The African Honey Bee

A Case Study Of A Biological Invasion

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One of the most amazing biological phenomena that has occurred in our life time is the introduction and spread of the African honey bee (AHB) in the western hemisphere. Within 50 years of its initial introduction into Brazil, the AHB has colonized much of the New World, giving rise to one of the most rapid and spectacular biological invasions humans have ever witnessed. A remarkable feature of the AHB is its ability to displace resident European honey bees (EHB). As the AHB migrated northward from Brazil, it largely or entirely replaced feral European honey bees in almost every area where it became established, often with dramatic consequences for apiculture, agriculture, and public safety. Indeed, a project that began 50 years ago as a means to improve honey production in Brazil ultimately changed beekeeping in 17 countries over two continents. The African bee arrived in the U.S. in 1990. It is now permanently established throughout the southwestern states and parts of California, where it is displacing feral EHB populations and creating public safety concerns in many regions. Recent reports of the AHB from Louisiana and Florida raise the possibility that the southeastern states will also be colonized by this highly invasive insect.

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Learning to control and cope with the African bee (and with the media attention that invariably follows it) requires knowledge of its biology, history, how it interacts with European bees, and the effects it is likely to have as it spreads in the U.S. To this end, we present a series of three articles that discuss the major aspects of the AHB in the Americas. The first article compares the biology and survival strategies of African and European bees and summarizes the history of the AHB in the western hemisphere, beginning with the initial introductions into Brazil. The second article discusses the different factors that influence the AHB's ability to displace European honey bees in the western hemisphere. In the final article, we examine the spread and economic impacts of the AHB in the U.S. and speculate about its possible future movements.

The Biology of African and European Honey Bees

The honey bee, *Apis mellifera*, is native to the Old World and occurs naturally from the southern tip of Africa to Russia. Within this enormous geographic range, populations have adapted to different environmental conditions giving rise to more than 20 different subspecies, or races. These races can be divided into two main groups, based on the type of environment they have evolved to inhabit. Temperate climate races occur in Europe and are collectively referred to as European honey bees. The primary factor shaping the survival strategy of these races is Winter. To survive Winter, colonies must amass huge honey stores, which require that they occupy large, well insulated cavities, build large amounts of comb, maintain large worker populations and foraging forces, and emphasize nectar over pollen collection (Fig. 1).

European honey bees swarm primarily in the Spring and early Summer, to provide new colonies with sufficient time to amass large food reserves, and typically produce only 1-3 swarms per year. In addition, European colonies tend to "stay put." They rarely abandon nest

Figure 1. *Unlike European bees, which must occupy large, insulated nest cavities for Winter survival, AHB colonies can occupy smaller cavities and are more likely to build exposed-comb nests.*

sites and never migrate to escape Winter conditions.

In contrast, tropical races of honey bees occur in Africa. They do not experience such cold Winters and can often forage year round. This reduces the benefit of amassing large food reserves and allows colonies to construct smaller amounts of comb and occupy smaller nest cavities. A major survival challenge faced by African races is a high level of predation, which selects for increased stinging responses and high rates of colony reproduction. African races are often more defensive than European races (Fig. 2). Also, they typically produce three to four times as many swarms (3-12 per year) and, because of the prolonged foraging season, often have several swarming periods throughout the year. High rates of swarming require increased brood production. African

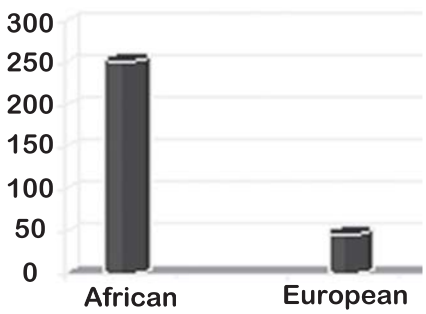

Figure 2. *The African honey bee race,* Apis mellifera scutellata*, is well known for its high degree of nest defense. Here are data comparing the number of bees captured while defending their colony in the first 30 seconds after a disturbance. About five times more African bees leave the colony than European bees in the same time interval. AHB workers also produce more alarm pheromone than EHB workers, which excites other workers to sting, and further contributes to greater colony defensiveness.*

bees devote two to four times more comb space to brood rearing than European bees and emphasize pollen over nectar collection to provide the proteins needed for increased brood production. Also, AHB workers have a shorter development time than EHB workers (18.5 versus 21 days, respectively), which further promotes the rapid build up of colony size and frequent swarming. Another challenge facing African colonies is the shifting pattern of floral resources that arises from the shifting, and often unpredictable patterns of rainfall of the African continent. Rather than hoard large amounts of food to survive dearth periods, African colonies often migrate long distances to follow seasonal changes in forage availability. African colonies will also readily abscond in response to disturbance and predation. As a result, African bees are much more mobile and more inclined to abandon nest cavities than are European honey bees.

Figure 3. *An AHB worker (left) and EHB worker (right). African and European bees look virtually identical, although African workers develop faster in the larval stage. AHBs sometimes have a darker color than EHBs, but color is too variable in both races to be used as a reliable identification mechanism. The two races differ more in how they behave rather than how they look.*

Thus, even though European and African honey bees belong to the same species, look virtually identical (Fig. 3), and have the same social structure, they have emphasized different aspects of their nesting biology, comb usage, foraging behavior, reproduction and movement patterns to adapt to their different environments.

The European races of honey bees are the most familiar to us. Their large population sizes, along with their emphasis on honey production, low swarming rates and reluctance to abscond make them ideal for beekeeping and agriculture. Starting in the 1600s or earlier, European honey bees were introduced repeatedly into North America by the first settlers that came from Europe. The bees thrived and quickly established large wild populations throughout the country.

European races were also continually introduced into Central and South America. Although some countries developed sizable beekeeping industries, European bees did not thrive in tropical habitats. They required extensive human management and never established large feral populations. Some nations therefore began to explore the possibility of importing African races of honey bees. Although the increased defensiveness, high swarming rates, absconding and migration behavior of African bees were recognized as detriments to beekeeping, it was thought that selective breeding could modify these undesirable traits, resulting in a gentle, tropically-adapted bee that would improve beekeeping and honey production in the Neotropics.

The differences in the biology of African and European bees that evolved over millennia therefore set the stage for the AHB invasion, and no one could have foreseen the enormous consequences that would arise from the subtle behavioral differences between these subspecies of honey bees.

