Spatial and Temporal Distribution and Nest Site Characteristics of Feral Honey Bee (Hymenoptera: Apidae) Colonies in a Coastal Prairie Landscape

KRISTEN A. BAUM,[1] WILLIAM L. RUBINK,[2] M. ALICE PINTO,[3] AND ROBERT N. COULSON

Knowledge Engineering Laboratory, Department of Entomology, Texas A&M University, College Station, TX 77843–2475

Environ. Entomol. 34(3): 610–618 (2005)

ABSTRACT We evaluated the distribution and abundance of feral honey bee, *Apis mellifera* L., colonies in a coastal prairie landscape by examining nest site characteristics, population trends, and spatial and temporal patterns in cavity use. The colony densities of up to 12.5 colonies per km^2 were the highest reported in the literature for an area including both suitable and unsuitable patches of nesting habitat. The measured cavity attributes were similar to those reported from other areas. The time occupied and turnover indices provided useful information about cavity quality, although none of the measured cavity attributes were correlated with these indices. Unmeasurable cavity characteristics, such as cavity volume, may provide a better estimate of cavity quality. Spatial patterns existed in cavity use by the feral colonies, with the colonies showing an aggregated pattern of distribution throughout the study. Colony aggregations probably resulted from the distribution of resources, especially cavities. Two years after the arrival of Africanized honey bees, cavities used by Africanized and European colonies were aggregated in distribution. During what seemed to be a transition period, both Africanized and European colonies were randomly distributed. After that time, European colonies remained randomly distributed, whereas Africanized colonies were aggregated. Therefore, the invasion of Africanized honey bees seemed to fragment the existing European population, corresponding to a decrease in the overall number of European colonies in the study area.

KEY WORDS honey bee, feral colonies, spatial distribution, nest site, cavity

THE LOCATION OF NEST sites, as well as structural and environmental characteristics of nest sites, influences the survival, growth, and reproduction of feral honey bee, *Apis mellifera* L., colonies (Seeley 1985, Ratnieks and Nowakowski 1989). Cavity volume constrains brood production and food storage and limits the number of adults in a colony (Seeley 1985, Winston 1987). Entrance size and orientation influence colony thermoregulation (Szabo 1983), whereas entrance size, height, and number influence a colony's ability to defend the nest against predators (Seeley 1985). Other factors, such as cavity exposure and visibility, also influence cavity quality for feral colonies (Winston 1987).

Several studies have identified and described the locations of feral honey bee colonies (Seeley and Morse 1976; Avitabile et al. 1978; Taber 1979; Visscher and Seeley 1982; Boreham and Roubik 1987; Schneider and Blyther 1988; Wenner 1989; Gambino et al. 1990; Morse et al. 1990; Schneider 1990; Ratnieks et al. 1991; Oldroyd et al. 1994, 1995, 1997; McNally and Schneider 1996). Many have simply estimated the density of feral colonies, whereas others have examined structural attributes of nest sites. However, few studies have evaluated spatial patterns of cavity use (Oldroyd et al. 1995, 1997; McNally and Schneider 1996), and no studies have evaluated spatial patterns through time.

Cavity selection by feral colonies has important implications for the dispersal of Africanized honey bees. Understanding nest site selection and the population ecology of feral colonies also provides insight into how Africanized honey bees will impact agricultural and beekeeping practices (McNally and Schneider 1996). Africanized honey bees, hybrids between European and African, *Apis mellifera scutellata* (Lepeletier), honey bees (Clarke et al. 2002, Pinto et al. 2005), first arrived in the United States in 1990 (Hunter et al. 1993, Rubink et al. 1996). For the purposes of this study, European refers to the existing honey bee population in the United States before the arrival of Africanized honey bees. The background population consisted of a variety of subspecies of *A. mellifera*, mostly from Europe (Sheppard 1989a, b).

The goal of this study was to evaluate spatial and temporal patterns in the distribution and abundance

[1] Current address: Department of Biological Sciences, 206 Life Sciences Bldg., Louisiana State University, Baton Rouge, LA 70803–1715.

[2] P.O. Box 2686, Edinburg, TX 78540.

[3] Current address: Departamento Florestal, Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 172, 5301-854 Bragança, Portugal.

