THE IMPACT OF MANAGED EUROPEAN HONEY BEE COLONIES ON THE COMPOSITION OF DRONE CONGREGATION AREAS IN REGIONS WHERE AFRICAN HONEY BEES ARE PRESENT

By

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To my loving family who has supported me through all of life’s inspirations

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

9-ODA	9-oxo-2-decenoic acid
cd	Cordovan
BP	Base Pair
DCA	Drone Congregation Area
DNA	Deoxyribonucleic Acid
FDACS	Florida Department of Agriculture and Consumer Services
mt	Mitochondrial
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

Abstract of Thesis Presented to the Graduate School
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Following introduction into Brazil, African honey bees (*Apis mellifera scutellata)* rapidly spread through the Americas, dramatically changing the South American beekeeping industry. I aim to determine if the management of European honey bees (*A. m. ssp*.) can mitigate the impact of African honey bee hybrids in the southeastern United States. I conducted a series of studies to access: (1) the frequency of European colony takeover (i.e. usurpation) by African matrilines, (2) the reliability of a phenotypic mutation, cordovan, as a marker of European ancestry, and (3) the extent to which the management of European colonies can modify the proportion of African matriline drones present at nearby drone congregation areas (DCAs). These studies require that DCAs be identified. Consequently, I developed an efficient method for locating DCAs. Results indicate that: (1) colony usurpation is a rare event and does not significantly contribute to the “Africanization” of European apiaries, (2) the cordovan phenotype is associated with both African and European mitotypes and therefore cannot be used as a reliable indicator of genetic ancestry, and (3) the management of European honey bee colonies can dramatically decrease the proportion of drones with African matrilines at nearby

DCAs. Additionally, the results support my assumption that a hybridization zone has developed in central Florida. Further experimentation in this region promises to offer valuable insight into the dynamics of African bee expansion into areas where European bee populations predominate and possible mechanisms contributing to the successful preservation of African phenotypes and matrilines in the population.

CHAPTER 1
LITERATURE REVIEW

African Honey Bees in the Americas

In the tropics, European-derived, temperate-race honey bees, *A. m. ligustica* Spinola, *carnica* Pollmann, *mellifera* Linnaeus, *causcasia* Pollmann, and *iberiensis* Engel (Sheppard 1989b, a), produce small honey yields. In an effort to optimize honey production in the tropics, African honey bees, *A. m. scutellata* Lepeletier (formerly adansonii (Ruttner 1976)), were imported to Brazil from Pretoria, South Africa in 1956. In the following year, a portion of the original African queens were introduced into the feral honey bee population near Rio Claro, São Paulo, Brazil (Kerr 1967). Subsequently, African-derived honey bees have rapidly colonized South, Central, and southern North America at an annual expansion rate of up to 300-400 km a year (Taylor 1977, Buchmann 1982, Hall and Muralidharan 1989). This rate dramatically declined when the bees arrived in the temperate regions of North America.

Beekeepers in the American tropics have adapted to the management of African-derived honey bees due to the exceptional performance of the bee. Subsequently, apiaries were forced into isolated, remote locations and hobbyist beekeeping became almost nonexistent (Michener 1975). However, European-derived honey bees will remain the primary subspecies managed by beekeepers in the United States (Taylor 1985). The ability to limit hybridization of managed European-derived honey bees with feral African-derived honey bees is of tremendous public concern due to animal and human safety issues. In the United States, efforts to minimizing hybridization of managed European-derived honey bees are very important.

Although it is recognized that African-derived honey bees in the Americas are not genetically identical to those in their native range (Schneider et al. 2004c), for conciseness I will refer to both African- and European-derived honey bees in the Americas as African and European, respectively.

Persistence of African Genetics

African honey bees promptly populate an area with densities of up to 120 feral colonies per square mile within 2-3 years of arrival in a new territory (Taylor 1985). Moreover, behavioral and molecular evidence suggest there has been little introgression of European genetics into the feral African honey bee population as the African population has expanded (Hall 1992a, Lobo and Krieger 1992, Schneider et al. 2004c). Typical African behavioral characteristics, such as aggressive behavior, increased swarm production, and propensity to abscond, are predominant in managed and feral colonies within 2 years of African bees reaching an area (Taylor 1985, Sheppard et al. 1991). Furthermore, sampled feral populations in Mexico had no European mitochondrial DNA (mtDNA ;Hall and Muralidharan 1989; Smith et al. 1989) after the arrival of African bees into the area. The lack of European mtDNA suggests an African population that has expanded through unbroken African matrilines, indicating that any introgression that has occurred is unidirectional, with the mating of virgin African queens to European drones being the only hybrids contributing to the expansion of the feral African population (Smith et al. 1989; Hall 1990).

Managed European colonies do become “Africanized” when European queens mate with African drones. However, monitoring managed apiaries most likely exaggerates the impact of hybridization on the total population (Hall and Muralidharan 1989). These crosses contribute little to the success of the African population and may

even be a dead end process (Hall 1992b). Several theories, including hybrid dysfunction, increased developmental stability, pairwise breeding, temporal isolation, increased drone production and drift, increased reproductive rate, and African patriline advantage have been developed to explain the predominance of African traits as the population expands into new areas hosting large European bee populations (Table 1-1).

Hybrid dysfunction

The disparity in gene flow between African and European populations in the Americas could be partially explained by the existance of dysfunctional hybrid offpring. Harrison and Hall (1993) found that pure African workers had higher mass-specific metabolic rates than pure European workers. Furthermore, hybrid offspring African queens mated to European drones and European queens mated to African drones had lower mass-specific metabolic rates that pure European crosses, with hybrids of the European matriline having the lowest mass-specific metabolic rates of all crosses. This increase in metabolic rate suggests that pure African honey bees have a greater flight capacity than European or hybrid bees. Which may play a role in the African honey bee's enhanced colony defense, foraging, and dispersal. Moreover, hybrids with a European matriline preformed worse than hybrids with an African matriline. Indicating that the European matriline is a selective disadvantage in the feral population (Harrison and Hall 1993).

Increased developmental stability

Variation in the developmental stability of African, European, and hybrid individuals has also been documented. Schneider et al. (2003) found evidence of differential developmental stability between pure African, European, and hybrid workers in the form of reduced fluctuating asymmetry of wing shape. African workers had the

least amount of asymmetry when compared to pure Europeans and both forms of hybrid. Unlike the metabolic rate findings, hybrids in this study had intermediate levels of wing asymmetry compared to that of the pure African and European crosses. These data did not indicate the presence of hybrid dysfunction. However, African honey bees were found to have increased developmental stability where compared to European and hybrid bees, that may result in increased fitness in African individuals.

Pairwise breeding

In addition to hybrid dysfunction, pairwise breeding has been hypothesized to contribute to the preservation of African genetics in the feral population. Kerr and Bueno (1970) discovered evidence that African and European queens were more likely to mate with drones of their own subspecies. They sampled brood 25-30 days after queen mating and determined that African queens mated with African drones 58% of the time, while European queens mated with European drones 64% of the time. Moreover, Taylor (1999) noted that in areas with abundant European drones, African queens mated primarily with African drones (96-100%). This tendency towards like species mating would further promote a feral African population with little introgression of European genetics. Pairwise breeding may be a direct preference or a result of other physiological factors. The only apparent difference in mating behavior Kerr and Bueno (1970) identified was that African drones have a faster ejaculation speed. They suggested this may desynchronize copulation between African and European reproductives by preventing European queen from receiving sperm from some African drones.

Temporal isolation

Temporal isolation of African and European mating flights, in the form of time of year and/or time of day, may further increase the likelihood of a queen pairing with a

drone of her own subspecies. In subtropical Mexico, differential drone concentrations at drone congregation areas (DCAs) were noted based on season (Quezada-Euan and May-Itza 2001). Higher frequencies of African drones were present in March and April. May had almost equal African and European proportions and European drones were the predominant subspecies in June and July. Moreover, Taylor and Spivak (1984) theorized that there might be partial time-of-day isolation between the subspecies. Although this has not been confirmed for reproductive castes, European workers in Brazil are most active from 830-1030 whereas African worker activity peaks in the afternoon (Michener 1975).

Drone production and drift

Together with the propensity of queens to breed with drones of their corresponding subspecies and potential temporal isolation, African colonies produce high numbers of drones even when resources are limited (Rinderer et al. 1987). In contrast, drone production of European colonies is dependent on resource availability, and very few drones are produced in times of resource scarcity.

Furthermore, African drones are prone to drift between colonies, exhibiting a tendency to enter European colonies more so than other African colonies (Rinderer et al. 1985). This is a reproductive advantage for African bees as there is a linear relationship between the number of migratory drones a colony is hosting and the number of drones that colony will produce. Each additional migratory drone gained by a colony suppresses drone production of the host colony by 0.65 drones (Rinderer et al. 1987). Conversely, the loss of a migratory drone by its parent colony increases the parent colony's drone production by 0.65 drones (losing a single migrating drone results in the production of 0.65 more drones in the parent colony and a reduction by 0.65

drones in the colony receiving the migrating drone – thus favoring the African parent colony a the DCA by 1.3 drones).

The African honey bee's propensity to produce drones earlier in the season and in times of resource scarcity, combined with the suppression of European drone production, could limit European drone production so severely that a small number of African colonies could produce the majority of drones in the reproductive population (Rinderer et al. 1985, Rinderer et al. 1987). In fact, Rinderer et al. (1987) found the majority of drones in European colonies to be African in Western Venezuela, further supporting the drift theory.

Reproductive rate

African colonies further dominate the reproductive population by generating more virgin queens than European colonies. African honey bees swarm more frequently and over a longer season than European honey bees (Winston 1979). The typical European colony seldom swarms its first year and swarms only once in the spring of each subsequent year, with an average annual production rate of 0.92 swarms per year (Taylor 1977). In contrast, African colonies have been known to create swarms throughout the year (Taylor 1977), generating smaller swarms every 50-90 days that travel great distances to new nest sites (Winston 1979). These swarms not only expand the feral African population into new territory but also increase the regional population size further increasing the prevalence of African genetics in that area.

African patriline advantage

Despite the factors detailed above, in the event that an European queen mates with both African and European drones, there is a developmental advantage for her daughter queens that have an African patriline (1998). When honey bee colonies

produce queens, several daughter queens are reared to adulthood simultaneously. In general, the first queen to emerge will chew holes into the sides of the cells of the developing queens, prompting the workers to abort the queens. Daughter queens with African patrilines develop faster and emerge before those with European patrilines (Degrandi-Hoffman et al. 1998b), thus giving African-patriline queens a clear competitive advantage. Moreover, African-patriline virgins have increased fighting success within the colony and were observed to pipe more frequently and kill more rivals than virgins with a European-patriline in the same colonies (Schneider and Degrandi-Hoffman 2003). It is hypothesized that African-patriline superiority may be an integral component of the asymmetrical nature of gene flow between African and European populations (Schneider et al. 2004c).

Hybridization Zones

Studies assessing the reproductive advantages of African Honey bees have occurred in tropical areas, such as in Brazil (Rinderer et al. 1985) and Southern Mexico (Hall and Muralidharan 1989), where European honey bee performance is suboptimal. However, a hybridization zone will develop as the northern and southern African migratory fronts approach more temperate climates where European honey bees are better suited (Taylor 1977).

European honey bees are adapted for temperate environments and survive long harsh winters by forming a thermoregulatory cluster and feeding on stored honey (Ratnieks 1991). Unlike European honey bees, African honey bees are adapted for semi-arid, sub-Saharan environments (Sheppard et al. 1991) and clustering has not been documented. Additionally, African colonies have small honey stores as a result of rapid reproductive swarming, (Michener 1975). Rather than overwinter in an area with

little or no resource availability, colonies abscond until they find an acceptable site or die (Michener 1975). African colonies migrate beyond their overwintering limit during warmer periods, but those colonies die or migrate back within their climactic limits during the colder months (Taylor 1985).

African honey bees already have reached this limit at the southern edge of their distribution in Argentina and Uruguay. In the Argentinian hybrid zone, a significant amount of introgression has occurred, and both European and African mitotypes are associated with a wide range of genetic markers and morphological characteristics (Sheppard et al. 1991). Furthermore, the predominate mitotypes behind and beyond the African overwintering border have remained predominantly African and European respectively (Sheppard et al. 1991), demonstrating an area of European advantage beyond the overwintering limit of the African population.