The History of the AHB in the Americas

The story of African honey bees in the New World begins in Brazil (Fig. 4). From 1954-1955, beekeeping agencies, government departments and private beekeeping cooperatives initiated projects in Brazil to increase the low honey production of the European colonies kept by commercial beekeepers. The best subspecies to introduce into Brazil was determined to be *A. m. adansonii* from southern Africa, a population now re-classified as *A. m. scutellata*. In 1956, Dr. W. E. Kerr traveled to Africa to select queens of the best stocks. Queens were selected from Angola, Mozambique and Bloemfontein, and Republic of South Africa, but none survived. Six queens were collected in Tanganyika, of which one survived introduction to Brazil. Of 132 queens collected from Pretoria, South Africa, 46 survived introduction into Brazil. These few surviving colonies are often viewed as the *only* colonies that gave rise to the African population in the New World. However, we now know that there were other introductions of AHB into South America, as well as human-assisted distributions of AHB queens to beekeepers. In combination, these colonies and queens formed the nucleus of the African population that would eventually colonize much of the western hemisphere.

Initially, it was thought that interbreeding between African and European bees would genetically dilute the undesirable AHB traits.

Although the EHB had never established wild populations in the Neotropics, many countries maintained large (sometimes enormous) populations of managed colonies. As the comparatively small African population interacted with the much larger managed EHB populations, it seemed logical that the AHB would become less-and-less "African like" as it spread northward. Early in the invasion process, it was widely believed that there was unrestricted mating and genetic exchange between the EHB and AHB populations. As a result, the feral bee in the Neotropics was given the common name, the *Africanized* honey bee, because it was thought to be a hybrid that arose largely from European queens mating with African drones. The population advancing northward was considered to be a "hybrid swarm" that consisted primarily of swarms escaping from managed European apiaries in which the queens had mated to some extent with African drones.

The development of molecular techniques allowed for increasingly detailed studies of the genetic structure of the African population. As these studies progressed, it became clear that the hybrid-swarm and genetic dilution concepts were incorrect. Studies of mitochondrial DNA were particularly important in this research. Unlike the DNA in the nucleus of cells, which is inherited 50/50 from the mother and father, mitochondrial DNA is inherited solely from the mother. Thus, if the feral honey bees in the Neotropics were Africanized (hybrids of European queens and Afri- ➢

TOOLS OF THE TRADE

Deborah Smith

Genetic Tools Used to Study Honey Bees

Allozymes are slightly different forms of the same protein, caused by mutations in the genes that produce them. This technique, the first used in honey bee genetic studies, is relatively cheap and provides high quality information: it is possible to detect whether individuals have inherited the same form of a protein gene from each parent (in which case they are called "homozygotes"), or a different one from each parent ("heterozygotes"). Unfortunately, honey bees show very little variation in allozymes. Just two, called MDH and HK for short, show pronounced differences between African and European honey bees. For more than 20 years, these have been the "work horses" of honey bee population genetic studies. However, proteins are relatively delicate molecules, so it is necessary to use fresh or well frozen tissues. This means that samples collected into alcohol and samples in poor condition cannot be analyzed, and dry ice, liquid nitrogen and -80°C freezers are not always easy to come by.

The DNA molecule is tougher than protein, so useable DNA can be extracted from samples that have been frozen, stored in alcohol, or freeze dried, making collection of samples much easier, and a variety of rapid and simple methods to extract DNA from tissues are available. Many genetic techniques detect differences in DNA sequence directly. These techniques reveal much more detail about the genome than allozymes can, but require specialized, and sometimes expensive, chemicals and equipment.

Restriction enzymes: Many different restriction enzymes are commercially available, each recognizing a different sequence of 4, 5 or 6 nucleotides in a strand of DNA. A DNA sample is incubated with a restriction enzyme; if the restriction enzyme detects its specific recognition sequence of 4-6 nucleotides, it will bind to the DNA strand and cut it in two at that point. If samples of DNA from two individuals are compared, one may possess a recognition sequence (e.g., GAATTC) and the other may have a different sequence not recognized by the enzyme (e.g., GGATTC). If these samples are incubated with the restriction enzyme, fragments of different length will be produced: **restriction fragment length polymorphisms,** or **RFLPs**.

RFLPs are used extensively to compare and classify honey bee mitochondrial DNAs, which are relatively small. African, west European and east European mitochondrial DNA are each characterized by unique patterns of presence or absence of particular restriction enzyme recognition sites.

Microsatellites are a form of what is loosely called junk DNA. A microsatellite "gene" contains many repeats of short, simple sequences, such as AATAATAAT, or GCGCGC. These repeats are a sort of "tongue-twister" for the cellular machinery that replicates DNA in the living cell—it is easy to accidentally delete or duplicate copies. Thus, different individuals in a population may have, for example, 90, 100, 101, 102 or more copies of a short sequence strung end to end. Although they do not code for any product, microsatellites are useful to a population biologist because they are abundant, highly variable, and rapidly evolving, and can be used as markers to distinguish populations or even individuals. A typical animal genome may contain thousands or even millions of microsatellites "genes" scattered around the chromosomes. In a population of organisms, there may be dozens or more variants or alleles for each microsatellite "gene," each consisting of different numbers of repeats (a microsatellite "gene" is more properly called a locus, or place, on the chromosome, since genes code for a product or function; each copy number variant is a different allele). Unlike allozymes, with only two genes loci that show differences between African and European bees, hundreds of microsatellite loci have been identified in honey bees, many of which show differences between African and European populations in the type and frequency of alleles. It is expensive and time consuming to develop a system to detect microsatellite loci in a new study organism, but once developed they are relatively quick and cheap to use (assuming one has invested in the appropriate lab equipment).

DNA sequencing determines the actual sequence of nucleotides in a particular gene or other piece of DNA. This provides the most detailed information, but within a single species most nucleotides in a gene will be the same and differences will be few. It is also too expensive and time consuming to carry out on the large numbers of individuals typically used in population studies. However, a new technique is on the horizon that will make it possible to screen large numbers of individuals for a polymorphism revealed by sequencing studies.

Single nucleotide polymorphisms, or SNPs. The recently completed Honey Bee Genome Project (HBGP, carried out at Baylor College of Medicine) sequenced the entire genome of a U.S. "Italian" honey bee. Parts of the genome of the European bee were compared with DNA sequences from Africanized honey bees from Texas, and more than 1500 places were found where the European and African sequences differed by a single nucleotide – SNPs (pronounced"Snips"). Thus SNPs represent just variable nucleotides, distillated out of the vast volume of DNA sequence data generated by the HBGP. These markers will provide a valuable set of markers for analysis of the Africanization process, detecting genes associated with desirable traits, and many other studies.