0046-225X/05/0610–0618$04.00/0

of feral honey bee colonies in a coastal prairie landscape by examining nest site characteristics, population trends, and cavity use. We studied feral colonies on the Welder Wildlife Refuge (San Patricio County, TX) over an 8-yr period, during which Africanized honey bees arrived and became established in the area. Specific objectives were 1) to compare the density of feral colonies in this study with densities reported in the literature, 2) to evaluate cavity suitability for feral honey bees based on structural and environmental attributes of the cavities, 3) to compare the structural and environmental attributes of cavities occupied only by Africanized or only by European colonies, 4) to examine spatial and temporal patterns in cavity use by the feral colonies, and 5) to examine the spatial and temporal distribution of Africanized and European colonies.

Materials and Methods

Study Site Description. The study site was located on the Welder Wildlife Refuge (San Patricio County, Texas), where Africanized honey bees were first recorded in 1993 (Pinto et al. 2004). The study area consisted of 6.25 km^2 of coastal prairie located in the western one-quarter of the refuge. The landscape was dominated by four vegetation communities that varied in the availability of important resources necessary for the survival, growth, and reproduction of feral colonies (Baum 2003). Cavities were abundant in the live oak and riparian woodland habitat patches but scarce in the brushland and grassland habitat patches. The distribution and abundance of food resources fluctuated throughout the year, with the live oak and brushland habitat patches providing the best overall sources of pollen and nectar. However, the grassland habitat patches were an important source of pollen and nectar during the winter (Baum 2003, Baum et al. 2004).

Colony Density. The study area was surveyed for feral honey bee colonies between 1993 and 2000. Surveys were conducted by evaluating the status of cavities identified during previous surveys to contain feral honey bees. We also searched for new cavities and colonies by inspecting all trees within the study area for honey bees (foragers leaving or returning to a colony) during periods of high honey bee activity. During this 8-yr period, 109 cavities containing a feral colony during at least one survey were identified. The density of feral colonies per square kilometer during each year was calculated based on yearly cavity surveys. The presence (active cavity) or absence (inactive cavity) of feral colonies in the identified cavities was recorded. Cavities were often surveyed multiple times during any given year, so the status (active or inactive) of each cavity during the survey from each year with the most active cavities was used for the calculations. The number of cavities surveyed increased through time as new cavities used by feral honey bee colonies were found. Densities are reported from 1993 through 2000, although densities before 1995 are probably underestimated because $<$30% of the cavities surveyed were found by then.

Cavity Attributes. Most of the feral colonies in the study area were located in tree cavities. Therefore, we collected detailed measurements for each cavity, including number of entrances, entrance orientation, entrance size, cavity height, tree species, tree diameter at breast height (dbh), tree height, basal area, canopy closure, ground cover, habitat type, and motte size. Mottes are clusters of woody vegetation that form around a nucleus (in this case a live oak tree, *Quercus virginiana* P. Miller) and may eventually expand and coalesce into a contiguous area of woody vegetation. Entrance number and tree species were obtained by visual inspection. Entrance orientation was recorded using a compass. The width and height of each cavity entrance were measured with a tape measure or estimated in centimeters when it was not possible to reach the cavity entrance. Entrance width and height were then converted into entrance area based on the area of an ellipse. Cavity height was measured in m from ground level by using a tape measure or visually estimated for high cavities. The dbh was calculated in centimeters by using a Spencer Original LoggersTape. Tree height was recorded in meters by using a Suunto clinometer with 15- and 20-m scales. Basal area was obtained using the five-factor option of a JIM-GEM Cruz-All. Canopy closure and ground cover (separated into monocot and dicot) were estimated at 10 m from the cavity tree in the four Cardinal directions. Estimates of percentage of cover were made by looking through a 5-cm-diameter by 10.5-cm-long hollow tube divided into four quadrants. Percentage of canopy closure and ground cover were averaged across all directions to obtain an overall value for each cavity tree. Habitat type was identified from a landscape classification of the study area based on vegetation communities, and motte size was obtained from a spatial database of the study area with a resolution of 0.25 m (Baum 2003). The boundaries of each live oak motte were digitized in ArcView GIS 3.2, and the area of the resulting polygons was calculated using an extension (area calculation for polygon) in ArcView GIS 3.2. Motte size was only measured for live oaks because none of the other cavity tree species formed distinct mottes.