In South America, African honey bees appear to be limited to ecotypes in which the average high temperature in January (Argentinian summer) is 19°C (Taylor 1985). By applying this temperature limit to the northern expansion, it has been speculated that the migratory border, and therefore the hybrid zone in North America, will develop around Clinton, North Carolina (Taylor and Spivak 1984).

However the African population does not appear to be advancing as far into the United States as predicted. Pinto et al. (2005) characterized the nuclear and mitochondrial frequencies of African and European honey bees over 11 years as African honey bees expanded into Texas. Their results describe a hybridized population where both African and European subspecies have made substantial genetic contribution, indicating the formation of the northern hybridization zone. Moreover, the African

expansion has been stable in the area of Orlando, Florida since 2005 (personal comm. David Westervelt, Florida Department of Agriculture and Consumer Services (FDACS) Apiary Division Assistant Chief). Therefore, other climatic factors such as average rainfall, total available water, and periods of dearth may play a role in limiting the African population.

Africanization

The dynamics of African and European subspecies in the feral population and in managed European honey bee apiaries are most easily conceptualized as two distinct events. As discussed above the feral African population has expanded throughout the Americas with little-to-no contribution by European queenlines. However, when the feral African population exists in regions where European queen lines are actively maintained in managed apiaries, the 'Africanization' of European colonies does occur (Hall and Muralidharan 1989; Smith et al. 1989). There are three mechanisms by which European apiaries become Africanized; usurpation, beekeepers collecting feral swarms and colonies, and hybridization of European queens (Table 1-2).

In the first mechanism, an African swarms, containing a mated queen, usurps a European colony by killing the European queen and introducing their own African queen as the laying queen of the colony (Michener et al. 1972). Usurpation is not well understood and has received little study (Schneider et al. 2004c). Moreover, the recorded annual frequencies of usurpation vary from 0-30.3% (Camazine 1986; Danka and Rinderer 1988; Danka et al. 1992; Vergara et al. 1993; Clarke et al. 2002; Schneider et al. 2004a; Schneider et al. 2004c). Usurpation occurs quickly and can be difficult to distinguish from a colony that has naturally reared a replacement queen (Taylor 1985). However, dramatic genetic change occurs in the event of colony

usurpation (Danka and Rinderer 1988). In fact, in this scenario no hybridization occurs and the colony is still truly African. Similarly, by the second mode, when beekeepers collect feral colonies and swarms in regions hosting an established feral African population, African colonies can be introduced into the European apiary (Michener 1975, Taylor 1985).

The third mode by which European apiaries become Africanized is through virgin European queens mating with African drones, producing hybrid Africanized offspring (Taylor 1985). Although European mtDNA does not contribute to the expansion of the African population (Hall 1992b), pairings of European queens with African drones do occur. In southern Mexico, managed European colonies were sampled fifteen months after the arrival of African Honey bees. Despite having European mtDNA, these colonies had a high frequency of African nuclear markers (Hall 1990), thus revealing that several generations of European queens had mated to African drones.

The term ‘Africanization’ implies that European honey bees are becoming more African. Of the three mechanisms described above hybridization of European queens with African drones is the only scenario in which truly ‘Africanized’ honey bees are produced. In regions of the United States where feral African populations exist, the management of European honey bees should focus on methods to minimize the chances of European queens mating with African drones.

Honey Bee Mating

Honey bee queens are polyandrous, mating with multiple drones in flight, during a series of flights that occur in the first weeks of her adult life. Numerous experiments have been conducted to determine the typical number of drones with which a virgin queen breeds. Estimates based on semen volume in the queen reproductive tract

(Woyke 1960) and genetic analysis of offspring (Jensen et al. 2005) both suggest that queen most often mate with 7 to 20 drones, with 11.6-12.1 matings being the average (Tarpy et al. 2004).

Drone Congregation Areas

Mature drones make daily flights to reproductive sites called drone congregation areas (DCAs). These DCAs persist throughout the reproductive season in the same locations and recur for many years (Ruttner 1985). In fact, drones of colonies brought to an area from great distances away immediately locate the regional DCAs (Tribe 1982).

Drone congregation areas were first observed in 1958 (Jean-Prost 1958), and comparatively little is known about the determination of DCA locations considering our advances in knowledge of other aspects of honey bee biology since that time. It generally is believed that drones leave their colony flying towards a depression in the horizon (Ruttner 1966) until they reach a vertical relief such as a tree line or building (Zmarlicki and Morse 1963, Strang 1970). Tribe (1982) suggested the locations of DCAs are based primarily on wind, stating that drones typically fly upwind until encountering a turbulent area that could be caused by buildings or trees. Thus, DCAs typically occur at breaks in the horizon like openings in forest areas, and along tree lines in fields (Ruttner 1985).

Formation of a DCA is not dependent on the drone population size or the presence of a virgin queen (Strang 1970). In fact, drones are more attracted to the location of the DCA than the virgin queen herself. Drones start their reproductive flights and assemble at DCAs about an hour prior to virgin queens beginning their mating flights. Moreover, Ruttner (1985) demonstrated that drones will stop following a tethered queen once she is moved beyond the boundary of a DCA.

Once a virgin queen enters a DCA, a drone comet of 20 to 41 drones will form and peruse her about 15 to 30 m above the ground (Gary 1963). Queen mandibular pheromone, specifically 9-oxo-2-decenoic (9-ODA), stimulates initial orientation of drones to the virgin (Gary 1962), but pursuit of the queen is primarily visual once she is within range of the drones (Gary 1963, Van Praagh et al. 1980, Gries and Koeniger 1996). In fact, drones have been observed to form small comets and chase other insects and animals in the DCA when queen mandibular pheromone is present. Surprisingly, there is very little physical contact between the drones in the pursuing comet (Gries and Koeniger 1996), and it is suspected that the queen mandibular pheromone may play a role in drones distinguishing the queen from other drones (Van Praagh et al. 1980).

Mating Flights

Drones prefer DCAs closer to their home colony. Few fly distances greater than 1.5- 2 km to a DCA (Tribe 1982, Rowell et al. 1992), and most common distance traveled is 0.5 km or less (Rowell et al. 1992), with a recorded maximum flight distance of 7 km. The drone's preference of closer DCAs leads to a concentration of nearby colony genetics and may be relevant in a virgin queens strategy for DCA selection (Koeniger et al. 2005).

Virgin queens fly further to a DCA than drones from the same apiary to avoid sibling breeding events (Rowell et al. 1992). Due to the haplo-diploid sex determination system in honey bees inbreeding results in fertilized individuals that are homozygous at the sex loci. These homozygous individuals dramatically decrease the productivity of a colony by developing into nonviable drone brood rather worker brood (Mackensen 1951).

Jensen et al. (2005) determined queens rarely mate with drones from the same apiary and half of the pairings occurred at mating distances (the cumulative distance from the queen's colony to the drone's colony) >2.5 km. Supporting observations made by Woyke (1960) in which queens housed in apiaries hosting large numbers of drones had no difference in mating efficiency when compared to queens house in an apiary 3 km from any drone source.

Population Structure of a DCA

The drones present at a DCAs are representative of the regional reproductive population because colonies that do not produce drones are unlikely to swarm (Moritz et al. 2003, Kraus et al. 2005). To date, reports of the number of colonies contributing drones to an individual DCA vary greatly. In Germany, one DCA was found to represent 238 colonies, although the DCA was located in close proximity to a large, managed apiary (Baudry et al. 1998).The estimated number of contributing colonies for each of seven DCAs in Brazil was between 8 and 58. Moreover, South African studies determined there to be between 29 to 36 contributing colonies at both of two DCAs (Shaibi et al. 2008), and another identified 12 during the winter and 72 in the summer (Jaffé et al. 2009). The Brazilian and African DCAs were influenced more strongly by feral populations than managed colonies. Collet et al. (2009) observed DCAs in Brazil to be distributed more continuously than in Europe, which may have led to a lower number of contributing colonies per DCA. Presently, it is unclear if the discrepancy between colony numbers is due to colony dispersal in the area of the DCA, difference in DCA formation by African and European honey bees or some combination of both.

Synopsis and Objectives

Northern expansion of the African honey bee has stopped in central Florida in the Orlando area (personal comm. David Westervelt, FDACS Apiary Division Assistant Chief). I suspect the northern hybrid zone has developed in this region as did the southern hybrid zone in Argentina. Hybridization zones are particularly interesting because they provide natural laboratories where genetic admixture and selection can be observed (Hewitt 1988), and the Florida honey bee population is ideal location to manipulate the reproductive population to ensure virgin queens are likely to mate with European drones.

Beekeepers have suggested that the management of European colonies in an area will help to minimize the impact of the feral African bee population. The FDACS states that gentle European honey bees are the best defense against feral African honey bees and has established best management practices for maintaining European honey bee colonies based on this untested assertion (FDACS 2013). European honey bees perform well in high population densities where food is the limiting resource (Ratnieks 1991), whereas African honey bees tend to abscond to areas with plentiful resources (Michener 1975). Considering these differences in strategy it is plausible that large European honey bee populations could minimize the size of the regional feral African population. Moreover, Pinto et al. (2005) noted that the portion of the population with more European ancestry was more successful, than those with African ancestry, in areas of Texas where competition for limited resources arose.

In addition to combating the size of the feral African population, methods to minimize the introgression of African genes into managed European colonies in the United States are necessary. Past research indicates this is possible by increasing the

probability of European drones breeding with virgin queens (Loper and Fierro 1991). However, Loper and Fierro (1991) employed 3 drone manipulations simultaneously and were therefore unable to determine which of the 3 had the most impact: (1) aggressive trapping of feral drones, (2) introduction of large numbers of European drones source colonies, and (3) strategic placement of drone source colonies.

The overall objective of this thesis is to determine the extent to which the management of European honey bees can mitigate the impact of African honey bees in the southeastern United States. I will address the overall objective with the following specific aims:

1. Develop an effective technique for locating DCAs.
2. Determine if the use of a recessive phenotypic color mutation, cordovan (*cd*), can identify European honey bees reliably.
3. Determine the frequency at which feral African honey bee swarms usurp managed European honey bee colonies.
4. Determine if the management of European honey bee colonies can increase the proportion of European drones present at nearby drone congregation areas.

Due to the dynamics of the honey bee mating system DCAs are ideal locations for assessing the influence of managed colonies on the total regional reproductive population. However DCAs are not readily apparent or easily identified. My first aim is to develop a technique that will allow me, and future investigators, to readily identify experimental drone congregation areas.

Half of the DCAs I will be sampling at are located close to 96 managed European honey bee colonies, and all of these colonies are headed by a *cd* queen. Cordovan is a recessive color mutation (Mackensen 1951; Laidlaw et al. 1953) that has been used in previous studies as an indicator of European ancestry (Rinderer et al. 1987; Degrandi-

Hoffman et al. 1998a; DeGrandi-Hoffman et al. 1998b; Schneider and Degrandi-Hoffman 2002; Schneider and Degrandi-Hoffman 2003; Schneider et al. 2003; Schneider et al. 2004a). However, I suspect that this mutation is also present in the African population, and therefor is not a reliable indicator of ancestry. I aim to determine if drones with both African and European matrilines express the *cd* phenotype in central Florida.

Within my overall goal of determining the impact of managed European honey bees on the African honey bee population, it is important that I verify the regional frequency of usurpation events. As discussed above, the reported frequencies of usurpation are highly variable, and if managed European colonies are frequently usurped by African queens, the experimental apiaries could be contributing to the African population rather than minimizing its influence.

Finally, I will determine the maternal ancestry of drones collected at DCAs located near by these managed apiaries and from DCAs distant to any managed colonies. These results are expected to determine if the management of European honey bees can significantly reduce the proportion of African drones present at regional DCAs. Thereby the risk of hybridization of European queens would be decreased by increasing the likelihood that virgin queens attending those DCAs will mate with European drones rather than African drones.