Figure 4. *Introduction of* Apis mellifera scutellata *into Brazil began in 1956. Within 50 years this tropically adapted race of bee colonized most of South America and all of Central America. It entered the U.S. in 1990 through south Texas and is now permanently established throughout the southwestern states and southern California. Throughout its range in the New World, the AHB shows a remarkable ability to displace resident EHB colonies.*

can drones), then their cells should contain only European mitochondrial DNA. However, in the late 1980s Dr. Deborah Smith of the University of Kansas and Dr. H. Glenn Hall of the University of Florida independently discovered that 97% of all colonies sampled in many regions of Latin America contained only African mitochondrial DNA. There was only one possible explanation: Rather than expanding northward as European-matriline hybrids, the feral honey bees in Central and South America were composed almost entirely of colonies that arose from African queens. Colonies arising from European queens were virtually absent. Although EHB queens in managed colonies were mating with African drones, European-matriline (Africanized) swarms escaping from apiaries were clearly not surviving in the wild.

This phenomenon is particularly striking in areas such as the Yucatán, Mexico and southern Texas, where the invading front encountered huge populations of European colonies. When the AHB arrived in Mexico in 1985-86, the Yucatán Peninsula supported one of the highest densities of European apiaries in the world. Yet, within 12 years, only about 20% of the managed colonies were still European and today wild European-matriline colonies are virtually nonexistent in the area. Recently, Dr. Alice Pinto, working at Texas A&M University, found a similar situation in southern Texas. When the AHB arrived in 1990, southern Texas contained large populations of both managed and feral European honey bee colonies. But, by 2001 only about 10% of feral colonies were of European maternity, suggesting that colonies arising from AHB queens had displaced most of the wild European population. Interpreting the results from Texas is complicated, because at the time of the AHB's arrival *Varroa* mites and tracheal mites had eliminated much of the feral European population. Thus, it is difficult to know the extent to which the loss of EHB colonies resulted from parasitic mites or displacement by African bees. Dr. Pinto's work also revealed that about 30% of newly established colonies in southern Texas are of European maternity, suggesting that managed European colonies are producing a sizable number of swarms that move into the wild. However, the evidence suggests that these colonies do not survive over time, resulting in a largely African population.

Although studies of mitochondrial DNA have taught us a great deal about the genetics of the African honey bee, they do not give us a complete picture. Because mitochondrial DNA is inherited solely from the mother, it can only tell us about the contributions of African and European queens to a honey bee population. To fully understand the genetic structure of a population, we must also examine the DNA of the nucleus of cells, which contains genetic material from both the queen and drones. A large number of genetic techniques have been used to determine the degree to which honey bee populations contain African and European nuclear genes (see Box). Each approach provides slightly different information, but all have revealed a consistent pattern: relatively little EHB genetic material moves into the African population and even less persists over time. Throughout Latin America and southern Texas, EHB nuclear genes rarely account for more than 30-35% of the genome (i.e. the bee's set of chromosomes containing all of its genes) of wild colonies, and the percentage is typically far less. Furthermore, the frequencies of these genes decline over time. After several decades, EHB genes usually account for only 10-20% of the feral genome, suggesting that hybrid colonies do not survive in the wild. Thus, feral honey bee populations in invaded areas are composed almost entirely of AHB matrilines, with predominantly African nuclear genomes.

African and European honey bees do hybridize and some European genes have become incorporated into the feral AHB population. It is now unarguable that the introduced bee in the western hemisphere is no longer genetically identical to the ancestral *Apis mellifera scutellata* population in Africa from which is was derived. The fact that AHB will interbreed with the EHB and produce hybrid colonies is not surprising, because they are races of the same species. What is much more surprising is how *little* European genetic material has become permanently incorporated into the African population, given the initially small size of the introduced African population, the large European population, and nearly 50 years of interbreeding.

Thus, studies of both mitochondrial and nuclear DNA have given the same results: The AHB has retained a largely African genome as it colonized the western hemisphere, despite continued interbreeding with European bees. Indeed, throughout much of its range in the New World, the invading honey bee has remained essentially African in its nesting biology, foraging, swarming and absconding behavior. For these reasons, we refer to the descendents of *Apis mellifera scutellata* in the Americas as African bees. We reserve the terms "Africanized bee" and the "Africanization process" to refer specifically to colonies that arise from European queens mated with African drones. The ability of the AHB to retain its African nature and displace European honey bees is one of the most amazing and mysterious aspects of the invasion process. In the next article, we explore a variety of factors that may contribute to this phenomenon.

The African Honey Bee II

The Displacement Of European Honey Bees by African Bees In The New World

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In our first article on the African honey bee (AHB), we explained how differences in the behavior and nesting biology of *A. m. scutellata* allowed it to thrive and spread at a phenomenal rate throughout Latin America, largely displacing European honey bees in all colonized regions. In addition, despite repeated interbreeding between African and European bees, in most areas the AHB has not blended with the EHB. Rather, it has largely retained its African genetic make up. This means that European traits brought into the African population through matings with European bees are being lost from the population. The failure to incorporate European characteristics is at the heart of the success of the AHB in the Americas and the biological and economic impacts it creates. Thus, to fully understand the African bee invasion and the effects it may have in the U. S., it is essential to understand how it interacts with *and replaces* European bees.

There is no *single* factor responsible for the displacement of European colonies and the loss of European characteristics in areas where African bees immigrate. Rather, at least six different mechanisms contribute to varying extents to the loss of European patrilines (lineages of the drones) and matrilines (lineages of the queens). Some of these factors may be of greater importance in managed apiaries, whereas others play a stronger role in feral honey bee populations.

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***Figure 1**. A swarm of African bees. African colonies swarm more often than European colonies and quickly establish large wild populations, which helps them out compete European colonies. African colonies are also much more likely to abscond (abandon a nest site) and migrate during dearth periods. This increased colony mobility further helps to spread the AHB quickly throughout a colonized region.*

Swarming Behavior

One of the major differences between European and African honey bees is the rate of colony population growth. While European bees have been selected primarily for honey production and storage to survive longer, colder winters, African colonies have a greater emphasis on pollen collection and a more rapid conversion of pollen into brood. African bees devote two to four times as much comb area to brood rearing compared with European bees. The resulting higher growth rates allow for increased African swarm production (Fig. 1). In the Neotropics, African colonies can increase 16-fold per year, while maximum increases in wild European colonies in temperate areas are only three- to six-fold. Consequently, the density of African colonies can increase quickly in the wild, especially in regions with small populations of European honey bees. This in turn gives the AHB a numerical advantage that helps it out compete and displace EHB colonies.