Cavity characteristics were evaluated in terms of time occupied and turnover indices calculated for each cavity. Time occupied refers to the proportion of the time surveyed that a cavity was active, whereas turnover reflects the number of changes in cavity status from active to inactive or inactive to active during consecutive surveys. Therefore, these indices provide an estimate of cavity quality based on honey bee use. Only cavities identified by 1995 were included in the analyses because the values for cavities surveyed only a few times may be biased, and 80% of cavities had been found by then. For example, a cavity surveyed only once (first identified during the most recent survey) would be occupied 100% of the time with 0% turnover.

Spatial and Temporal Patterns. The spatial coordinates for each cavity tree used by feral colonies during the 8-yr study period were recorded to a submeter

Table 1. Spatial and temporal patterns of cavity use and colony density for each year based on a 6.25-km^2 study area

Yr	Sample size[a]	Mean nnd ±	Mean rand	Mean disp	Nn index	Z	Distribution	Density[a] (no./km^2)
1993	25	112.46 ± 126.09	192.45	413.58	0.584	−3.976	aggregated	3.84 (24)
1994	62	76.14 ± 68.75	142.55	306.34	0.534	−7.018	aggregated	9.60 (60)
1995	81	64.60 ± 65.34	124.71	268.02	0.518	−8.299	aggregated	12.16 (76)
1996	32	120.71 ± 112.33	177.64	381.76	0.680	−3.468	aggregated	4.80 (30)
1997	35	133.18 ± 142.17	182.46	392.11	0.730	−3.057	aggregated	5.28 (33)
1998	38	117.11 ± 128.02	174.5	375.01	0.671	−3.879	aggregated	6.56 (41)
1999	61	83.15 ± 94.43	141.61	304.33	0.587	−6.169	aggregated	7.68 (48)
2000	76	82.71 ± 86.5	127.5	274.01	0.649	−5.859	aggregated	12.48 (78)

[a] Colony density was calculated based on the status (active/inactive) of each cavity during the survey from each year with the most active cavities. Honey bee samples for mtDNA analysis were collected from colonies from any cavity active at anytime throughout the year (Pinto et al. 2004), so sample sizes differ from those used to calculate colony density.

Abbreviations are as follows: nearest neighbor distance (nnd), nearest neighbor distance expected for a random distribution (rand), nearest neighbor distance expected for a dispersed distribution (disp), the nearest neighbor index (nn index); a Z statistic was used to test for significance; resulting distributions can be random (nn index values close to 1.0), dispersed (nn index values >1.0), or aggregated (nn index values <1.0).

accuracy by using a Trimble GPS Pathfinder receiver and TSC1 Asset Surveyor data logger. When cavities were located in areas with dense canopy cover, an Advantage Laser Rangefinder was used to calculate the offset from the cavity to where spatial coordinates were obtained.

Mitochondrial DNA (mtDNA) Analysis. mtDNA is maternally inherited and does not recombine during sexual reproduction, passing directly from queen to offspring. Thus, mtDNA provides a historical perspective on the invasion of Africanized honey bees, highlighting what happened to the existing and invading colonies and their subsequent offspring (Schneider et al. 2004). Within our study site, the pattern of mtDNA is closely followed by the nuclear pattern (Pinto et al. 2005), suggesting that mtDNA also may reflect what happened at the nuclear level (Pinto et al. 2005). However, mtDNA only represents the maternal side of the Africanization process, and nuclear DNA may provide additional insights into the invasion process that were not available for this study (Schneider et al. 2004). Honey bee samples for mtDNA analysis were collected from colonies from any cavity active at anytime throughout the year (Pinto et al. 2004). Therefore, sample sizes differ from those used to calculate colony density. After extraction of total DNA from the thorax of a single adult worker per colony, as detailed in Pinto et al. (2004), a 485-bp section of the cytochrome *b* gene (Crozier et al. 1991) was amplified in a 5-μl total volume containing 0.5× *Taq*DNA polymerase buffer (Promega, Madison, WI), 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 2 pM of each primer, 0.5 μl of template DNA, and 0.25 U of *Taq*DNA polymerase (Promega). The polymerase chain reaction (PCR) temperature profile was 94°C for 3 min followed by 30 cycles of 94°C for 15 s, 50°C for 15 s, and 68°C for 5 s. After the final cycle, an additional 10 min at 72°C was performed. After PCR, samples were digested with the restriction enzyme *Bgl*II by using the temperature and buffer conditions recommended by the supplier. The total digestion volume was then electrophoresed on a 2% agarose/Tris borate-EDTA gel, stained with ethidium bromide, and visualized under UV light. Based on the visualization of a one-band pattern or two-band pattern on the gel, the type of mtDNA (mitotype) was scored as *A. m. scutellata* (referred to as Africanized throughout this article) or non-*A. m. scutellata* (referred to as European throughout this article; Pinto et al. 2003).