Cumulatively, the work presented in this thesis will offer insight into the dynamics of the African and European honey bee populations within, what I suspect to be, the hybridization zone at the northern front of the African population in the southeastern United States. My results will provide a foundation from which management practices

may be developed to efficiently utilize European honey bees to combat the introgression of African traits into managed European colonies.

Table 1-1. Descriptions of the factors hypothesized to contribute to the preservation of African genetics in the feral population in the face of expansion into regions hosting established European honey bee populations.

Factors involved in preserving African Genetics	Description	References
Hybrid dysfunction	Hybrid individuals have lower mass-specific metabolic rates that either pure African or European honey bees suggesting some level of hybrid dysfunction	Harrison and Hall (1993)
Increased developmental stability	Fluctuating wing asymmetries suggest that African honey bees have the highest developmental stability. While pure Europeans have the least and hybrid individuals are intermediate	Schneider et al. (2003)
Tendency towards pairwise breeding	African queens are more likely to mate with African drones even in regions that have an abundance of European drones	Kerr and Bueno (1970); Taylor (1999)
Temporal isolation of African and European reproductives	In Mexico African drones were most abundant in March and April and European drones were predominant in June and July. Further isolation may occur in daily drone flight time by subspecies, as is seen in the worker caste in Brazil.	Michener (1975); Taylor and Spivak (1984);Quezada-Euan and May-Itza (2001)
Heightened drone drift and production	African drones are prone to drift into non-parent colonies, and they are more likely to drift into European colonies than African ones. When hosting drifting drones, a colonies own drive to produce drones is suppressed.	Rinderer et al. (1985); Rinderer et al. (1987)
Advantages for virgin queens with an African patriline	When rearing new virgin queens, those with African patrilines emerge earlier, pipe more frequently, and kill more rival virgins than queens with European patrilines.	Degrandi-Hoffman et al. (1998b); Schneider and Degrandi-Hoffman (2003) Schneider et al. (2004c)
High reproductive rate	African colonies swarm year round, up to every 50 days. Allowing for increased production of colonies and virgin queens compared to European colonies.	Michener (1975); Taylor (1977); Winston (1979)

Table 1-1. Continued.

Factors involved in preserving African Genetics	Description	References
Usurpation of European colonies	African swarms (containing a mated queen) invade European colonies, kill the resident queen, and introduce their own queen who begins to lay eggs	Michener et al. (1972); Taylor 1985; Danka and Rinderer (1988)

Table 1-2. Descriptions of methods by which managed European honey bee colonies become ‘Africanized’.

Methods of 'Africanized'	Description	References
Usurpation of European colonies	African swarms invade European colonies, kill the resident queen, and introduce their own queen who begins to lay eggs	Michener et al. (1972); Taylor 1985; Danka and Rinderer (1988)
European Queens mating with African drones	Virgin European queens mate with African drones. Over successive generation offspring can be produced that have a high level of African genetics despite European maternal ancestry.	Taylor (1985); Hall and Muralidharan (1989); Hall (1990); Hall (1992b)
Advantages for virgin queens with an African patriline	Queens with African patrilines emerge earlier, pipe more frequently, and kill more rival virgins than queens with European patrilines in the same colony	Degrandi-Hoffman et al. (1998b); Schneider and Degrandi-Hoffman (2003); Schneider et al. (2004c)
Introduction of captured African swarms and colonies	Beekeepers capture feral colonies and swarms and introducing them into their apiaries	Michener (1975); Taylor (1985)

CHAPTER 2
SCIENTIFIC NOTE ON A SINGLE-USER METHOD FOR IDENTIFYING DRONE CONGREGATION AREAS

Throughout the breeding season, drones of Western honey bees, *Apis mellifera*, congregate on calm, sunny days with little to no wind at DCAs (Zmarlicki and Morse 1963; Tribe 1982). Drone congregation areas are specific locations that typically occur in an open area, free of trees and/or buildings, and protected from wind by a vertical relief such as a tree line (Zmarlicki and Morse 1963; Ruttner 1966; Tribe 1982). Moreover, DCAs occur in the same location from year to year and they are found by successive generations of drones (Tribe 1982; Ruttner 1985).

Drone congregation areas are extremely useful in numerous aspects of honey bee research. Identification of a DCA is a prerequisite for many reproductive studies of the honey bee, as this behavior cannot be induced in artificial conditions (Schmolke 1977). Drones typically attend the closest DCA to their parent colony (Koeniger et al. 2005), resulting in a mixture of managed and wild (or feral) colonies at DCAs located close to managed apiaries. The drones present at any DCA are representative of the regional population, allowing for population level analysis (Baudry et al. 1998). However, the determining factors for DCA formation are still unclear (Scheiner et al. 2013).

Previously published methods for locating DCAs are often labor intensive, require multiple researchers, and are less effective in areas with low colony population densities (i.e. feral populations). These techniques include listening for the loud humming of drone flight (Ruttner 1985), monitoring tethered queens or queen dummies (Ruttner 1985; Muerrle et al. 2007), and radar monitoring (Loper et al. 1987). We expanded upon

these methods to develop an efficient technique in which a single investigator can locate a DCA regardless of the regional honey bee population density.

Locations meeting the known attributes of DCAs are identified using satellite imaging (for example, Google Earth Pro 7.1) and on-site inspection. Once probable locations are selected, up to 6 balloon stations (Fig. 2-1) are evenly distributed throughout the location (between 75-150 m apart) for the duration of the regional drone flight period (see Scheiner et al. 2013 for methods to determine regional drone flight time). Each balloon station consists of a 1.2 m chloroprene balloon equipped with a Williams (1987) drone trap (baited by a queen lure containing 1 mg synthetic 9-oxo-2-decenoic (9-ODA; Gary 1962; Gries and Koeniger 1996) suspended below the trap opening (Fig. 2-2). The drone trap is attached 5m below the balloon and the bottom of the trap is between 10-30 m above the ground (Fig. 2-3). The tether line is attached to a cinder block (Fig. 2-4). A balloon height of 10 m is ideal for initial detection of congregation areas for several practical reasons: (1) drones may not fly at higher elevations in less-than-ideal weather conditions, (2) a trap at a height of 10 m can be determined visually as empty without lowering the trap, and (3) there is less opportunity for tangling 10 m of line opposed to 30 m when lowing the trap to collect drones.

A single investigator can easily visit 6 stations within a single, 20-30 minute interval. During inspections, the traps are lowered and all drones present collected (Fig. 2-5). As areas of increased drone activity (more drones being trapped) become apparent, the balloon stations are relocated towards those areas. Final designation as a DCA can be made when drone comets are observed (Fig. 2-6), drones are present

immediately at balloon elevation and > 50 drones are collected in a 20 minute interval (Tribe 1982; Muerrle et al. 2007).

The presented method proved to be effective for a single investigator to readily identify DCAs in regions with low population densities, hosting only feral honey bee colonies, and near large managed apiaries. Further benefits of the current method are that (1) each station actively traps drones while unattended, (2) no modification of the balloon station is necessary to collect samples, and (3) a single investigator may collect samples at multiple DCAs simultaneously.

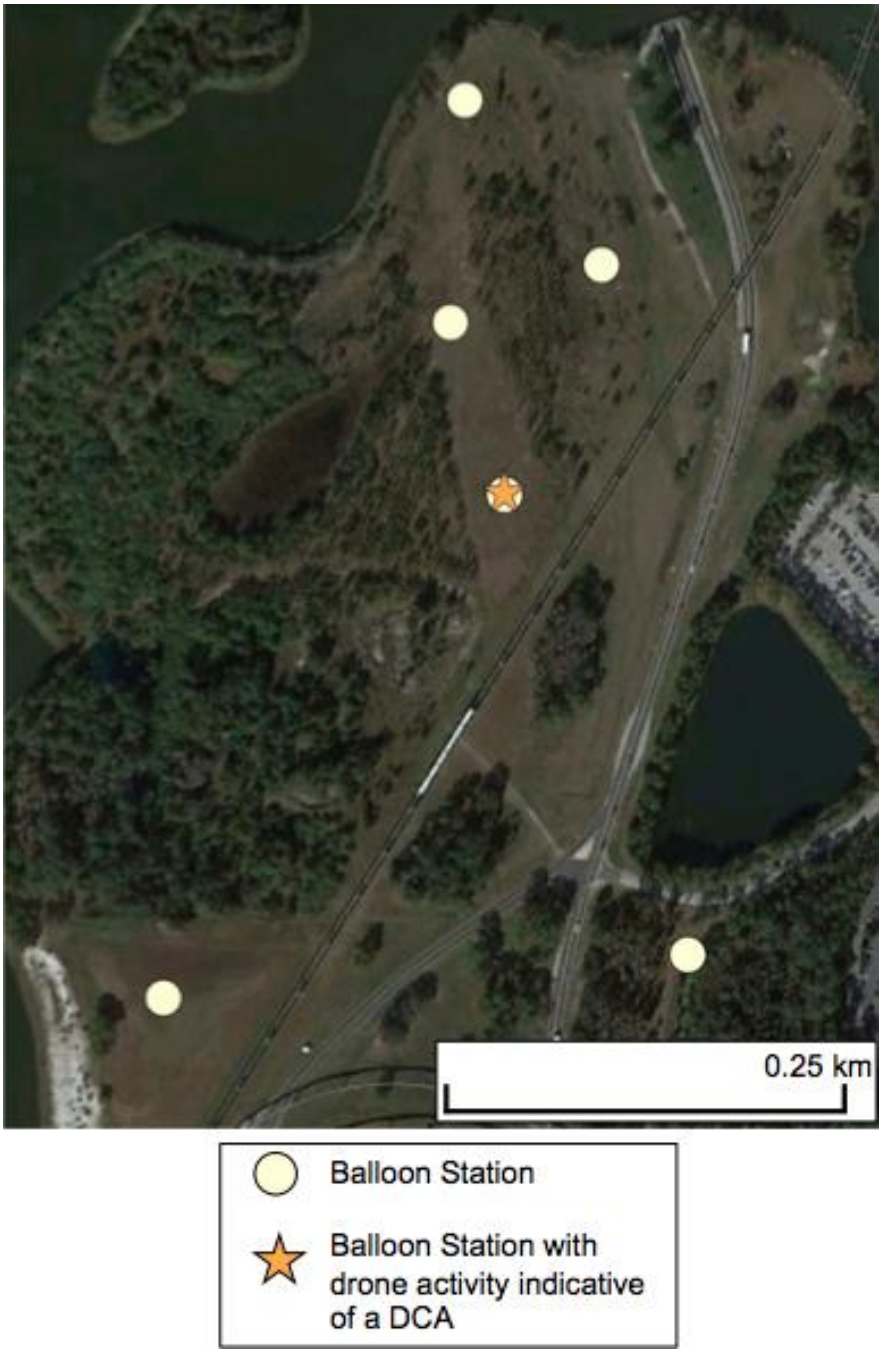

Figure 2-1. Satellite image map demonstrating the distribution of balloon stations within a potential DCA. Little-to-no drone activity was observed at all balloon stations except for the station marked by the orange star. This location was determined to be a DCA due to drone comet formation and >50 drones being trapped in 30 minutes. Satellite imaging provided by Google Earth Pro 7.1.