Negative Heterosis in Hybrid Bees

A factor that has been proposed repeatedly to explain the loss of European traits from African populations is reduced fitness and survival of hybrid bees (Fig. 2). Several researchers have proposed that there may be incompatibilities between European and African genes that make it difficult for hybrid colonies to persist unless they are managed by humans. In particular, it is thought that European maternal and African paternal genes may be especially incompatible. This would help to explain the drastic loss of European matrilines in feral

Figure 2. *Worker, drone and queen honey bee. AHB/EHB hybrid workers and queens have lower metabolic rates than African bees. Also, the left and right fore wings of EHB/AHB hybrid workers are less symmetrical when compared to one another than are the wings of African workers. This may further reflect incompatibilities between African and European genes that negatively affect larval development. In combination, the physiological and developmental differences might make hybrid bees less competitive and less efficient at foraging, swarming and mating. As a result, hybrid colonies may not survive well in the wild, which would contribute to the loss of European traits.*

honey bee populations, despite repeated opportunities for "Africanized" European swarms to move from commercial apiaries into the wild. The reduced fitness in hybrids (referred to as **negative heterosis**) is a controversial topic among honey bee researchers, but there are two main lines of evidence to suggest that it occurs.

First, European/African hybrids have lower metabolic rates than "pure" African or European bees. This was first demonstrated for hybrid workers in 1993 by Dr. Jon Harrison of Arizona State University and Dr. H. Glenn Hall of the University of Florida. More recently, Dr. Harrison's lab has shown that hybrid queens also have lower metabolic capacities. The lower metabolic rates could reduce flying ability, which in turn could negatively affect foraging, swarming, and mating flights.

A second line of evidence for reduced fitness in hybrid bees comes from studies of the shape of the honey bee wing. Dr. Stan Schneider of the University of North Carolina at Charlotte and Dr. Gloria Degrandi-Hoffman of the USDA Carl Hayden Bee Research Center in Tuscon, AZ used instrumental insemination to create crosses between European queens and African drones (i.e. EHB/AHB hybrid workers), "pure" African workers (African queens mated with African drones), and "pure" European workers (European queens mated with European drones) and then raised them in the same colony. On the day that the workers emerged as adults, they were collected and the shape of the left and right forewing of each bee was compared using a mathematical process. The wings of hybrid workers were slightly less symmetrical that those of the pure African workers. The ability to build left- and right-side structures during an animal's embryonic development is genetically controlled. If the genetic programs for development are stable, then the two sides of an animal's body are more symmetrical. Conversely, if the genetic programs have less stability, then the animal often has more asymmetrical structures. Thus, the greater asymmetry in the wings of hybrid workers suggests that they have lower developmental stability than "pure" African bees, which may further indicate incompatibilities between European and African genes. In combination, the lower metabolic rates, possible developmental problems, and greater asymmetry of hybrid workers would contribute to the loss of European characteristics and might help to explain why hybrid colonies do not persist over time in the wild.

Mating Advantages for African Drones

When African bees colonize areas with resident European populations, queens will mate with both European and African drones (Fig. 3). However, matings with European drones decline over time resulting in a reduction of European patrilines in managed and wild colonies. There are several factors that may contribute to an African drone mating advantage in invaded areas. First, AHB colonies produce more drones than EHB colonies of the same size. Second, the more numerous AHB drones will drift into European colonies, which suppresses the production of EHB drones. Third, AHB colonies experience high rates of queen loss and the resulting queenless colonies rear large numbers of worker-produced drones. Fourth, there may be differences in mating-flight times that increase the chance of EHB

Figure 3. *AHB drones may have a mating advantage over EHB drones. African drones are more abundant, take mating flights at times that may increase their chances of mating with European queens, and will drift into EHB colonies and suppress the rearing of European drones. Also, even if queens mate with an equal number of AHB and EHB drones, they may preferentially use African sperm to fertilize their eggs. AHB drone mating advantages result in the rapid spread of African genes and the loss of European genes.*

queens mating with African drones, but decrease the chance of AHB queens mating with European drones. Finally, in a recent study, Dr. Gloria DeGrandi-Hoffman, Dr. Stan Schneider and Dr. David Tarpy of the North Carolina State University instrumentally inseminated queens with semen from an equal number of African and European drones and then monitored the number of AHB and EHB workers produced for six months. They found that significantly more AHB workers than expected were produced, which suggests that African sperm may have an advantage over European sperm, even if queens mate with the same numbers of drones of both types. An African drone mating advantage would result in the rapid loss of European paternal genes and may be an important factor in the AHB's ability to displace European bees.

African-patriline Advantages During Queen Replacement

European genes are also lost when colonies in invaded regions raise new queens. When a colony swarms or supersedes its queen, workers rear up to a dozen or more virgin queens (VQs) in specially constructed cells. Virgin queens are highly aggressive toward one another and battle to the death until there is a sole survivor, who then becomes the new laying queen of the colony. A virgin queen can eliminate her "rival" sister queens in two ways. First, she will attack queen cells and sting her rivals to death before they emerge. Second, emerged VQs seek out each other and attempt to sting one another. Workers often attempt to influence the interactions of virgin queens and may play a major role in determining which VQ will "win" the elimination process.

"To fully understand the African bee invasion and the effects it may have in the U.S., it is essential to understand how it interacts with *and replaces* European bees."

In colonies where queens mate with a combination of African and European drones, the colony population is made up of African- and European-patriline bees. During queen replacement, these "mixed" colonies will rear VQs from both patrilines. Recent research conducted by Dr. Gloria DeGrandi-Hoffman and Dr. Stan Schneider has shown that African-patriline VQs have a strong advantage during queen elimination.

Workers in mixed colonies raise similar numbers of African- and European-paternity queen larvae and devote equal care to both queen types. However, African-patriline VQs develop faster and emerge sooner, which may give them more opportunities to eliminate rivals confined in queen cells. In 70% of the mixed colonies studied, an African-patriline VQ was the first queen to emerge, and the first-emerging queen usually became the new laying queen of the colony.

