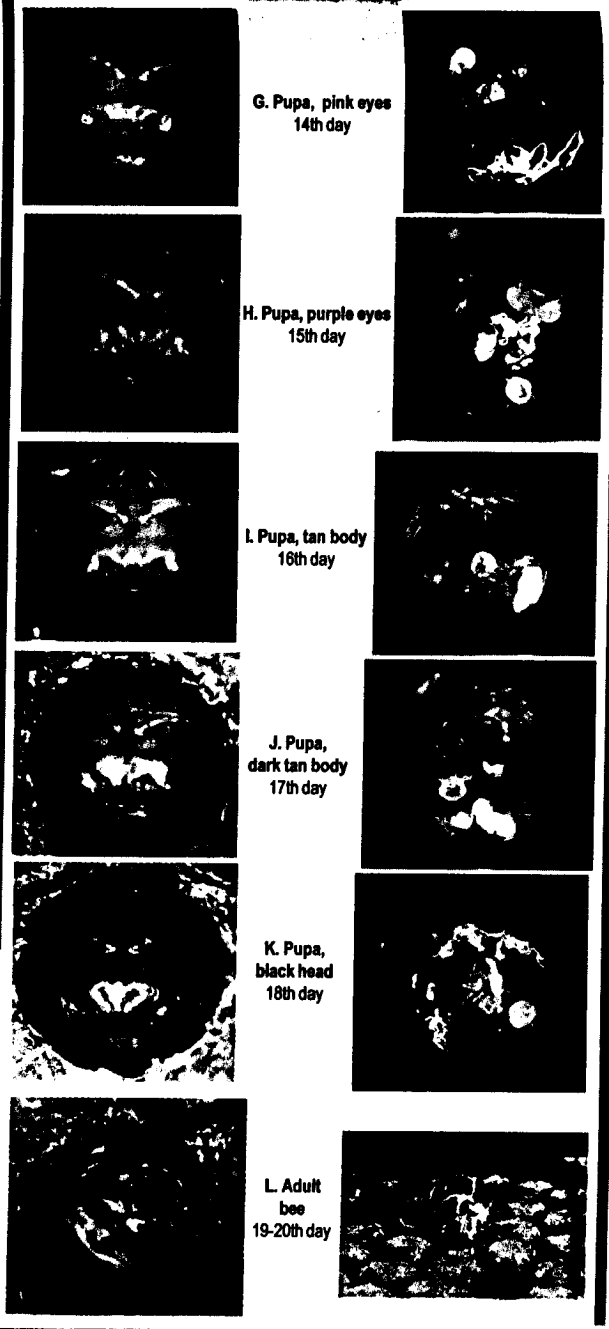


Figure 1: *Stages in the development of bees are given in the left column, and the corresponding mite family (or activities of the mite) is shown in the right column of the table. The center of each row is labeled by a description of the bee's stage and approximate age of development (days). The mite photos in* ***D—J*** *show the progressive growth of the mite family. The mite in* ***C*** *is covered by brood food, and an extended peritreme can be seen between the 3rd and 4th legs on each side of the body. The right side of* ***K*** *shows adult male and female mites with their shed skins from the final molt (see also Fig 2E).*

bee during metamorphosis. The young adult bee remains in the capped brood cell for another 10-18 hours. Her cuticle continues to harden during this time, and she frequently moves her legs and wings. Eventually she chews away the cell cap and emerges from the brood cell to begin her life as an adult member of the colony (Fig 1L).

Varroa Mites Reproduce in Capped Brood Cells

Two distinct phases comprise the life cycle of a *Varroa* mite: (1) a phoretic phase when the mite lives on adult bees, and (2) a reproductive phase when she enters a brood cell to lay eggs. Potential attacks from adult bees make phoretic life more perilous than when a mite lives in a capped brood cell. The phoretic phase lasts from several days to more than a month, but the average is about one week. Mites prefer young nest bees to older workers and drones during the phoretic period. Reproduction by *Varroa* mites requires bee brood, and a colony of honey bees usually has brood except during the late fall and early winter. Mites must live solely on adult bees during broodless periods.

A *Varroa* mite may attempt to reproduce as many as 7 times during her life[1], but the average is about three reproductive cycles per mite[10]. The reproductive phase must fit within the 12-day postcapping period of the worker bee because mites cannot enter or leave a cell while it is capped. A foundress mite (or mother mite) begins reproduction by invading a brood cell. She does this by riding the belly-side of a nurse bee and running into an open cell that contains an old larva (Fig 1C). The mite probably chooses an appropriate cell by detecting the chemicals that the bee larva emits to stimulate the sealing of the cell by attendant bees. The mite moves down the cell wall and immerses herself in the brood food beneath the larva.