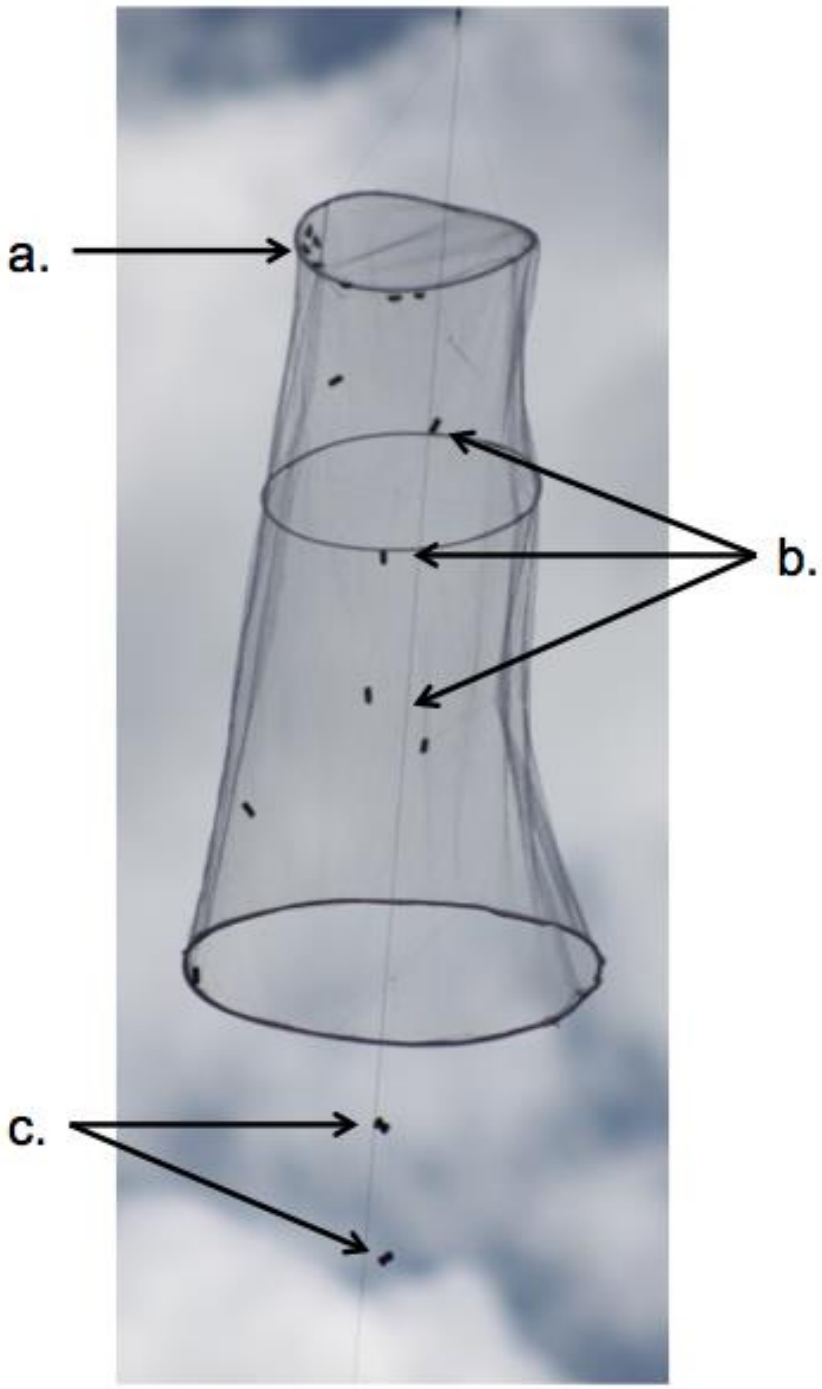

Figure 2-2. Williams (1987) drone trap equipped with visual queen dummies (b.) and pheromone queen dummies (c). Pheromone queen dummies are suspended 20 and 30 cm below the middle of the trap opening to attract drones to the area of the trap. Visual dummies are attached throughout the interior of the trap to draw drones (a.) up to the top of the trap. Photograph by Ashley N. Mortensen.

Figure 2-3. Drone trap elevated 10 m above the ground using a 1.2 m chloroprene balloon at a DCA. Note that this DCA occurs along the edge of an open area, near the surrounding tree line. Photograph by Ashley N. Mortensen.

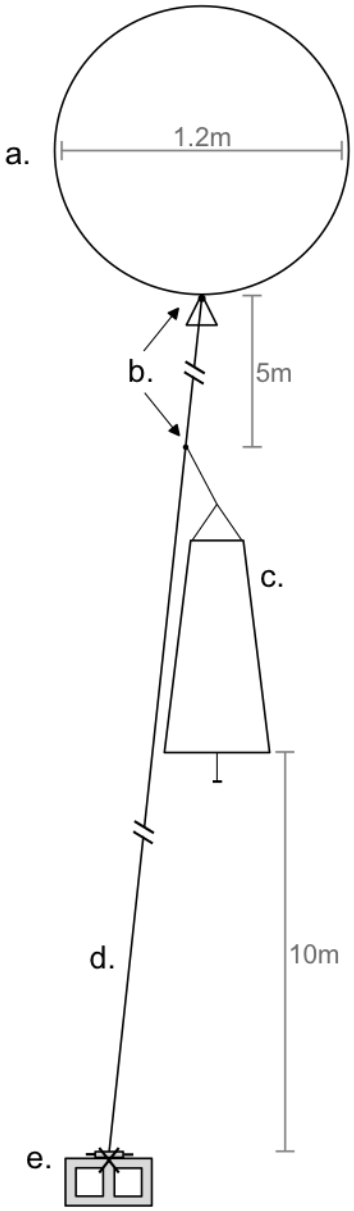

Figure 2-4. Balloon station diagram with parts labeled: (a) chloroprene balloon inflated with helium, (b) swivel hook attachment to kite line for balloon and trap, (c) Williams (1987) drone trap with queen pheromone dummy suspended below, (d) 22.7 kg kite line, (e) kite reel secured to a cinder (cement) block, weighing 15 kg, with 2 bungee tie cords.

Figure 2-5. Collecting trapped drones into a 50ml centrifuge tube of 95% ethanol. Suspension of the trap by a helium balloon allows the trap to be quickly and easily lowered for collections. Photograph by Ashley N. Mortensen.

Figure 2-6. Drone trap elevated in a DCA. A comet of drones can be seen entering the trap. Approximately 200 drones are trapped in the top of the trap. Photograph by Ashley N. Mortensen.

CHAPTER 3
A SCIENTIFIC NOTE ON THE PREVALENCE OF THE CORDOVAN PHENOTYPE IN THE AFRICAN-DERIVED HONEY BEE POPULATION IN THE SOUTHEASTERN UNITED STATES

Readily identifiable phenotypic markers, such as color, are useful research tools in an array of disciplines. In the Western honey bee, *Apis mellifera* Linnaeus, a recessive phenotype know as cordovan (*cd,* Mackensen 1951; Laidlaw et al. 1953) has been used in numerous genetic and behavior studies ranging from the discovery of haplodiploid sex determination to kinship recognition, and spatial dynamics of the mating system (Mackensen 1951; Rowell et al. 1992; Breed et al. 1994).

Cordovan individuals express red to brown cuticular coloration in all areas that otherwise would be black (Mackensen 1951). There is considerable variation in the amount of black cuticular color expressed ordinarily in all subspecies of the Western honey bee. However, even in the most yellow of wild-type workers and drones, the sixth abdominal tergum is always black (Tucker 1986). Thus, *cd* mutants are easily differentiated from their wild-type counterparts, allowing them to be informative in laboratory and field studies.

With the introduction of African-derived honey bees, *A.m. scutellata* Lepeletier, into the Americas (Kerr 1967), investigators have used this marker in a variety of comparative studies on African and European honey bees (Rinderer et al. 1987; DeGrandi-Hoffman et al. 1998a; DeGrandi-Hoffman et al. 1998b; Schneider and Degrandi-Hoffman 2002; Schneider and Degrandi-Hoffman 2003; Schneider et al. 2003; Schneider et al. 2004a). However, I suggest that the *cd* phenotype be used cautiously, particularly as an indicator of European decent, as African- and European-derived populations in the United States have become increasingly admixed, .

In June and July of 2012 I conducted a survey in which 400 drones were sampled from each of 6 drone congregation areas for a total of 2,400 drones collected in Orange and Osceola counties, Florida. Three of the DCAs were located within 0.25 km of 96, 10-frame, commercial European honey bee colonies headed by *cd* queens. The 3 remaining DCAs were located > 2.8 km from any managed honey bee colonies. Since drones typically travel < 2.0 km to a DCA (Rowell et al. 1992), drones trapped > 2.8 km from managed colonies were considered representative of the feral population.

Drones were trapped via an aerial Williams (1987) trap, baited with a synthetic 9-oxo-2-decenoic (9-ODA) queen lure (Gary 1962), and suspended 10-30 m above the ground by a chloroprene balloon (see Chapter 2). They then were preserved in 95% ethanol for transport to the laboratory where they were preserved at -80°C until molecular processing. Total DNA was extracted from each drone with 10% Chelex extraction (Walsh et al. 1991), and maternal ancestry determined using a PCR-RFLP technique for a mitochondrial Cytochrome *b* gene diagnostic marker (Crozier et al. 1991; Pinto et al. 2003). Additionally, drones were determined visually to be wild type or *cd*.

In total, 829 *cd* drones were collected.125 drones were trapped at DCAs >2.8 km from any managed honey bee colonies (Table 3-1). Fourteen of the 125 individuals had African mtDNA (or 3.4%). Moreover, 7 of 22 (or 31.82%) of the African matriline drones trapped at DCAs near managed colonies (0.25 km) were *cd*. These data indicate that the *cd* mutation has been integrated into the feral population. Furthermore, the data show that the *cd* phenotype is associated with both African and European matrilines (Fig. 3-1).

In the state Florida the African honey bee population not expanded northward beyond its current limit in the central region of the state since 2005 (personal comm. David Westervelt, FDACS Apiary Division Assistant Chief). I suspect the feral population is highly hybridized in this region, as seen at the southern front in Argentina (Taylor 1977; Sheppard et al. 1991). Moreover, *cd* queens are becoming more popular in commercial and hobbyist apiaries, cumulatively leading to the incorporation of the *cd* phenotype into the feral African population in the southeastern United States.

Under carefully controlled experimental designs, the *cd* allele will continue to be an informative research tool. However, I advise that care be taken to ensure feral *cd* individuals are not misinterpreted within experimental conditions in regions where beekeepers maintain *cd* queens and feral African colonies exist.

Table 3-1. The characteristics of drones trapped at DCAs sampling the feral population (> 2.8 km to *cd* colonies) and DCAs proximal to apiaries of 96 commercial colonies headed by *cd* queens (0.25 km to *cd* colonies). Total drone counts are presented for drones with the *cd* phenotype, an African matriline, and with *cd* phenotype and African matriline. The percentage of collected African matriline drones that were *cd* is presented in the % African *cd* column.

DCA Proximity to managed colonies	no. drones collected	Total no. collected that were *cd*	Total no. collected having an African Matriline	Total no. *cd* collected having an African Matriline	% total no. having an African matriline collected that were *cd* (column 5/column 4)
>2.8 km to *cd* colonies	1200	125	412	14	3.40%
0.25 km to *cd* colonies	1200	704	22	7	31.82%
Overall Totals	2400	829	434	21	4.84%