African-patriline VQs also appear to be better fighters than their European-paternity sister queens (Fig. 4). African VQs kill significantly more of their emerged rivals then do the European VQs. African-paternity VQs also produce more bouts of "piping" (a high-pitched, pulsed sound) than European-paternity queens. The function of piping is unclear, but it is somehow related to fighting ability. VQs that pipe more survive longer, kill a greater number of rivals and are more likely to become the new laying queen. Workers in mixed colonies also interact with African-patriline VQs more often than they do with European-patriline VQs. In particular, workers perform more "vibration signals" on African VQs (Fig. 4). The vibration signal may promote queen fighting success, because queens that receive more signals kill more rivals and are more likely to survive the queen elimination process. In combination, because of their earlier emergence, greater fighting ability, piping activity and vibration signals received, African-patriline VQs are five times more likely to survive the queen-elimination process than are their European-paternity sister queens. When the African-paternity queens take mating flights in invaded areas, they will mate largely or entirely with AHB drones, resulting in increasing Africanization with each successive queen replacement event. A survival advantage for African-patriline virgin queens may therefore play a major role in the rapid loss of EHB characteristics in invaded regions.

Figure 4. *Two virgin queens fighting to the death (photo courtesy of Ken Lorenzen). When African and European-paternity VQs are present in the same colony, the African queens kill more of their rivals and produce more bouts of "piping" (a sound signal that may promote fighting success). African VQs also receive more "vibration signals," which consist of workers grabbing a VQ and rapidly vibrating their bodies up and down for one to two seconds (see drawing). Queens can be vibrated hundreds of times an hour and VQs that receive more signals survive longer and kill more rivals. In combination, the greater fighting ability, piping activity and vibration signals received results in African-patriline queens winning the rival elimination process and becoming the new laying queens of their colonies. This in turn results in the rapid loss of EHB characteristics.*

Dominance of African Genes

In invaded areas, European colonies headed by open-mated queens often exhibit African behavioral traits, even if they mate with a relatively high proportion of European drones. African genes may therefore be dominant for some characteristics. Dominance of African genes has been most thoroughly studied for defensive behavior. African honey bees exhibit a more intense nest defense behavior than European bees. Colonies composed of workers that are crosses between European queens and African drones show levels of defensiveness that do not differ from those of 'pure' African bees (i.e., workers having an African matriline and patriline). These findings suggest that African defensive behavior may be genetically dominant. Because a honey bee queen mates with an average of 12 drones, the level of defensiveness exhibited by a colony will depend upon the number of workers sired by African drones. As explained above, queens in invaded areas mate disproportionately with African drones and may preferentially use African sperm to fertilize their eggs, which can increase the level of defensiveness in a relatively short period of time. In addition to genetic dominance, colony defense behavior also is affected by alarm pheromone. When African-patriline workers defend their colony, they release more alarm pheromone and this could stimulate European-paternity workers that otherwise would not respond to the initial disturbance to exhibit defensive behavior. Thus, the presence of African-patrilines in

Figure 5. *An African swarm usurping a European colony. Usurpation swarms are small reproductive or absconding swarms that invade an EHB nest, replace the European queen, and take over the colony. Queenless EHB colonies and those with a caged queen are particularly susceptible to usurpation. In southern Arizona, annual usurpation rates can reach 20-30%, suggesting that usurpation is an important factor in the displacement of European in parts of the southwestern U.S. In southern Arizona, peak usurpation activity occurs from October - December, which corresponds to the absconding season for African bees in the Tucson basin.*

a colony could increase its defensive response even if many of the workers have European paternity.

Nest Usurpation

One of the most unique behaviors of African honey bees is their ability to usurp European colonies. Nest usurpation is a form of social parasitism where small African swarms invade European colonies and replace the resident queens (Fig. 5). Nest usurpation results in the complete and instantaneous loss of European matrilines and may partially explain the rapid loss of European characteristics in regions where African bees are established. Dr. Stan Schneider, Dr. Gloria DeGrandi-Hoffman and Dr. David Gilley of the Carl Hayden Bee Research Center in Tucson studied nest usurpation in southern Arizona and found that annual usurpation rates can reach 20-30%. Furthermore, there are strong seasonal patterns for nest usurpation. A minor peak of usurpation activity occurs during the spring-summer months, which corresponds with the reproductive swarming season for honey bees in southern Arizona. However, the greatest usurpation activity occurs during the fall-winter months, which corresponds to the period of seasonal absconding when African colonies in southern Arizona abandon their nests to search for better foraging conditions. Usurpation swarms may be reproductive or absconding swarms that are too small to successfully establish a nest, and instead adopt a strategy of invasion and parasitism. Nest usurpation may be an important part of the annual colony cycle in southern Arizona, and play an important, although seasonally and regionally variable role in the spread of African bees.

How African swarms find and invade host colonies is unclear. Pheromone cues associated with the presence and condition of a queen may be involved in the location of susceptible hosts. We have found that queenless colonies and those with a caged queen are particularly susceptible to invasion. This raises important concerns about the manner in which European colonies are maintained in Africanized areas. The most commonly used method to maintain European characteristics is to annually requeen colonies with new European queens. The old European queen is removed and the new queen is confined in small cage for several days and then released. We have found that if requeening is conducted during the swarming or absconding season, it may actually increase the chance of colonies becoming African, rather than help to retain European characteristics.

Queenless and caged-queen conditions are not the only factors that make colonies susceptible to usurpation. Queenright colonies can also be invaded, suggesting that cues other than those emanating from the state of a colony's queen are involved in host location. While some overt aggression occurs during the invasion process, pheromone signals may also help usurpation swarms gain entry into EHB colonies. Invading African swarms may produce pheromone signals that circumvent the defensive responses of EHB colonies and alter worker-queen interactions in a manner that contributes to the loss of EHB queens. However, the mechanisms that regulate nest usurpation represent one of the least understood aspects of the African bee invasion process.

Conclusions

In summary, there is an array of behavioral, physiological and genetic factors that contribute to the AHB's ability to displace European honey bee colonies in invaded areas. Throughout much of Latin America, these factors have helped to preserve the genetic structure of the African honey bee population despite repeated interbreeding with European bees. The available evidence also suggests that these same factors are contributing to the spread and success of the AHB in the southwestern U.S. However, what the African bee will do in the U. S. in the future is far from clear. In the final article of this series, we explore the past and possible future spread of the AHB throughout the U.S., the factors that may contribute to its distribution and the impacts it is likely to have on beekeeping and agriculture. BC

The African Honey Bee III

The African Honey Bee Has Arrived – So Where Do We Go From Here?

Gloria DeGrandi-Hoffman, Mona Chambers, Stan Schneider, David Tarpy, Deborah Smith

In the first two parts of this series, we discussed the history of African honey bees (AHB) in the New World and the biological and behavioral bases for their successful establishment. In this third and final part of the series, we address the practical aspects of the AHB migration into the U.S. We begin with a brief overview of the history of AHB in the U.S. and then discuss some behavioral features that might indicate that a colony is in the early stages of Africanization. We then review the current methods of determining whether or not a sample of bees is AHB. We also discuss the potential future range of AHB and how they might spread throughout the U.S., as well as address public perceptions of AHB, beekeepers and white boxes, and the role of the media. Finally, we provide some advice on what to do if you suspect that a colony is Africanized, including some guidelines concerning what beekeepers can do to prevent or reverse Africanization.