The mite becomes immobile while in brood food. She probably breathes from an air bubble that she holds around the bases of her legs while lying on her back in the brood food[12]. Varroa mites inhale and exhale air through a pair of tiny spiracles or stigma. One stigma is located on each side of the body near the base of the third leg (mites have 4 pair of legs). A fingerlike tube or peritreme extends from between the third and fourth leg on each side of the body when a mite is immersed in brood food (Fig 1C). Each peritreme connects to the mite's airway near the stigma. Scientists believe that the two peritremes eliminate excess CO_2 from the blood and retain water in the blood during respiration[12].

The bee larva inadvertently liberates the foundress mite by eating the remaining brood food (before spinning her cocoon). Bee blood is the only food for adult and immature mites. An awakened mite usually sucks the blood of her host beginning in the late larval stage, and these early meals are necessary for the development of her eggs.

Feeding sites usually occur on the abdomen of the bee, but mites can feed anywhere on the host. The mouthparts of immature mites are too short and soft to puncture the cuticle of the host, and the mouthparts of adult males are modified for transferring sperm during mating. Therefore, immature mites and adult males must feed from a wound made

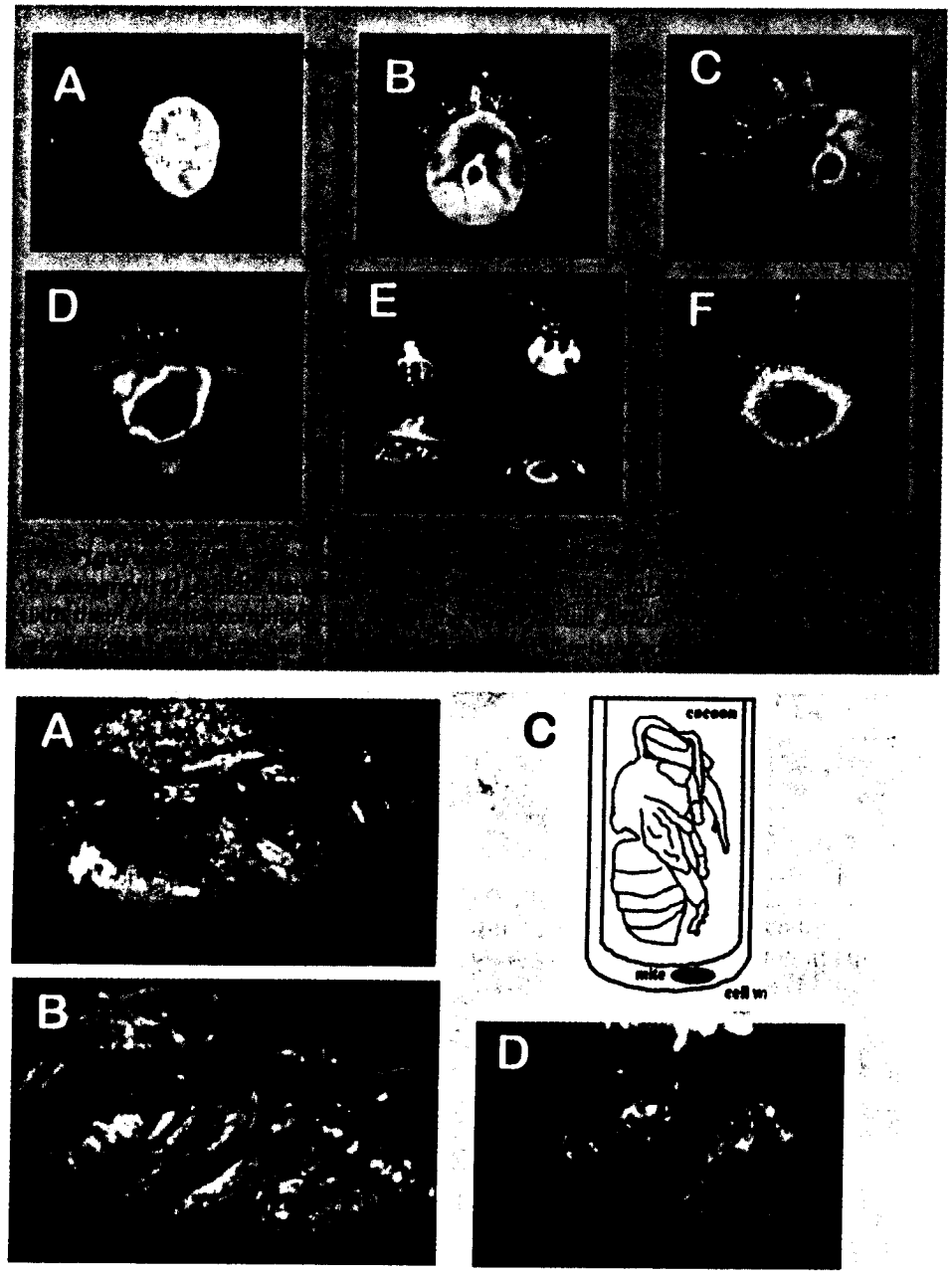


Figure 3: *Typical nonreproduction.* ***A****, a mite places her fecal patch on the bee rather than on the cell wall where it is normally placed;* ***B****, sometimes nonreproductive mites place several small fecal patches on the bee. For some reason, defecating on bees is often associated with mites that lay no eggs;* ***C****, diagram showing a mite entrapped between the cocoon (top layer) and cell wall (bottom layer);* ***D****, an entrapped mite as seen through the silk cocoon.*

by their mother[2]. All members of the family tend to defecate on the cell wall near the feeding site. The white mite excreta contrast sharply with the dark wall of the brood cell (e.g. Fig 1D).

Varroa mites develop through gradual metamorphosis like grasshoppers. The immature mites, or nymphs, have the general shape of an adult. Both sexes of mites have four life stages: egg → protonymph → deutonymph → adult (Fig 2). Males and females need 6.5 and 5.5 days, respectively, to develop from egg to adult.