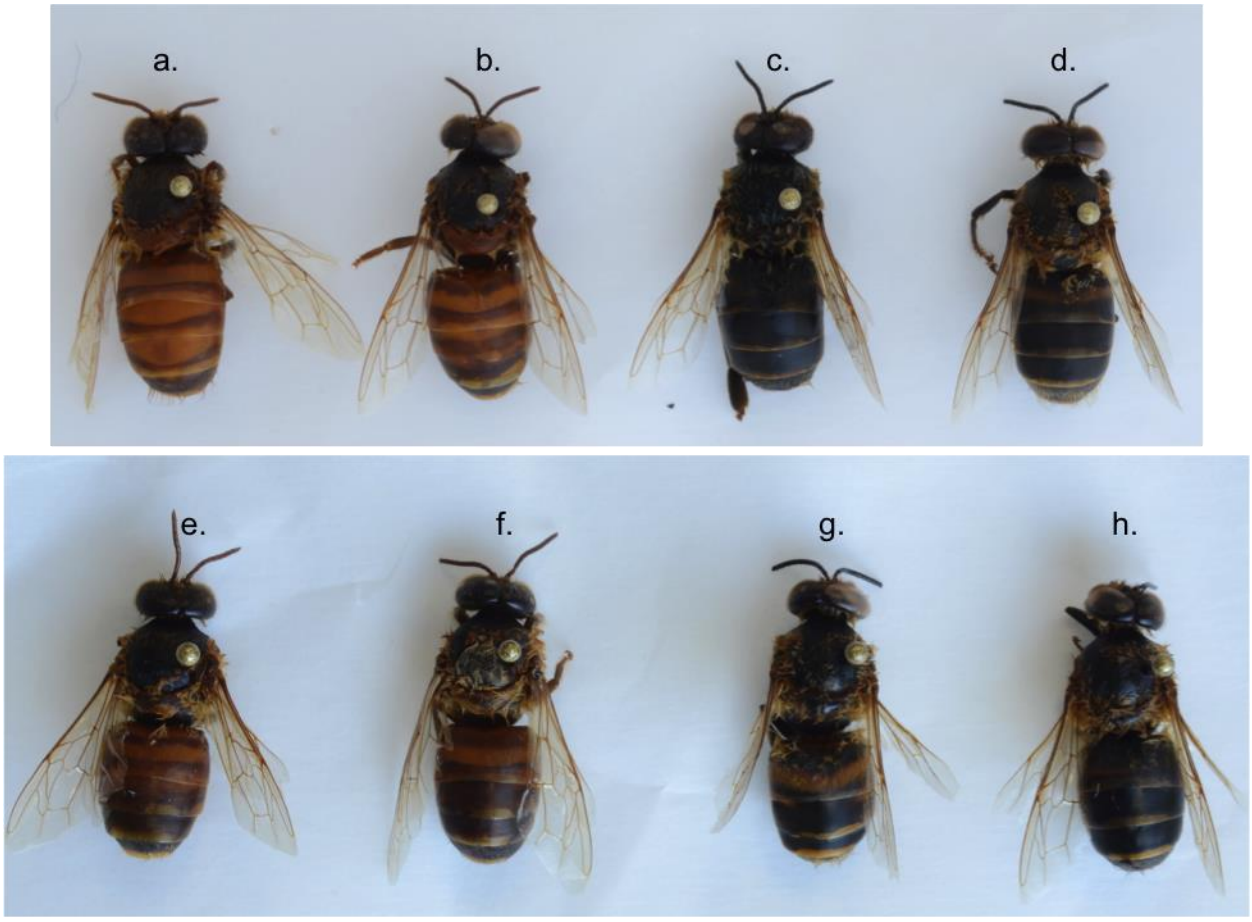

Figure 3-1. Feral drones trapped at DCAs in central Florida. European (a., b., c., d.) and African (e., f., g., h.) drones demonstrating *cd* mutation (a., b., e., f.) and wildtype (c., d., g., h.) cuticular colorations. Photograph by Lyle Buss.

CHAPTER 4
USURPATION OF MANAGED EUROPEAN-DERIVED HONEY BEE COLONIES VIA AFRICAN MATRILINE SWARMS IN THE SOUTHEASTERN UNITED STATES

Introduction

Since their introduction in 1957 (Kerr 1967), African honey bees have rapidly colonized the western hemisphere. This expansion has occurred via unbroken African matrilines (Hall and Muralidharan 1989; Smith et al. 1989), with African bees replacing feral European honey bees in many regions. Probable mechanisms contributing to the dominance of the African matriline are thought to be rapid colony growth, high swarming rates (Schneider et al. 2004b), frequent absconding, long distance swam movement (Ratnieks 1991), hybrid dysfunction (Harrison and Hall 1993), queen developmental time (Taylor 1999), drone mating advantage (Schneider et al. 2004b; Schneider et al. 2004c), and nest usurpation (Michener 1972; Taylor 1985). The latter occurs when an intruding swarm (containing a mated queen) enters an occupied colony, kills the resident queen, and introduces their own queen who begins to lay eggs (Michener 1972).

Usurpation is not well understood. There has been little systematic study of the behavior (Schneider et al. 2004c) and annual frequencies are reported to vary from 0-30.3% (Camazine 1986; Danka and Rinderer 1988; Danka et al. 1992; Vergara et al. 1993; Clarke et al. 2002; Schneider et al. 2004a; Schneider et al. 2004c). Moreover, usurpation events typically are subtle and difficult to distinguish from hybridization of a colony's naturally reared replacement queen (Taylor 1985). However, dramatic genetic change occurs in the event of colony usurpation opposed to hybridization of a naturally reared replacement queen (Danka and Rinderer 1988).

In the present study, we aim to determine the extent to which usurpation of managed European colonies contributes to the perpetuation of African matrilines at the northern front of the African honey bee expansion in Florida, USA. The African expansion has been stable in central Florida since 2005 (personal comm. David Westervelt, FDACS Apiary Division Assistant Chief), and mtDNA analysis has confirmed the African matriline in this region (unpubl. data). We expect that usurpation of managed European colonies by African matrilines occurs at low frequencies in commercially managed apiaries, and does not perpetuate African matrilines in this region significantly.

Materials and Methods

We utilized a combination of morphometric and mtDNA analysis (Danka et al. 1992) to monitor up to 288 commercial European honey bee colonies distributed between 5 apiaries in Orange and Osceola Counties (two apiaries of 96 colonies each and 3 apiaries of 32 colonies each) from December 2011-December 2012. During this time period, all colonies were managed in accordance with FDACS best management practices for maintaining European honey bees (FDACS 2013). This includes requeening colonies in instances of queen failure, excessive defensive behavior, poor colony productivity, etc.

In December of 2011, approximately 50 workers were collected from the comb of the uppermost box (or super) of each colony. Samples were collected in 95% ethanol and transported to the laboratory where forewing lengths of 10 randomly selected individuals per colony were morphometrically analyzed via the Fast African Bee Identification System (FABIS, Sylvester and Rinderer 1987). Any colony that received a FABIS score of $\geq$80% likely to be African was requeened to begin the experimental

period. In March, June, September, and December of 2012, all colonies were resampled and assigned a FABIS score as described above. No requeening was directed by 2012 FABIS results and apiary managers were blind to the results.

Each colony receiving a FABIS score of ≥80% likely African was analyzed further for African matriline using a Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique (Crozier et al. 1991; Pinto et al. 2003). Colonies exhibiting African mtDNA were considered 'usurped' by another colony with an African matriline. Colonies exhibiting European mtDNA were considered 'hybridized' via virgin European-derived queens from the original colonies mating with African drones. Finally, colonies exhibiting both African and European mitotypes were considered 'mixed' (an inconclusive category). The numbers of usurpation and hybridization events for each sample period were totaled and the frequency of each calculated based on the total number of queenright. If a colony showed hybridization or usurpation in successive sampling periods, its status was counted at its first discovery.

Results

The following data are detailed in Table 4-1: (1) the total number of live, queenright colonies, (2) the number of those colonies that received a FABIS score of ≥ 80% likely to be African, and (3) the mtDNA designation of the colonies in point 2 for each sampling period. All African-derived colonies identified at the June, September, and December sampling periods (27 total colonies) were classified as hybridization events rather than usurpation by a swarm headed by a queen with an African matriline. In March, 1 colony had both African and European mtDNA present at the time of sampling. However, this colony received a FABIS score of 16.8% likely African at the next sample period in June (well below the experimental threshold of ≥ 80%). The other

22 African colonies from the March sampling were classified as hybridized. Hybridization rates for each sample where as follows; March - 7.67%, June - 4.10%, September - 3.96%, and December - 1.99%. No colonies showed evidence of usurpation.

Discussion

Previous usurpation research has focused on areas with established African honey bee populations (Danka and Rinderer 1988, Danka et al. 1992, Vergara et al. 1993, Schneider et al. 2004a, Schneider et al. 2004c). The role of usurpation in the African population's invasion of new territories and the subsequent 'Africanization' of the regional European population are not well understood. We found no evidence that usurpation plays a significant role in the perpetuation of African matrilines at the northern front of the African honey bee expansion in the Southeastern United States. In fact, our study indicates that apiary management practices and the lack of usurpation events resulted in no perpetuation of the African matriline in our studied apiaries.

Hybridization of European matrilines was identified at each sample period, but no clear evidence of usurpation by an African matriline was found. It is possible that the 'mixed' mtDNA result seen in March (Table 4-1) could have represented a recently usurped colony that had not fully replaced the worker population at the time of sampling. Potentially, the colony was requeened by the commercial apiary managers in accordance with the FDACS best management practices as it began to express more phenotypically African behavior, i.e. heightened defensive behavior (Michener 1972). This would have resulted in the low FABIS score recorded at the next sample collection in June.

During the study period, the hybridization rate appears to follow the honey bee swarming season with the highest rate in March. Then the frequency of hybridized colonies declines through June, September, and December. European colonies typically swarm once a year in the spring (Taylor 1977). At which time the original colony produces a vigin qeen to mate with regional drones. As more virgin qeueens are likely to be reared during the swarming season it is likely that the instances of hybridization would also increase.

Interestingly, Quezada-Euan and May-Itza (2001) found a similar temporal pattern in the abundance of African drones present at drone congregation areas in subtropical Mexico. They found the ratio of African to European drones present at drone congregation areas was highest in March and decreased over the swarming season until the end of their sampling period in July. It is possible that higher African drone abundance during the early months of the swarming season may have led to an increased rate of hybridization of virgin queens produced by swarming colonies in our apiaries. Further investigation, incorporating multiple swarming seasons, is needed to determine if seasonal variation in subspecies abundance exists at DCAs in the southeastern United States as does in the Yucatan.

In the present study, it is possible that usurpation by hybridized queens possessing a European matriline were classified as hybridized rather than usurped. However, the focus of our study was to determine the contribution of usurpation specifically to the perpetuation of the African matrilines. Further studies integrating queen marking and monitoring of supersedure, swarming, and beekeeper-mediated

requeening should be conducted to expand our understanding of usurpation's role in the Africanization process.

The central Florida honey bee population offers a unique opportunity for understanding the interactions of African and European honey bee populations and the Africanization process. We suspect substantian hybridization is occurring in this region, comparable to the southern front in Argentina (Taylor 1977; Sheppard et al. 1991). Further experimentation promises to offer valuable insight into the dynamics of the African and European honey bee populations and possible mechanisms contributing to the successful preservation of African phenotypes and matrilines in the population.

Table 4-1. Number of colonies that received a FABIS score of > 80% likely African and their designation as hybridized, usurped, or mixed based on mtDNA anaylsis by sample period. Colonies declared as hybridized, usurped, or mixed are only reported at their first discovery.

Sample Date	Colonies Sampled	≥ 80% FABIS	Hybridized	Usurped	Mixed	% of colonies hybridized
March	287	23	22	-	1	7.67
June	268	11	11	-	-	4.10
September	278	11	11	-	-	3.96
December	251	5	5	-	-	1.99

CHAPTER 5
MITIGATING THE IMPACT OF AFRICAN HONEY BEES: DETERMINING HOW MANAGED EUROPEAN HONEY BEE COLONIES AFFECT THE PROPORTION OF AFRICAN DRONES AT CONGREGATION AREAS

Introduction

Following their introduction into Brazil, African honey bees rapidly spread through the Americas, dramatically changing the South American beekeeping industry (Michener 1975). In South America, managed African honey bees outperform European honey bees and quickly became the predominate subspecies upon entering new territory that was suitable for their habitation (Michener 1975, Hall and Muralidharan 1989). African honey bees are better adapted to the tropical environment of south and central America than European honey bees. Furthermore, they outperform European honey bees in terms of survivability and honey production (Michener and al. 1972). However, due to undesirable traits, like the African honey bee's heightened defensive behavior, hobbyist beekeeping became nonexistent and managed apiaries were relocated to isolated locations (Michener 1975). African genetics now predominate honey bee populations in the American tropics despite the efforts of beekeepers and local agencies to conserve favorable European traits (Hall and Muralidharan 1989; Smith et al. 1989). As seen in South America, it is important to the United States beekeeping industry that the introgression of African traits into European populations be limited (Taylor 1985).

In Argentina, at the southern edge of the African expansion, a hybridization zone has formed. In the Argentinian hybridization zone substantial admixture occurs between the African and European populations (Sheppard et al. 1991). The northern expansion of the African honey bee population has halted in central Florida and been stable in this

region since 2005 (personal comm. David Westervelt, FDACS Apiary Division Assistant Chief). I suspect a hybridization zone, comparable the one in South America (Taylor 1977, Sheppard et al. 1991), has developed in central Florida. Techniques to limit the introgression of African genetics into the managed European honey bee population in the United States should be focused in this region.