Statistical Analyses. We used a Spearman rank correlation coefficient to identify cavity characteristics correlated with the time occupied and turnover indices. A χ^2 test applied to circular distributions (Batschelet 1965) was used to examine whether there were any patterns in entrance orientation, by using eight groups at equal intervals. We used a Kruskal–Wallis test to compare the time occupied and turnover indices between habitat types. A Mann–Whitney *U* test was performed to compare cavity characteristics between cavities used only by Africanized and only by European honey bee colonies (Sokal and Rohlf 1995, SAS Institute 1998).

We used a nearest neighbor analysis to compare observed patterns of cavity occupancy with those expected by chance. The nearest neighbor index (NNI) was calculated by comparing the mean observed nearest neighbor distance with the mean expected nearest neighbor distance for spatially random points (Clark and Evans 1954). We used CrimeStat version 2.0 (Levine 2002) for the calculations. Values close to 1.0 indicate observed average distances do not differ from random, whereas values <1.0 indicate aggregation and values >1.0 indicate dispersion. A Z test was used to identify significant values of the NNI. We performed the nearest neighbor analysis for all occupied colonies and then separately for Africanized and European colonies.

Results

Colony Density and Cavity Attributes. Colony density ranged from 3.8 to 12.5 colonies per km^2 from 1995 through 2000 (Table 1). Densities were lowest in 1993 and 1996 and highest in 1995 and 2000. Cavities used by feral colonies were located in live oak (*Q. virginiana;* $n = 93$), hackberry (*Celtis* spp.; $n = 8$), anacua [*Ehretia anacua* (M. Terán & J. Berlandier) I. M. Johnston; $n = 3$], cedar elm (*Ulmus crassifolia* T.

Table 2. Descriptive statistics for the measured structural and environmental attributes of cavities occupied by feral honey bee colonies on the Welder Wildlife Refuge

	Mean ± SE	Min.	Max.	Sample size
Entrance ht (m)	2.52 ± 0.18	0	7.6	92
Entrance no.	1.15 ± 0.05	1	5	104
Entrance size[a] (cm^2)	42.10 ± 8.73	0.8	544.3	91
dbh (cm)	74.96 ± 2.73	30	184.5	106
Tree ht (m)	11.80 ± 0.32	7	25	106
Basal area (m^2/ha)	35.09 ± 1.48	5	80	107
Canopy closure (%)	50.94 ± 2.20	0	91.3	107
Ground cover monocot (%)	28.22 ± 1.95	0	88.5	108
Ground cover dicot (%)	16.03 ± 0.95	0	48.8	108
Motte size (m^2)	351.23 ± 41.67	20.2	2,115.7	79

[a] Based on the area of an ellipse using entrance width and height.

Nuttall; $n = 1$), and mulberry (*Morus rubra* C. Linnaeus; $n = 1$) trees. Cavity entrance height varied from ground level to 7.6 m (Table 2). Most cavities only had one entrance, although 12 cavities had two to five entrances. The entrances of cavities used by feral colonies most often faced the northeastern and southwestern directions and differed significantly from random ($\chi^2 = 16.92$, df = 7, $P = 0.018$). Entrance size was extremely variable but on average was 42.10 cm^2 (Table 2). The dense live oak habitat patches contained 56 cavities (51%), the open live oak habitat patches contained 36 cavities (33%), the woodland habitat patches contained 16 cavities (15%), and the brushland–grassland habitat patches contained only one cavity (1%; Fig. 1).

Nine cavities were occupied for >80% of the surveys (Fig. 2). Five cavities were occupied only during the survey in which they were first found. Turnover was relatively low, ranging from 5 to 30% of surveys. However, no cavities were occupied continuously throughout the surveys (Fig. 3).

None of the measured cavity characteristics was significantly correlated with the time occupied or turnover indices ($P \geq 0.082$). None of the measured cavity site characteristics was significantly different between cavities only used by Africanized colonies and only used by European colonies ($P \geq 0.140$).