Eggs and protonymphs of the two sexes look alike, but the deutonymphs and adults of the sexes are easily differentiated (Fig 2). The duration of each stage varies between the sexes. A male lives 30 hours as an egg, 52 hours as a protonymph, and 72 hours as a deutonymph. A female lives 20-24 hours as an egg, 30 hours as a protonymph and 75-80 hours as a deutonymph. Immature mites actively feed, or they walk on the host bee or cell wall when not preparing to molt. Mites of both sexes molt and shed their outgrown skins twice during development; between protonymph and deutonymph, and then between deutonymph and adult. A mite becomes immobile during the 16 hour period preceding its first molt, and for a 50 hour period (30 hours for males) preceding the second molt. The dried skin from the second molt is easily seen with a dissecting microscope (Fig 2E). Immature mites of both sexes are white. Newly-molted adult mites are tan. Bodies of females darken to reddish brown within a couple of days of the final molt (e.g. Fig 3A); males remain light tan (Fig 2E).

The reproductive success of a foundress mite depends on her ability to lay eggs so that 1-2 daughters have time to mature fully before the host bee leaves the cell. A mite produces as many as 5 eggs, but she cannot lay all 5 at once because each egg is large relative to her body. Many blood meals provide the nutrition needed to produce a single egg.

Evidence suggests that either the first blood meals or chemicals from the bee (larva or prepupa) stimulate *Varroa* mites to produce and lay eggs. The stimulus synchronizes a mite's reproduction to the metamorphosis of the host bee and ensures that all eggs will be laid along a schedule that maximizes the number of adult daughters. A mite lays her first egg, which is usually male, about 60 hours after attendant bees seal the brood cell. She places the male egg on the cell wall near the head of the prepupa (Fig 1D). The mite lays each subsequent egg (females) at 30-hour intervals, placing each egg on the cell wall near the abdomen of the host pupa. The typical mite will stop laying eggs on or before the 15th day.

The staggered sequence of egg laying causes the development and maturation of the offspring to be staggered relative to each other. For example, a typical mite family on a pupa that has purple eyes and a white body consists of a male deutonymph, an immobile female deutonymph (1st daughter), a mobile female deutonymph (2nd daughter), a female protonymph (3rd daughter) and an egg (4th daughter) (Fig 1H). Although the foundress mite lays the male egg about 30 hours before the first daughter egg, the longer development time of a male causes him to mature less than half a day before his oldest sister. Theoretically, a foundress mite can produce three adult daughters in a worker cell, but most mites only produce 1-2 mature daughters. The foundress mite and her adult daughters will exit the brood cell and survive after the young bee emerges (Fig 1L). Adult males and immature mites cannot survive after the host bee matures.