As the African population expanded into Texas it was noted that when competition for limited resources arose the portion of the population with more European ancestry was favored over those with African ancestry (Pinto et al. 2005). European honey bees are adapted to and perform well in high population densities where food is the limiting resource (Ratnieks 1991), while African honey bees tend to abscond to areas with plentiful resources (Michener 1975). It has therefore been hypothesized that the management of European honey bees may itself be a method of control for the African honey bee population in the United States.

In addition to creating unfavorable environmental conditions for African colonies, saturation of the environment with European colonies may also modify the regional reproductive population so that European queens are most likely to mate with European drones rather than hybridizing with African drones. Hybridization of managed European queens is the primary source of African genetics in managed European apiaries (Hall and Muralidharan 1989). Methods to minimize the 'Africanization' of United States' apiaries should focus on techniques to modify the abundance of African drones present at DCAs, thereby increasing the probability that virgin European queens will mate with European drones (Loper and Fierro 1991)

Loper and Fierro (1991) monitored drones attending DCAs in Chiapas, Mexico and determine that the simultaneous implementation of three drone techniques effectively manipulated the regional reproductive population. In the, Loper and Fierro (1991) study, they; (1) aggressively trapped and removed feral drones prior to introduction of European drone source, (2) introduced a large number of European drones to flood the regional population, and (3) strategically placed introduced drone source colonies. However, due to the experimental design, they were unable to ascertain which factor, if any, was most impactful.

In the present study, I aim to determine if the management of European honey bees can increase the proportion of African drones present at nearby drone congregations areas dramatically. I expect that the management of European colonies will result in significantly more European drones present at nearby DCAs.

Materials and Methods

Drones were collected in June and July of 2012 from 6 DCAs in Orange and Osceola counties, Florida. Three DCAs were located within 0.25 km of 96, 10-frame, commercial European colonies that had been established in March of 2011, and the 3 remaining DCAs were > 2.8 km from any managed colonies (Fig. 5-1). Since drones typically travel < 2.0 km to a DCA (Rowell et al. 1992), drones trapped > 2.8 km from managed colonies were considered representative of the feral population.

Drone collections occurred through the duration of the flight time via a Williams (1987) drone trap equipped two queen lures baited with 1 mg of synthetic 9-oxo-2-decenoic (9-oda, Gary 1962, Gries and Koeniger 1996). The trap was suspended below a white, 1.2 m, helium-inflated, chloroprene balloon and elevation was determined by the distance from the bottom of the trap opening to the ground (see Chapter 2). Four

hundred drones were collected from each of the 6 DCAs. Within each DCA, drones were caught at 2 heights: 200 drones from 10 m and 200 from 30 m.

Collected drones were preserved in 95% ethanol and transported to the laboratory for storage at -80°C. Total DNA was extracted individually from a hind leg of each drone with 10 % Chelex technique (Walsh et al. 1991), and maternal ancestry determined by digesting an amplified portion of the mitochondrial cytochrome *b* gene with a *Bgl* II restriction enzyme (Crozier et al. 1991, Pinto et al. 2003).

In individuals of European descent the *Bgl* II digestion cleaves the amplified 485 BP (base pair) cytochrome b fragment into two, 194 and 291 BP, fragments. African matriline lindividials do not have a *Bgl* II restriction site in the cytochrome *b* fragment resulting in an intact 485 BP fragment that is easily discriminated from the banding pattern of European individuals when visualized under UV light on an ethiduim bromide stained 2% agarose/Tris acetic acid EDTA gel (Fig. 5-2).

The proportions of African and European drones collected were analyzed by proximity to managed colonies and height within the DCA using Chi Square Analysis.

Results

Mitochondrial results indicated that DCAs distant to managed European apiaries had significantly higher proportion ($p<0.0001$) of African matriline drones (34.33% of the collected individuals) than did DCAs located close to managed European apiaries (1.83% of the collected individuals; Table 5-1). However, there was not a detectible difference in the vertical distribution of African or European drones within the DCA (Fig. 5-3).

Discussion

The data suggest that the management of European colonies can influence the proportion of drones at DCAs that have an African matriline. This is most likely the result of managed European colonies producing large numbers of drones that flooded the proximal DCAs. However, it is possible that the management of large European apiaries saturated the environment, encouraging feral African colonies to abscond to areas with more resource availability. The latter cannot be determined by mtDNA alone. However, further investigation incorporating nuclear analysis of closely linked microsatellites will allow for quantification of the regional population (Kraus et al. 2005). This will be useful for determining if there are fewer African colonies in the proximity of the managed apiaries.

Regardless of the underlying cause for the changes seen in DCA composition, the data suggest that the management of European colonies can be considered a viable option for limiting the introgression of African genetics. Paternal gene flow by way of European queens mating with African drones is the form of “Africanization” in managed European populations (Hall and Muralidharan 1989). Increasing the proportion of European drones present at a DCA will increase the likelihood that virgin queens at that DCA will mate with European, rather than African, drones (Loper and Fierro 1991). It appears this can be accomplished managing European bees in an area.

Virgin queens fly to more distant DCAs than do drones from the same apiary. Drones typically travel $\leq$ 0.5 km to a DCA whereas queens are estimated to travel 2.0 km to a DCA (Rowell et al. 1992, Jensen et al. 2005). Further research is needed to determine the concentration and distribution of managed European colonies needed to

modify the regional reproductive population to the extent that virgin queens produced in apiaries would, themselves, attend a DCA with a high proportion of European drones.

Past studies have indicated the existence of a slight tendency towards like-subspecies mating where African and European queens mated with drones of their same subspecies 58% and 64% of the time respectively (Kerr and Bueno 1970b). Several theories, including partial physiological barriers and temporal isolation, exist to explain their tendencies (Kerr and Bueno 1970a, Taylor and Spivak 1984, Hall 1992, Quezada-Euan and Jesus May-Itza 2001). In the present study, drones were collected from both 10 and 30 m elevations to determine if there is a vertical distribution of African and European subspecies within the DCA. However, no detectable difference in vertical flight behavior of African and European drones was observed.

The frequency of African mtDNA, 34.3% (Table 5-1), observed in the feral population (drones collected at DCAs >2.8 km from managed colonies) supports the belief that significant hybridization is occurring in central Florida. African honey bees were confirmed in Orange and Osceola counties in 2005 via morphometric analysis (personal comm. David Westervelt, FDACS Apiary Division Assistant Chief), but have not expanded northward since that time. Studies of African mtDNA frequencies in predominantly African regions have reported between 83-88% African mtDNA (Sheppard et al. 1991, Diniz et al. 2003), and 69% of samples from the southern edge of the hybridization zone in the southwestern United States had African mtDNA (Pinto et al. 2005). I suspect the southeastern hybridization zone may exist in central Florida. However, since the African population in Florida is not a part of the continuous African expansion, further investigation is required to determine if this is truly a hybridization

zone or if south/central Florida's feral honey bee population is simply a hybridized population.

The central Florida honey bee population offers a unique opportunity for understanding the interactions of African and European honey bee populations and the Africanization process. Furthermore, experimentation in this region promises to offer valuable insight on possible population manipulation techniques to limit the introgression of undesirable African genetics into the manage European honey bee population.

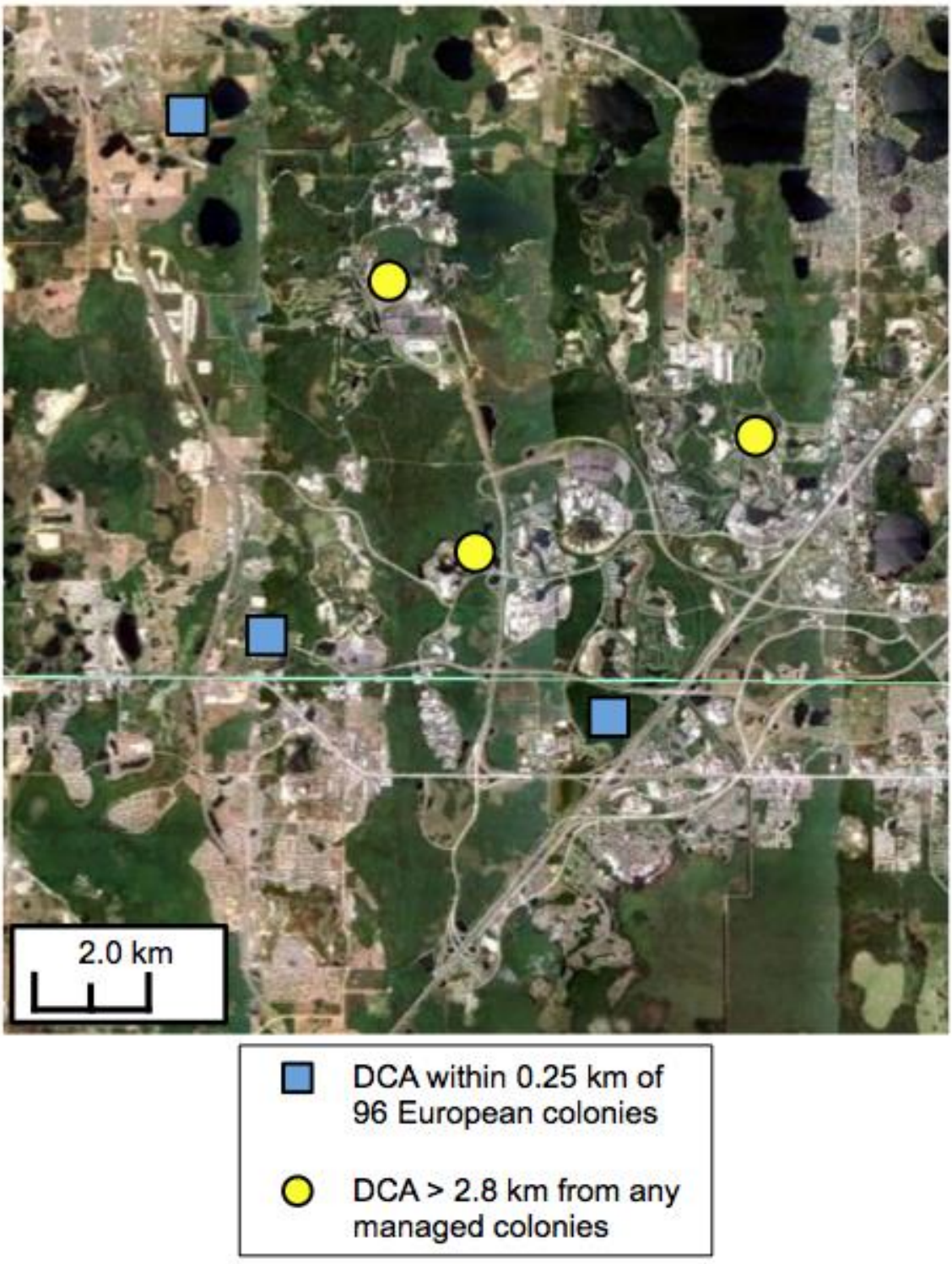

Figure 5-1. Satellite image of DCA locations in Orange and Osceola counties Florida. Satellite imaging provided by Google Earth Pro 7.1.