Spatial and Temporal Patterns. Occupied cavities were aggregated in distribution for all years examined (Table 1). An analysis of spatial patterns by mitotype yielded different results (Table 3). The first colony of *A. m. scutellata* maternal descent (Africanized honey bee colony) in the study area was identified in 1993. At that time, colonies of non-*A. m. scutellata* maternal origin (European honey bee colonies) were aggregated in distribution. In 1994, three Africanized colonies were found and European colonies were aggregated in distribution. The distributions of European and Africanized colonies were aggregated in 1995, when 82.7% of the colonies were European. In 1996, there were equal numbers of European and Africanized colonies, both with aggregated distributions. By 1997, 62.9% of the colonies were Africanized, and both European and Africanized colonies were randomly distributed. From 1998 through 2000, 73.7, 80.3, and 80.3% of colonies were Africanized. For each of these years, the distribution of Africanized honey bee colonies was aggregated and the distribution of European honey bee colonies was random (Table 3).

Discussion

Colony Density. The densities of up to 12.5 colonies per km^2 observed for this study were the highest reported to date for an area including both suitable and unsuitable patches of nesting habitat (see review of colony density in Table 1 of Ratnieks et al. 1991, as well as more recent data in Oldroyd et al. 1994, 1997 and McNally and Schneider 1996). The live oak and riparian woodland habitat patches were the only areas providing suitable cavities, and these habitat patches comprised 44% of the study area (Fig. 1). Oldroyd et al. (1994) reported a density of 77.1 colonies per km^2 but only considered a narrow swath of suitable nesting habitat 100 m in width. When considering a square area (1 km by 1 km), the density is actually 7.71 colonies per km^2, with suitable habitat comprising only 10% of the total area. Data reported in Kerr (1971) were omitted for similar reasons (Ratnieks et al. 1991) and because detailed information was not available to convert the data to a comparable format. Based on these considerations, the previously reported highest densities were 7.8 (Schneider and Blyther 1988), 7.7 (Oldroyd et al. 1994), and 7.1 (Boreham and Roubik 1987) colonies per km^2. Therefore, the highest density reported for this study is higher than previously reported densities of feral colonies.

In general, the study area represents highly suitable habitat for feral honey bee colonies. Cavity density is high in certain areas, and pollen and nectar sources are abundant throughout most of the year (Baum 2003, Baum et al. 2004). Conservative estimates of annual pollen and nectar production for plants in the study area based on abundance and growth form suggest that 1,895 feral colonies could be supported by pollen sources and 244 feral colonies could be supported by nectar sources within the study area, based on the annual resource requirements of a typical feral colony (Baum 2003). These estimates increase to 3,161 feral colonies for pollen sources and 407 feral colonies for nectar sources when the area is expanded beyond the study site boundaries to include the entire potential foraging range of the feral colonies, based on the spatial locations of cavities and a foraging radius of 800 m (Baum 2003). The densities reported in this study are high for natural areas (Ratnieks et al. 1991, Oldroyd et al. 1994, McNally and Schneider 1996). High densities also may occur in urban landscapes where honey bee colonies nest in human made structures and landscaping practices provide pollen and nectar during natural periods of resource dearth (K.A.B, M. D. Tchakerian, S. C. Thoenes, and R.N.C., unpublished data).

Cavity Attributes. The most common tree genus used by feral colonies in this study, *Quercus*, also was frequently used in other areas (Seeley and Morse 1976, Avitabile et al. 1978, Gambino et al. 1990). Feral