Varroa mites do not have eyes, so they depend on touch and smell to navigate through their environment. The mite excreta attract immature mites, which probably helps them find the feeding site. Mite excreta also attract newly-molted adult mites of both sexes, and mating occurs on or near the feces[2,3]. An adult male mates with a female shortly after she becomes an adult. He uses specialized mouthparts to transfer sperm from the genital opening of his chest to one of the two genital openings of the female. Each of her openings is located between the bases of her third and fourth legs. A typical female mates many times, but only a few sperm are transferred with each mating[3]. A fully-mated female mite stores 40-70 sperm within her spermatheca. She will use the stored sperm to produce eggs after she enters a cell in a future reproductive cycle.

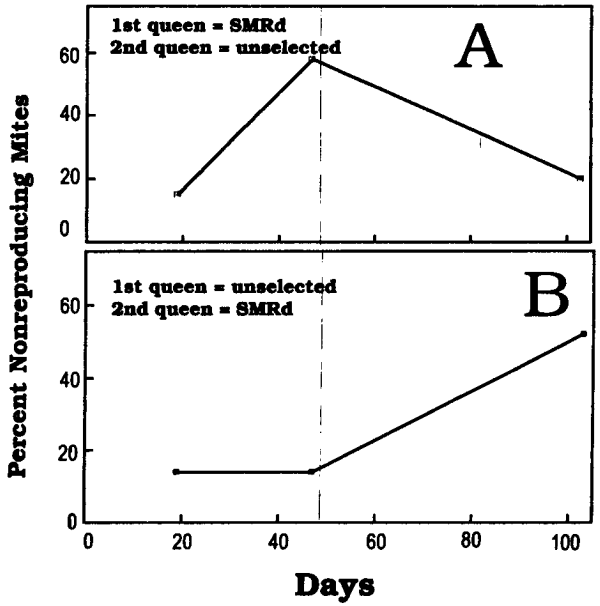


Figure 4: *Changes in the percent nonreproducing mites in the queen exchange experiment. **A**, plot of the average percent nonreproduction for 10 colonies that began with a queen selected for SMRd and were given an unselected queen after 48 days; **B**, a plot of the opposite group - 10 colonies that began with an unselected queen and were given a SMRd queen after 48 days. Generally, the percent nonreproducing mites increased in all colonies within a few weeks of installing the SMRd queens, and it decreased within a few weeks of installing unselected queens. The dotted line indicates the day that queens were exchanged.*

Suppression of Mite Reproduction

Not all *Varroa* mites in a colony of bees attempt to reproduce at the same time. During periods of active brood rearing by the bees, about 1/3 of a population of *Varroa* mites lives on adult bees, and 2/3 of them live within capped brood cells. In normal colonies of bees, about 15-25% of the mites that enter worker brood cells do not produce even one mature daughter[11]. Four categories of these

nonreproductive foundress mites can be described: (a) mites that die before laying eggs, (b) mites that live but do not lay eggs, (c) mites that produce only a male, and (d) mites that produce progeny too late so that none is able to mature. All four categories may exist in a typical colony of bees.

The percentage of nonreproducing mites is estimated by uncapping brood cells and recording the reproductive success for each foundress mite. To measure mite reproduction in a colony, we examine about 30 singly infested brood cells containing tan-colored pupae (Fig 1 I—J). Each mite is then classified as reproductive or nonreproductive, and we record the percentage of mites that are nonreproductive (%NR).

Various environmental factors affect the percentage of nonreproductive mites (%NR) in colonies of bees. High temperatures and relative humidity increase the %NR[8,9]. The %NR varies with season, and levels of nonreproductive mites are highest during late summer and when mites first enter brood cells after the winter. A higher %NR occurs in colonies from tropical climates than in those from moderate climates.

However, if we are to succeed in selective breeding, some of the nonreproduction must have a genetic basis in the bees. We showed that there is a heritable trait in bees that affects mite reproduction[4]. Therefore, it is possible to enhance this trait with selective breeding. Generally, high percentages (50 —100%) of living mites that had not laid eggs occurred in colonies of our selected line[6]. These non-laying mites often placed their feces on the bee (Fig 3A—B) rather than in the normal position on the cell wall. We also found that many non-laying mites had no stored sperm, which suggests that nonreproduction could be related to nonmating. With continued selective breeding, we began to find dead mites that were sandwiched between the cocoon that is spun by the host larva and the cell wall (Fig 3C—D)[6]. We term this condition "entrapped by the cocoon". Few entrapped mites (1-2%) are found in unselected colonies, but > 50% of the mites in colonies of bees bred for SMR are entrapped.