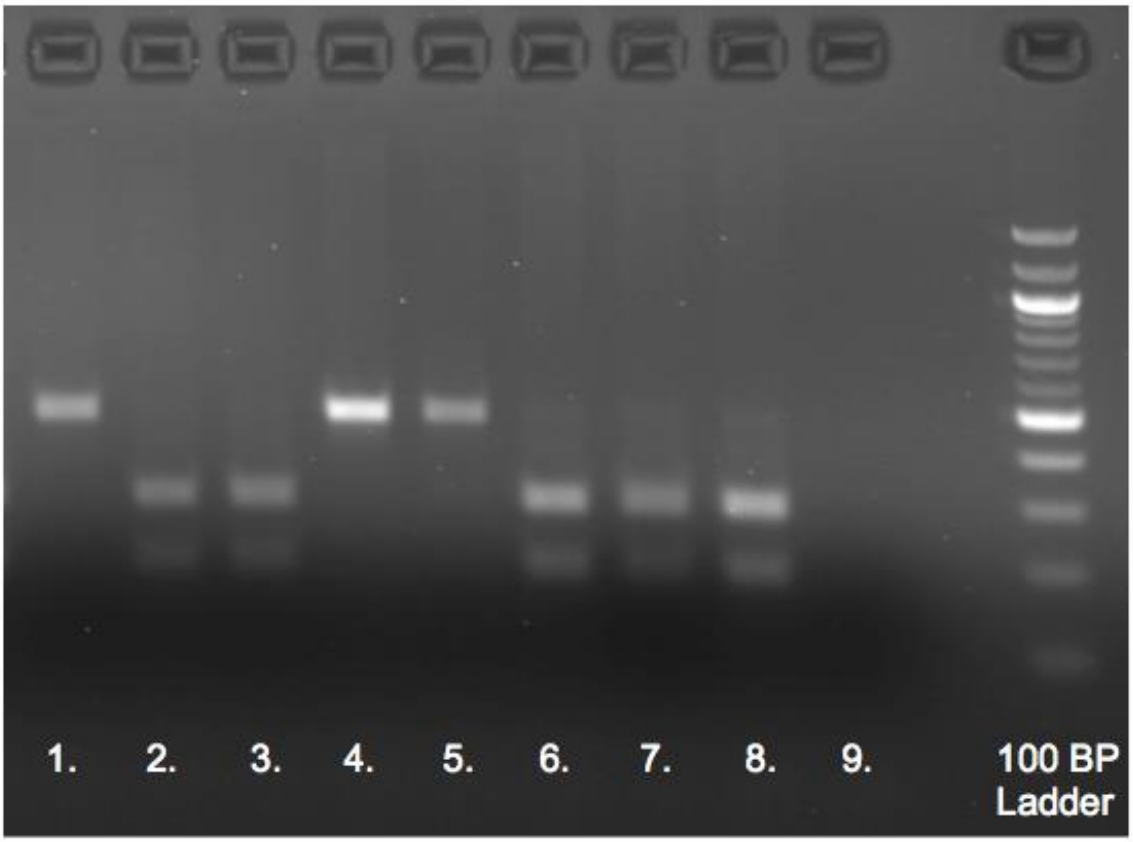

Figure 5-2. 2% agarose gel of the PCR-RFLP results of 8 drones collected at a DCA >2.8 km from managed colonies. A single band of approximately 485 BP indicates African mtDNA (lane numbers: 1., 4., 5.), and 2 bands of approximately 194 and 291 BP indicate European mtDNA (lane numbers: 2., 3., 6., 7., 8.). 100 BP size marker can be seen on the far right lane, and lane 9 represents a negative control.

Table 5-1. Mitochondrial results for drones trapped at DCAs ~ 0.25 km of managed European honey bees or > 2.8 km from any managed colonies, and relative proportions of African and European matriline drones present at each DCA location type are presented. Columnar data followed by different letters are significantly different at P≤0.05 (Chi-Square test).

DCA locations	Total no. drones collected	Total drones collected with European mtDNA	Total drones collected with African mtDNA	% of total drones collected with European mtDNA	% of total drones collected with African mtDNA
0.25 km from apiary	1200	1178	22	98.17%	1.83% a
>2.8 km from apiary	1200	788	412	65.67%	34.33% b

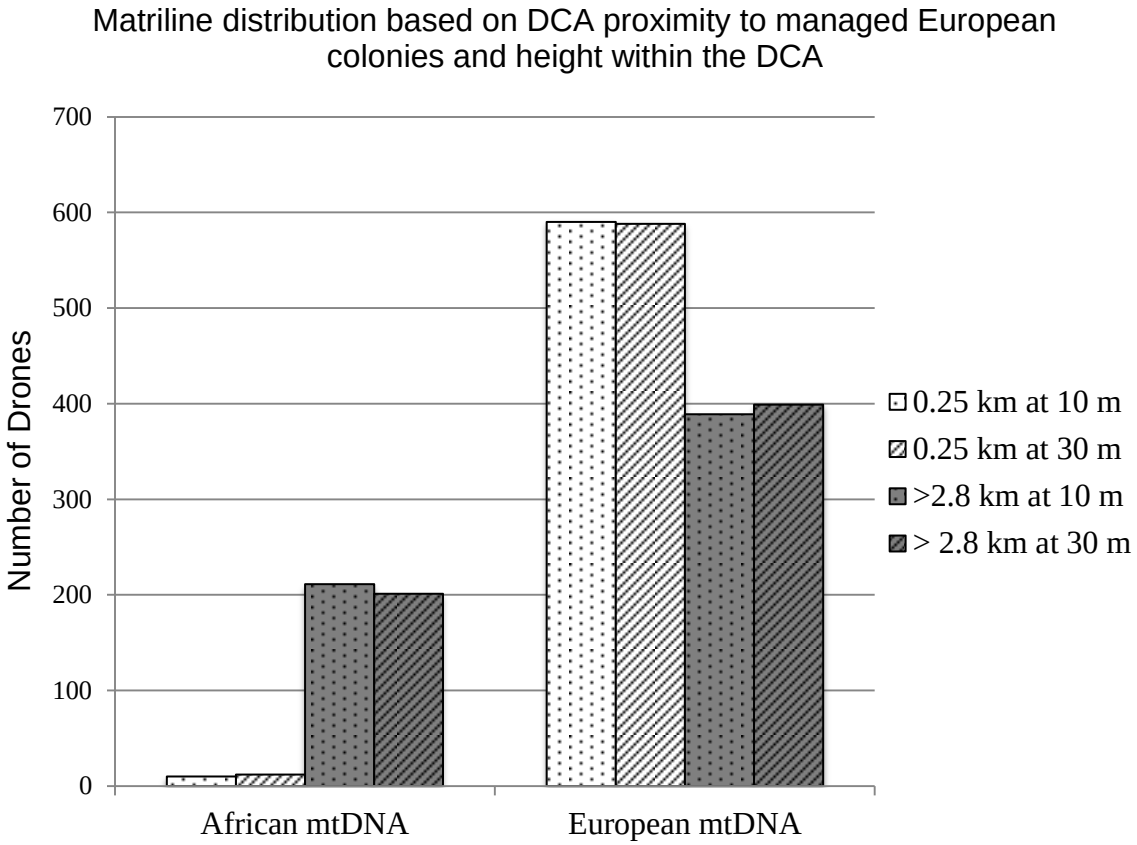

Figure 5-3. Distribution of African and European matrilines. Total number of drones expressing an African or European matriline are presented based on the proximity of the DCA to managed European colonies and vertical distribution within that DCA from which they were trapped.

CHAPTER 6
CONCLUSION

Cumulatively, the experiments presented in this thesis confirm morphometric indications that the feral African honey bee population is established as far north as Orange and Osceola counties in central Florida. Furthermore, the frequency of African mtDNA in the feral population and the occurrence of the *cd* phenotype in individuals with African matrilines suggest high levels of hybridization in the feral population. This region promises to provide a natural laboratory in which introgression of the African and European honey bee populations can be studied (Hewitt 1988). Moreover, DCAs provide distinct sites where population level samples can be collected, and the methods described herein allow for relatively simple identification of these locations.

The studies revealed that the maternal introgression of African genetics into managed European colonies, by way of usurpation, does not appear to contribute to the Africanization of managed European apiaries. It is more likely that African matrilines are incorporated into managed colonies by beekeepers collecting feral colonies and swarms (Michener 1975, Taylor 1985). In the case that beekeepers do not introduce African queenlines into their apiaries, paternal gene flow is the primary source of African genetics observed in managed European colonies (Hall and Muralidharan 1989). Therefore techniques to limit the probability of virgin European queens mating with African drones will be the most influential means of limiting ‘Africanization’ of managed European honey bees in the United States.

Furthermore, the data suggest that the management of European honey bees can decrease the proportion of African drones present at nearby DCAs. However the minimum density and most effective distribution of European colonies to duplicate these

results on a large scale are still unclear and may, in fact, be unrealistic. Most reasonably European colonies could be used to mitigate the African population in areas that are most sensitive to the presence of African honey bees, such as popular tourist destinations or areas in which honey bee producers are rearing and breeding virgin queens, rather than as a first line of defense throughout the state.

At this point it is unclear what the minimum density of colonies needed to alter the proportion of African drones present at nearby DCAs is. The large European apiaries in these studies were most likely saturating the environment. However, seemingly high numbers of managed European colonies may be necessary to modify the proportion of African drones present at nearby DCAs due to the African honey bee's drone production advantages: (1) African colonies begin producing drones earlier in the breeding season (Quezada-Euan and May-Itza 2001), (2) African drones have a higher tendency to drift to non-parental colonies, (3) African drones are most likely to drift into European colonies, and (4) hosting drifter drones suppresses a colony's drive to produce their own drones (Rinderer et al. 1985). These factors cumulatively create an environment where a small number of African colonies could produce the majority of the regional drones and European colonies could host only African drones (Rinderer et al. 1985, Rinderer et al. 1987).

Moreover, further study is necessary to determine what this ideal distribution of these European apiaries would be. Ideally, apiaries should be established in a way to ensure that virgin queens produced in drone source apiaries would themselves attend a DCA with a high proportion of European drones. Previous research has demonstrated that the majority of drones fly less that 0.5 km from their colony to a DCA (Rowell et al.

1992). It is likely that an appropriate distribution of colonies would be approximately 1 km between apiaries so the area of dispersal from one apiary would reach to the area of dispersal of the next. However, additional investigation is needed to determine the actual area over which a European apiary can modify the reproductive population.

The high level of population manipulation this type of management would likely result could further decrease the influence of African genetics by increasing the probability of virgin African queens mating with European drones, and/or causing European genetics to be selected for in the population due to resource limitation (Pinto et al. 2005).

I used a mitochondrial technique to diagnosis maternal ancestry in the present studies. Regrettably, mtDNA provides no information on the paternal genetic contribution, and no hybridization can be identified. Recognizing that these experiments occur in a hybridization zone, where one expects to see high levels of introgression, further analysis of the nuclear genome promises to offer more insight into the interactions of the regional African and European populations.

LIST OF REFERENCES

Baudry, E., M. Solignac, L. Garnery, M. Gries, J. Cornuet, and N. Koeniger. 1998. Relatedness among honeybees (Apis mellifera) of a drone congregation. Proceedings of the Royal Society of London. Series B: Biol. Sci. 265: 2009-2014.

Breed, M.D., C. K. Welch, R. Cruz. 1994. Kin discrimination within honey bee (Apis mellifera) colonies: an analysis of the evidence. Behav. Process. 33: 25-39.

Buchmann, S. 1982. Africanized bees confirmed in Panama. Am. Bee J. 122: 322.

Camazine, S. 1986. Queen rearing in São Paulo State, Brazil: a beekeeping experience of over 20 years. Am. Bee J. 126: 414-416.

Clarke, K. E., T. E. Rinderer, P. Franck, J. G. Quezada- Euán, B. P. Oldroyd. 2002. The Africanization of honeybees (Apis mellifera L.) of the Yucatan: a study of a massive hybridization event across time. Evolution. 56: 1462-1474.

Crozier, Y., S. Koulianos, R. Crozier. 1991. An improved test for Africanized honeybee mitochondrial DNA. Cell. Mol. Life Sci. 47: 968-969.

Danka, R., R. Hellmich, T. Rinderer. 1992. Nest usurpation, supersedure and colony failure contribute to Africanization of commercially managed European honey bees in Venezuela. J. Apic. Res. 31: 119-119.

Danka, R. G., T. E. Rinderer. 1988. Social reproductive parasitism by Africanized honey bees, pp. 214-222. *In* G. R. Needham, R. E. Page Jr, M. Delfinado-Baker, C.E. Bowman (eds.), Africanized Honey Bees and Bee Mites. Halsted Press, Ney York, USA.

DeGrandi-Hoffman, G., A. Collins, J.H. Martin, J.O. Schmidt, H.G. Spangler. 1998a. Nest defense behavior in colonies from crosses between Africanized and European honey bees (Apis mellifera L.)(Hymenoptera: Apidae). J. Insect Behav. 11: 37-45.