Stan Schneider received his PhD in Animal Behavior from the University of California at Davis and is now at the University of North Carolina at Charlotte. His main areas of research are honey bee communication and the biology and behavior of the African honey bee.

Gloria DeGrandi-Hoffman received her PhD from Michigan State and is now Research Leader at the USDA Bee Lab in Tucson. Studies include pollination, African honey bee genetics and biology.

Deborah Smith received her PhD in Neurobiology and Behavior from Cornell University and post-doctoral training in molecular biology at the Laboratory for Molecular Systematics at the University of Michigan. She is now at the University of Kansas, studying mitochondrial DNA analysis, honey bee biogeography and honey bee population biology.

David Tarpy received his PhD in Entomology from the University of California at Davis and is now the Extension Apiculturist in the Department of Entomology at North Carolina State University. His research interests address the biology and behavior of honey bee queens – using techniques including field manipulations, behavioral observation, instrumental insemination, and molecular genetics.

Mona Chambers is a Research Technician at the Carl Hayden Bee Research Center in Tucson, Arizona, and Coordinator of the Honey Bee Morphometrics Laboratory. She received her Bachelors degree from the University of Arizona.

The AHB in the U.S.

The African honey bee was first detected in the U.S. in 1990 in south Texas (Fig. 1). For the next three years, their population was confined to southern Texas. In 1993, however, the AHB was detected in Arizona when a dog was severely stung by honey bees. Over the following year, feral honey bees were sampled in the southern region of Arizona, and AHB were found across the state. In 1995, AHB were detected for the first time in New Mexico and southern California. AHB then spread northward in Arizona and New Mexico and, by 1998, they were detected in Nevada. The bees continued their migration northward in every state where they became established. By 2004, the bees had migrated through Texas and were detected in the southern most counties of Oklahoma.

For reasons that are yet unclear, the spread of AHB eastward out of Texas stalled in the Houston region for several years. One explanation for why the population did not expand east was that areas with more than 55 inches of yearly rainfall could not support AHB populations. The reasoning for this was that the higher humidity might enable *Varroa* mites to have greater survival rates in colonies so that most feral AHB would succumb to *Varroa* infestations. The propensity of AHB colonies to increase brood production in response to rainfall also was given as a possible explanation for the failure of AHB to expand eastward. Since Winter rains in east Texas are not followed by blooms on flowering plants, it was assumed that the AHB colonies would starve.

Despite their temporary inability to spread east of Texas, by 2005 the AHB expanded eastward from Houston. Most recently, the AHB has become established in Florida, western Louisiana, and in the southwest region of Arkansas. It remains unclear how Florida became populated, but it seems likely that the bees entered on cargo ships through commercial shipping ports.

How do I know if my bees are Africanized? Behavioral Attributes

Extreme nest defense has been the calling card of AHB; a stinging incident usually is the first indication that AHB are present. In such cases, an inordinate number of bees – usually from a colony in a structure like a shed or garage rather than a managed hive – sting an unsuspecting person or pet that inadvertently disturbed their nest. Stinging incidents from confirmed AHB colonies tell us several things about the local feral honey bee population. First, the colony is probably not the only AHB nest in the

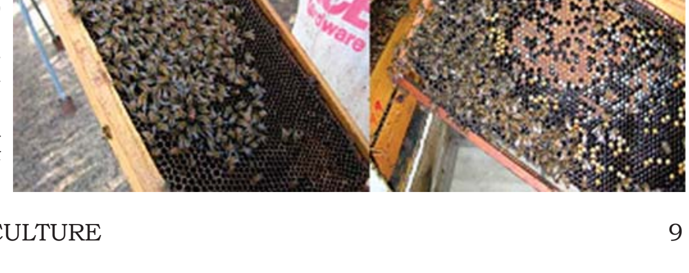

Figure 2. *Comparison of European (left) and African bees (right) on a brood frame. Notice how the European bees cover the brood, while the African bees leave it exposed.*