High levels of nonreproducing mites become apparent about 6 weeks after placing a queen with the SMR trait into a colony of bees. This delayed suppression of mite reproduction is called SMRd. Another type of mite suppression occurs in the first brood produced by a queen with the SMR trait. The acronym for immediate suppression is SMRi. Our past breeding work has been with the SMRd trait. We have only recently found the SMRi trait at high levels.

Queen Exchange Experiment

We demonstrated the effect of SMR in an experiment where queens with and without the SMRd trait were exchanged between colonies[7]. We established 20 uniform colonies, each with about 2 pounds of bees and 600 mites. Ten colonies started with queens having the SMRd trait, and the other ten colonies began with unselected queens that did not have the trait. After 48 days, we exchanged queens between colonies so that each colony was given the opposite type of queen. Bee and mite populations grew for 103 days. The %NR was measured at the beginning of the test (day 19), just before queens were exchanged (day 47), and at the end of the test (day 103) (Fig 4).

It was clear from this study that mite reproduction changed when queens were exchanged. Mite populations became more or less reproductive in response to the type of queen. Suppression was the delayed type (SMRd) because mite reproduction was nearly identical in all colonies during the first reproductive cycle (the observation on the 19th day). Differences were not apparent until after several weeks (day 47).

Based on our studies and that of others, we are confident that honey bees will become resistant to *Varroa* mites. SMR is only one of the possible mechanisms that can help our bees become *Varroa*-resistant. Our plan is to insert *Varroa*-resistant traits into our honey bees so that the bees will need fewer chemical treatments to control mites. Eventually they will need none. BC

Jeffrey Harris and John Harbo are research scientists at the USDA Honey Bee Breeding Lab in Baton Rouge, Lousiana.

Sources Consulted

1. de Ruijter A. 1987. *Reproduction of Varroa jacobsoni during successive brood cycles of the honeybee.* Apidologie 18: 321-326.
2. Donzé G. and P.M. Guerin. 1994. *Behavioral attributes and parental care of Varroa mites parasitizing honeybee brood.* Behavioral Ecology & Sociobiology 34: 305-319.
3. Donzé G., Herrmann M., Bachofen B. and P.M. Guerin. 1996. *Effect of mating frequency and brood cell infestation on the reproductive success of the honeybee parasite Varroa jacobsoni.* Ecological Entomology 21: 17-26.
4. Harbo J.R. and J.W. Harris. 1999. *Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to Varroa jacobsoni (Mesostigmata: Varroidae).* Journal of Economic Entomology 92: 261-265.
5. Harbo J.R. and J.W. Harris. 1999. *Selecting honey bees for resistance to Varroa jacobsoni.* Apidologie 30: 183-196.
6. Harris J.W. and J.R. Harbo. 1999. *Low sperm counts and reduced fecundity of mites in colonies of honey bees (Hymenoptera: Apidae) resistant to Varroa jacobsoni (Mesostigmata: Varroidae).* Journal of Economic Entomology 92: 83-90.
7. Harris J.W. and J.R. Harbo. 2000. *Changes in reproduction of Varroa destructor after honey bee queens were exchanged between resistant and susceptible colonies.* Apidologie 31: 689-699.
8. Kraus B. and H.H.W. Velthuis. 1997. *High humidity in the honey bee (Apis mellifera L.) brood nest limits reproduction of the parasitic mite Varroa jacobsoni Oud.* Naturwissenschaften 84: 217-218.
9. Le Conte Y., Arnold G. and P. Desenfant. 1990. *Influence of brood temperature and hygrometry variation on the development of the honey bee ectoparasite Varroa jacobsoni (Mesostigmata: Varroidae).* Environmental Entomology 19: 1780-1785.
10. Martin S.J. and D. Kemp. 1997. *Average number of reproductive cycles performed by Varroa jacobsoni in honey bee (Apis mellifera) colonies.* Journal of Apicultural Research 36: 113-123.
11. Martin S., Holland K. and M. Murray. 1997. *Non-reproduction in the honeybee mite Varroa jacobsoni.* Experimental & Applied Acarology 21: 539-549.
12. Pugh P.J.A., King P.E. and M.R. Fordy. 1992. *The respiratory system of the female Varroa jacobsoni (Oudemans): its adaptations to a range of environmental conditions.* Experimental & Applied Acarology 15: 123-139.