Degrandi-Hoffman, G., J. C. Watkins, A. M. Collins, G. M. Loper, J. H. Martin, M. C. Arias, and W. S. Sheppard. 1998b. Queen developmental time as a factor in the Africanization of European honey bee (Hymenoptera: Apidae) populations. Ann. Entomol. Soc. Am. 91: 52-58.

Diniz, N. M., A. E. E. Soares, W. S. Sheppard, and M. A. Del Lama. 2003. Genetic structure of honeybee populations from southern Brazil and Uruguay. Genet. Molec. Biol. 26: 47-52.

(FDACS) Florida Department of Agriculture and Consumer Services. 2013. Best management practices for maintaining European honey bee colonies http://www.freshfromflorida.com/pi/plantinsp/apiary/apiary.html

Gary, N.E. 1962. Chemical mating attractants in the queen honey bee. Science. 136: 773.

Gary, N. E. 1963. Observations of mating behaviour in the honeybee. J. Apic. Res. 2: 3-13.

Gries, M. and N. Koeniger. 1996. Straight forward to the queen: pursuing honeybee drones (Apis mellifera L.) adjust their body axis to the direction of the queen. J. Comp. Physiol. A. 179: 539-544.

Hall, H. 1990. Parental analysis of introgressive hybridization between African and European honeybees using nuclear DNA RFLPs. Genetics 125: 611-621.

Hall, H. G. 1992a. Further characterization of nuclear DNA RFLP markers that distinguish African and European honeybees. Arch. Insect Biochem. 19: 163-175.

Hall, H. G. 1992b. DNA studies reveal processes involved in the spread of New World African honeybees. Fla. Entom. 75: 51-59.

Hall, H. G., and K. Muralidharan. 1989. Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. Nature. 339: 211-213.

Harrison, J. F., and H. G. Hall. 1993. African-European honeybee hybrids have low nonintermediate metabolic capacities. Nature. 363: 258-260.

Hewitt, G. M. 1988. Hybrid zones-natural laboratories for evolutionary studies. Trends Ecol. Evol. 3: 158-167.

Jaffé, R., V. Dietemann, R. Crewe, and R. Moritz. 2009. Temporal variation in the genetic structure of a drone congregation area: an insight into the population dynamics of wild African honeybees (Apis mellifera scutellata). Molec. Ecol. 18: 1511-1522.

Jean-Prost, P. 1958. Queen mating. Apimondia. XVII Int. Beekeep. Congr: 404-408.

Jensen, A. B., K. A. Palmer, N. Chaline, N. E. Raine, A. Tofilski, et al. 2005. Quantifying honey bee mating range and isolation in semi-isolated valleys by DNA microsatellite paternity analysis. Conserv. Genet. 6: 527-537.

Kerr, W. E., and D. Bueno. 1970. Natural crossing between Apis mellifera adansonii and Apis mellifera ligustica. Evolution. 24: 145-148.

Koeniger N., G. Koeniger, M. Gries, S. Tingek. 2005. Drone competition at drone congregation areas in four Apis species. Apidologie 36: 211-221.

Koeniger, N., G. Koeniger, and H. Pechhacker. 2005. The nearer the better? Drones (Apis mellifera) prefer nearer drone congregation areas. Insect. Soc. 52: 31-35.

Kraus, F. B., N. Koeniger, S. Tingek, and R. F. A. Moritz. 2005. Using drones for estimating colony number by microsatellite DNA analysis of haploid males in Apis. Apidologie. 36: 223-229.

Laidlaw H., M. Green, W. Kerr. 1953. Genetics of several eye color mutants in the honey bee. J. Hered. 44: 246-250.

Lobo, J. A., and H. Krieger. 1992. Maximum likelihood estimates of gene frequencies and racial admixture in Apis mellifera L. (Africanized honeybees). Heredity. 68: 441-448.

Loper, G., and M. Fierro. 1991. Use of drone trapping and drone release to influence matings of European queens in an Africanized honey bee area (Hymenoptera, Apidae). J Apic Res 30: 119-124.

Loper, G. M., W. W. Wolf, O. Taylor. 1987. Detection and monitoring of honeybee drone congregation areas by radar. Apidologie. 18:163-172.

Mackensen, O. 1951). Viability and sex determination in the honey bee (Apis mellifera L.). Genetics. 36: 500.

Michener, C. D. 1975. The Brazilian bee problem. Annu. Rev. Entomol. 20: 399-416.

Michener, C. D. 1972. Final Report Committee on the African Honey Bee, National Academy of Sciences, Washington, D.C., USA.

Moritz, R., H. Scharpenberg, H. Lattorff, and P. Neumann. 2003. A technical note for using microsatellite DNA analyses in haploid male DNA pools of social Hymenoptera. Insect. Soc. 50: 398-400.

Muerrle, T., H. Hepburn, S. Radloff. 2007. Experimental determination of drone congregation areas for Apis mellifera capensis. J. Apic. Res. 46: 154-159.

Pinto, M. A., J. S. Johnston, W. L. Rubink, R. N. Coulson, J. C. Patton et al. 2003. Identification of Africanized Honey Bee (Hymenoptera: Apidae) Mitochondrial DNA: Validation of a Rapid Polymerase Chain Reaction-Based Assay. Ann. Entomol. Soc. Am. 96: 679-684.

Pinto, M. A., W. L. Rubink, J. C. Patton, R. N. Coulson, and J. S. Johnston. 2005. Africanization in the United States: replacement of feral European honeybees (Apis mellifera L.) by an African hybrid swarm. Genetics. 170: 1653-1665.

Quezada-Euan J. J. G., W. D. J. May-Itza. 2001. Partial seasonal isolation of African and European-derived *Apis mellifera* (Hymenoptera: Apidae) drones at congregation areas from subtropical Mexico. Ann. Entomol. Soc. Am. 94: 540-544.

Ratnieks, F. L. W. 1991. Africanized Bees: Natural Selection For Colonizing Ability, pp. 119-136. *In* M. Spivak, D. J. C. Fletcher and M. D. Breed (eds.), The "African" honey bee. Westview Press, Inc., Boulder, Colorado.

Rinderer, T. E., R. L. Hellmich, R. G. Danka, and A. M. Collins. 1985. Male reproductive parasitism: a factor in the Africanization of European honey-bee populations. Science. 228: 1119.

Rinderer, T. E., A. M. Collins, R. L. Hellmich II, and R. G. Danka. 1987. Differential drone production by Africanized and European honey bee colonies. Apidologie. 18: 61-68.

Rowell, G. A., O. R. Taylor, Jr., and M. A. Long-Rowell. 1992. Spatial Dynamics of the Honey Bee Mating System (Apis mellifera L.). J. Kan. Entomol. Soc. 65: 218-222.

Ruttner, F. 1966. The life and flight activity of drones. BeeWorld 47: 93-100.

Ruttner, F. 1976. African races of honeybees. Proc. Int. Beekeeping Congr 25: 325-252.

Ruttner, F. 1985. Reproductive behavior in honeybees, pp. 225-236. *In* B. Holldobler. and M. Lindauer (eds.), Experimental behavioral ecology and sociobiology. Springer, New York, USA.

Scheiner, R, C.I. Abramson, R. Brodschneider, K. Crailsheim, W.M. Farina et al. 2013. Standard methods for behavioural studies of *Apis mellifera,* in: Dietemann,V., Ellis, J.D., Neumann, P. (Eds.), The Coloss Beebook, Volume I: Standard Methods for *Apis mellifera* Research, J. Apic. Res. (in press).

Schneider, S., and G. Degrandi-Hoffman. 2002. The influence of worker behavior and paternity on the development and emergence of honey bee queens. Insec. Soc. 49: 306-314.

Schneider, S., and G. Degrandi-Hoffman. 2003. The influence of paternity on virgin queen success in hybrid colonies of European and African honeybees. Anim. Behav. 65: 883-892.

Schneider S., T. Deeby, D. Gilley, G. DeGrandi-Hoffman. 2004a Seasonal nest usurpation of European colonies by African swarms in Arizona, USA. Insect. Soc. 51(4): 359-364.

Schneider S., G. DeGrandi-Hoffman, K. Hartfelder, D.d. Jong, R. Pereira, et al. 2004b. Mechanisms that favor the continuity of the African honeybee genome in the Americas, Proceedings of the 8th IBRA International Conference on Tropical Bees and VI Encontro sobre Abelhas, Ribeirão Preto, Brasil, pp. 232-240.

Schneider, S., G. DeGrandi-Hoffman, D. R. Smith. 2004c. The African honey bee: Factors contributing to a successful biological invasion. Ann. Rev. Entomol. 49: 351-376.

Schneider, S., L. Leamy, L. Lewis, G. DeGrandi- Hoffman. 2003. The influence of hybridization between African and European honeybees, Apis mellifera, on asymmetries in wing size and shape. Evolution. 57: 2350-2364.

Schmolke, M.D. 1977. Mating honey-bees in confinement., in: Fletcher D.J.C. (Ed.), African Bees: Taxonomy, Biology and Economic Use: Proceedings of an Apimondia International Symposium, Apimondia, Pretoria, South Africa, pp. 169-175

Seeley, T. D. 1985. Honeybee ecology: a study of adaptation in social life. Princeton University Press, Princeton, N.J.

Shaibi, T., H. Lattorff, and R. Moritz. 2008. A microsatellite DNA toolkit for studying population structure in Apis mellifera. Mol. Ecol. Resour. 8: 1034-1036.

Sheppard, W. S. 1989a. A history of the introduction of honey bee races into the United Sates: Part I. Am. Bee J. 129: 617-619.

Sheppard, W. S. 1989b. A history of the introduction of honey bee races into the United Sates: Part II. Am. Bee J. 129: 664-667.

Sheppard, W. S., T. E. Rinderer, J. A. Mazzoli, J. A. Stelzer, and H. Shimanuki. 1991. Gene flow between African- and European-derived honey bee populations in Argentina. Nature. 349: 782-784.

Smith, D. R., O. R. Taylor, and W. M. Brown. 1989. Neotropical africanized honey bees have African mitochondrial DNA. Nature. 339: 213-215.

Strang, G. E. 1970. A study of Honey Bee Drone attraction in the Mating Response. J. Econ. Entomol. 63: 641-645.

Sylvester, H. A. and T. E. Rinderer. 1987. Fast Africanized bee identification system (FABIS) manual. Am. Bee J. 127: 511-516.

Taylor, O. R. 1977. The past and possible future spread of Africanized honeybees in the Americas. Bee World 58: 19-30.

Taylor, O. R. 1985. African bees: potential impact in the United States. B. Entomol. Soc. Am. 31: 15-24.

Taylor, O. R. 1999. Displacement of European honey bee subspecies by an invading African subspecies in the Americas, pp. 38-46. *In* R. Hoopingarner and L. Connor (eds.), Apiculture for the 21st Centry. Wicwas Pr, Cheshire, CT.

Taylor, O. R. and M. Spivak. 1984. Climatic limits of tropical African honeybees in the Americas. Bee World 65.

Tarpy, D. R., R. Nielson, D. I. Neilson. 2004. A scientific note on the revised estimates of effective paternity frequncy in *Apis*. Insec. Soc. 51: 203-204

Tucker, K. 1986. Visible Mutants, pp. 57-77. *In* T. E. Rinderer (ed.), Bee Genetics and Breeding, Academic Press, INC., Orlando, FL, USA,

Tribe, G. 1982. Drone mating assemblies. S. Afr. Bee J. 54: 99-100.

Van Praagh, J., W. Ribi, C. Wehrhahn, and D. Wittmann. 1980. Drone bees fixate the queen with the dorsal frontal part of their compound eyes. J. Comp. Physiol. 136: 263-266.

Vergara, C., A. Dietz, A.d. Leon. 1993. Female parasitism of European honey bees by Africanized honey bee swarms in Mexico. J. Apicult. Res. 32: 34-30.

Walsh, P. S., Metzger D. A., Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506.

Williams, J.L. 1987. Wind-directed pheromone trap for drone honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 80: 532-536.

Winston, M. L. 1979. Intra-colony demography and reproductive rate of the Africanized honeybee in South America. Behav. Ecol. and Sociobiol. 4: 279-292.

Zmarlicki, C., and R. Morse. 1963. Drone congregation areas. J. Apic. Res 2: 64-66.

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