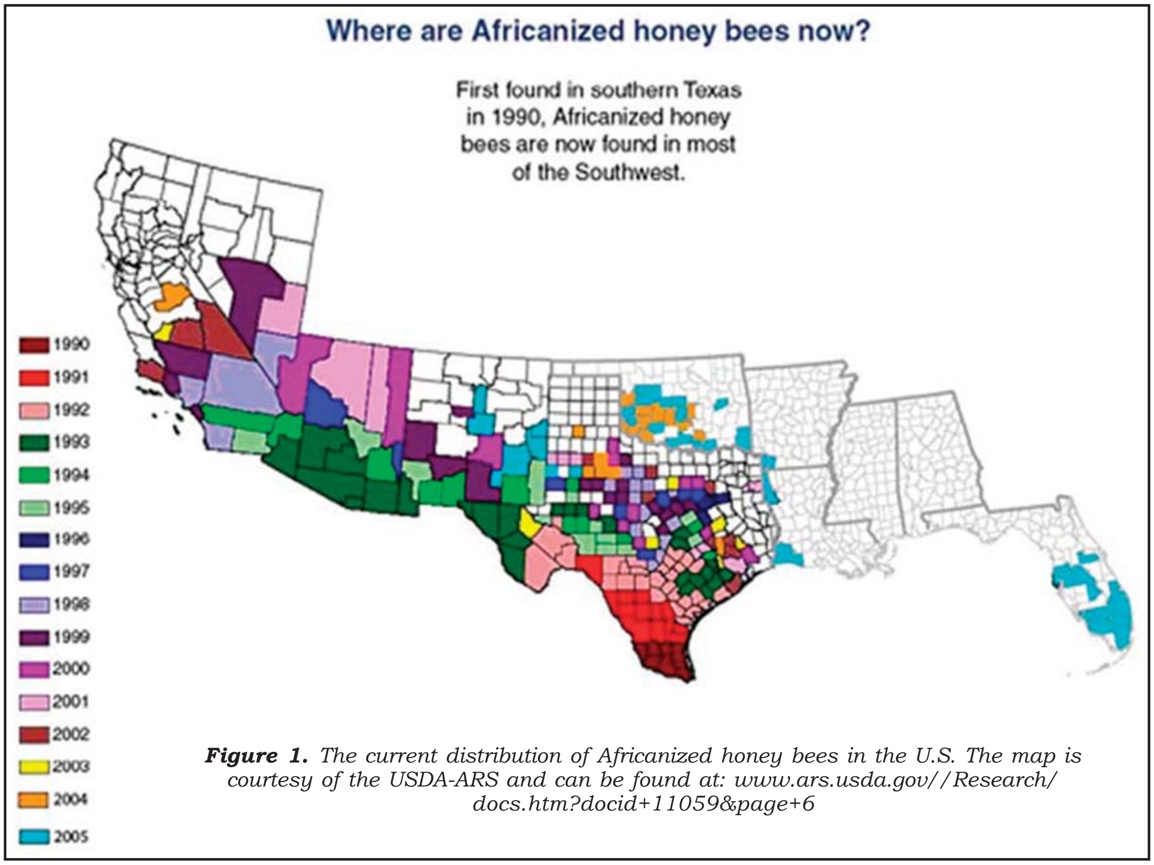

Figure 1. *The current distribution of Africanized honey bees in the U.S. The map is courtesy of the USDA-ARS and can be found at: www.ars.usda.gov//Research/docs.htm?docid+11059&page+6*

area. The drones that mated with the queen likely derived from other AHB colonies. The stinging incident also indicates that colonies of AHB in the area are numerous and successful enough to rear drones. Finally, there are sufficient numbers of AHB colonies so that queens are mating with enough drones that workers are expressing nest defense characteristics that are unlike those of EHB colonies.

While the general public might notice that a colony is Africanized only when a severe stinging incident occurs, beekeepers might notice more subtle changes in colony behaviors that could indicate lower levels of Africanization. First, AHB workers may persistently fly into the helmet or mesh of the bee veil when a colony is first opened. Second, AHB workers will move to the corners of the comb and expose the brood when a frame is removed from a hive (unlike EHB, which usually remain on the frames and cover the brood) (Fig. 2). Populous AHB colonies will also form clumps of bees on the sides of frames and attach to surfaces against which the frame is set. Third, AHB colonies often construct a single queen cell on a honey frame, particularly in populous colonies. Finally, beekeepers might also see workers with shiny black abdomens that look like small virgin queens (Fig. 3). Dr. Gloria DeGrandi-Hoffman of the USDA Carl Hayden Bee Research Center in Tucson has recently demonstrated that these shiny black bees are "intermorphs" that have characteristics of both workers and queens. How and why intermorphs are reared in colonies remains unclear, but they are signs that AHB colonies are in the area.

Perhaps one of the most extraordinary behavior exhibited by African bees is invading and usurping colonies of EHB (see Part 2: **The Displacement of European Honey Bees by African Bees in the New World)**. While the behaviors described above are characteristic of Africanized bees (i.e., those arising from European matriline queens mated with African drones), nest usurpation behavior appears to be associated with "pure" African bees (i.e., African matriline queens mated with African drones). Researchers have yet to collect a swarm that invaded an EHB colony that does not have African mitochondrial DNA, which means the workers in the swarm were produced by an African queen (see Part 1: **The African Honey Bee: A Case Study of a Biological Invasion**). Usurpation swarms can look like a clump of bees hanging out at the entrance of a colony that is over crowded especially during the warmest part of the day. However, upon closer examination, bees might be seen fighting and dead bees might be found at the colony entrance or on the ground around the hive. The definitive characteristic of a usurpation swarm is that one or more queens are found in the center of the swarm (Fig. 4). Usurpation swarms can present serious problems to managed colonies: if queens are not marked, and the swarm is misdiagnosed as simply an overcrowded colony, an EHB colony will be invaded and will immediately become converted to an African colony.

Methods of Identification

Despite behavioral differences, the only way to be certain if bees are Africanized is to have them analyzed by an objective test. There are two general categories of tests to determine Africanization: morphometrics and DNA analysis. We have previously reviewed the

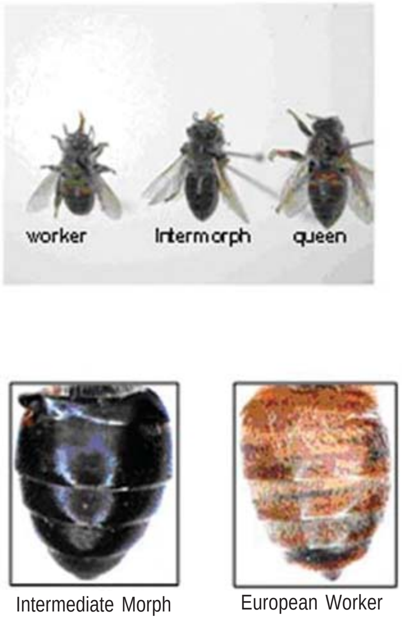

Figure 3. *Examples of worker bees, intermorphs from African colonies and an African queen; note the differences between the abdomens of a European worker and an intermediate morph. Notice the differences in the amount of branched hairs on the worker abdomen, and the absence of them on the intermorph.*

various DNA analyses that are currently available to distinguish AHB from EHB (see Part 1: **The African Honey Bee: A Case Study of a Biological Invasion**). Here, we briefly describe the morphometric analyses that are commonly used to detect AHB.

Any analysis that differentiates groups of bees based upon their morphology is called morphometrics. Morphometric analysis uses a system of measuring morphological characteristics to determine honey bee type by a statistical technique called discriminant analysis. The technique determines whether bees are EHB or AHB based on body measurements. While they are difficult to distinguish with the naked eye, AHB workers are, on average, smaller than EHB workers. By carefully and accurately measuring different body parts, researchers can determine the likelihood that a particular sample of bees are AHB or EHB (see box on next page).

The benefit of morphometric analyses is that they are relatively inexpensive and can be accomplished fairly rapidly (particularly by using a protocol called FABIS that measures only the wings). A limitation of morphometrics, however, is that it does not provide information on whether Africanized traits are derived from the queen (matriline), the drones she mated with (patriline), or both. Knowing whether a colony has African matrilines or patrilines provides insights into the state of the AHB population in the area. If the bees have EHB matrilines and African patrilines, the invasion is probably in the early stages, but if African matrilines are detected, the invasion probably has been ongoing for some time. While DNA analyses can provide this valuable genetic information, they can be relatively expensive and time-consuming compared with morphometric tests.

Many states now perform morphometric or genetic analyses of bee samples. In addition, the USDA Africanized Honey Bee Identification Laboratory housed in the Carl Hayden Bee Research Center (CHBRC) in Tucson, Arizona will analyze honey bee samples for state apiary inspectors, maritime port authorities, and other USDA agencies such as Animal Plant and Health Inspection Service (APHIS) and Plant Protection and Quarantine (PPQ). Samples for morphometric analysis should be collected by placing 30 to 50 adult honey bees in a small container or vial containing just enough 70% ethyl alcohol to cover the bees. The name of the collector, collection date, and location need to be included with the sample.

What beekeepers can do to minimize the Africanization process

Be vigilant

1. Mark all queens; **no** exceptions.
2. Regularly check hives for unusual external clumping of bees, as these may be parasitic AHB swarms.
3. **Requeen** any colony that is unacceptably defensive or contains an unmarked queen; use only queens from a known EHB source.
4. Inspect hives for behavioral signs of AHB, particularly after they are transported in and out of known AHB areas.
5. Send suspect samples to authorities for morphometric or genetic testing; place 30 to 50 adult bees in a small container, fill with enough 70% ethyl alcohol to cover the bees, and label with contact information, collection date, and location.
6. In an Africanized area, attempt to make all potential AHB nesting sites "bee tight"; avoid storing empty beehives outdoors.

Be responsive

1. Keep AHB incidents in an appropriate context during media interviews. **DO NOT** include box hives in filming about stinging incidents, as this promotes a negative perception of **all** honey bees. **DO** include managed hives in filming about the benefits of beekeeping.
2. **Avoid speculation** and answer only those questions to which you know the answer. Parts 1 & 2 of this series of articles were designed to provide the background information necessary for explaining the AHB to the media and public.
3. **Don't sensationalize** defensive behavior by using terms like "aggressive" or "vicious."
4. Make clear the relative risk of the AHB; the number of deaths each year from stinging incidents are far fewer than dog attacks, food allergies, even lightening strikes.

Be proactive

1. Emphasize that beekeepers are on the front lines of defense—**beekeepers are part of the solution**, not the problem.
2. Be a good neighbor and inform anyone who may be in close proximity to your hives; educating them about the benefits of honey bees and the relative risks of AHB should lessen their fears.
3. Establish and maintain lines of communication between local beekeepers, first responders, and local officials.
4. Make people aware of the distinction between yellow jackets and bees, as many people mistake wasps for honey bees. Increased public awareness of the different types of stinging insects will reduce the number of erroneous AHB reports.

Figure 5. *European queen introduced in an Africanized colony behind a push-in cage. The arrow is pointing to the marked queen.*

the AHB cannot survive a prolonged Winter, which will slow or prevent its movement into northern states. However, we now know that feral AHB populations are established in areas above 5000 ft in Arizona and New Mexico and can survive through the Winter. Thus, at this point we do not know the extent to which the AHB will spread in the U.S. or how quickly the invasion process will proceed. Furthermore, we cannot predict in which regions the AHB will become permanently established or be a "seasonal visitor," in which colonies may migrate in during Spring and Summer but die out during the Winter. The ultimate distribution of the African bee in the U.S. will depend on a combination of its inherent ability to spread and survive in new areas and human assisted movements that might transport the bee past barriers that otherwise would halt its progression.

What should beekeepers do to minimize Africanization?

The first step in minimizing Africanization is for beekeepers to be vigilant. Queens in colonies that are transported into or reside in Africanized areas should be marked and their hives should be inspected on a regular basis (see Box 2). Any hive that contains an unmarked queen should be requeened with a new queen of known European descent. The new queen should be introduced behind a push-in cage over emerging brood (Fig. 5). AHB workers often kill EHB queens introduced in shipping cages. The push-in cage should be moved every two to three days, and this should be repeated at least three times before releasing the queen. After the queen is released, the colony should be examined every 10-14 days for six weeks to ensure that the introduced queen has been accepted, and to destroy any supersedure queen cells that the workers may construct.

Another important way that beekeepers can minimize the impact of the AHB is to respond to reports of AHB in an appropriate and timely manner. In many areas of the U.S. where AHB have already become established, the initial detection occurred from a stinging incident. It is likely that the same will happen in newly established areas. These events are sure to make the local TV news and grace the front pages of local newspapers. Such incidents are indeed serious, but they can be easily sensationalized. If they are, the public may become fearful of all bees and the beekeeper might be perceived as part of the problem. To mitigate the damage from sensationalized news reports, beekeepers and beekeeping organizations need to have sound and coherent information concerning the AHB. They must also adopt strategies to work effectively with the media. After a stinging incident, a reporter might want to interview a local beekeeper. If this occurs, avoid conducting interviews with managed beehives in the background, because the image conveys to the public that all managed colonies contain AHB. Instead, the background for news stories about AHB should have a structure, such as a carport or shed, where an AHB colony is or can become established. Also, avoid having reporters film bees on frames, as a large number of bees may be taken out of context when the story appears. Instead, emphasize the importance of honey bees to U.S. agriculture, the benefits of local honey and pollination, and the relative risks of bee stings.

Finally, beekeepers can help by being proactive. The main message that beekeepers need to communicate to the public is that managed colonies are part of the solution and not part of the problem. Because beekeepers manage the genetics of their colonies, they are ensuring that genes associated with reduced nest defense behavior remain in the honey bee population. Thus, managed colonies are the genetic buffer between the public and the feral AHB population. By projecting the AHB issue in an accurate and rational manner, beekeepers will be able to adjust to the changing landscape of beekeeping in the country and address the AHB in the coming years.

General Conclusions

In closing, the goal of this series of three articles was to provide beekeepers with the background information and management tactics necessary for coping with the AHB, and for handling the media and public attention it invariably generates. The African honey bee is now a permanent resident throughout the southwestern states and California, and seems destined to become part of the landscape in the southeastern states as well. As has occurred throughout Latin America, the AHB is likely to displace feral EHB colonies and have a substantial impact on the management of European colonies throughout the southern tier of the U.S. The ultimate distribution of this highly invasive honey bee cannot be predicted at present, and only time will tell the economic and public welfare impacts that it will have in our country. The only certainty is that the AHB is now a feature of U.S. beekeeping. But, just as we have learned to cope with introduced mites and beetles, we will also learn to cope with this latest challenge to beekeeping – and perhaps in the long run we will realize benefits from doing so. Studies in Mexico and Central and South America have suggested that under some conditions the AHB is a highly efficient pollinator, and might be more resistant to *Varroa* mites than the EHB. Whether these benefits will be realized in the U.S. remains to be seen. Nevertheless, by being prepared and managing our colonies effectively, we can ensure the continuation of a viable beekeeping industry in the U.S. that controls, and perhaps some day incorporates, this new arrival. BC