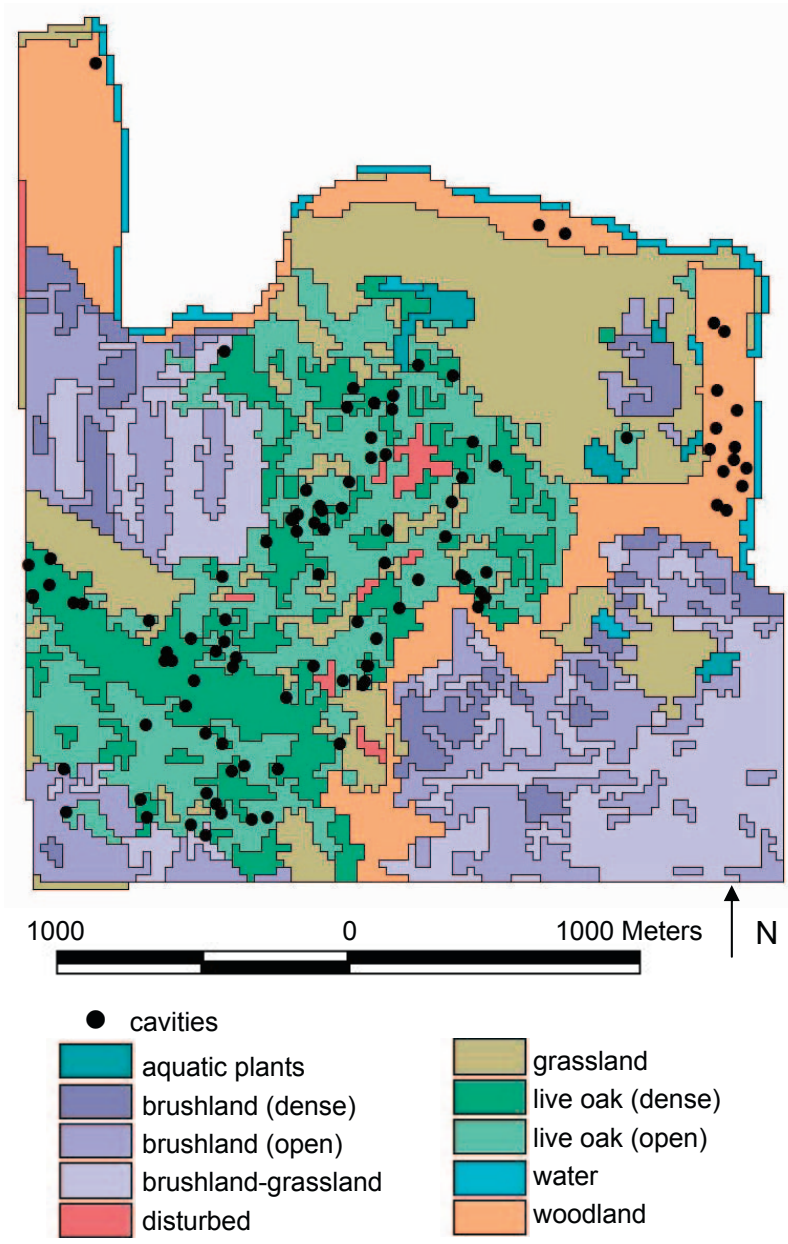

Fig. 1. Location of all identified cavities used by feral honey bee colonies within each habitat patch type on the Welder Wildlife Refuge.

colonies usually occupied cavities located in living trees (Avitabile et al. 1978, Gambino et al. 1990, Oldroyd et al. 1994), although Seeley and Morse (1976) reported 25% of colonies using cavities in dead trees. Most occupied cavities had a single entrance, which also was reported in other studies (Seeley and Morse 1976, Avitabile et al. 1978, Gambino et al. 1990). Entrance height varied, but may be more a function of available options than a preference for the reported heights. Entrances recorded in this study were smaller than those reported by others (Avitabile et al. 1978, Gambino et al. 1990).

Usually, the observed cavity attributes were similar to those reported from other areas. However, cavity constraints on feral colonies vary depending on geographic location. For example, tropically adapted Africanized honey bees typically have smaller colony sizes and store less honey than temperately adapted European honey bees (Winston et al. 1981). Therefore, Africanized colonies often use smaller cavities than European colonies (Seeley and Morse 1976, Seeley 1977). These differences highlight selection pressures faced by feral honey bees in different geographic locations (Winston et al. 1983).

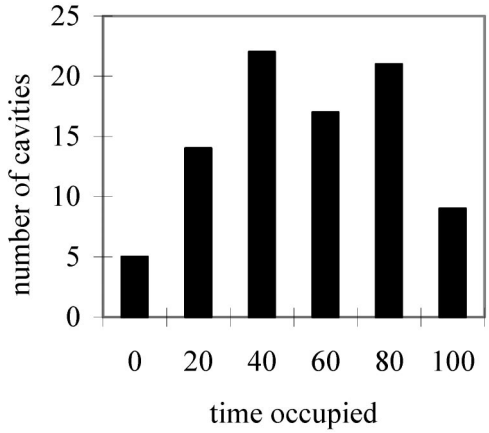

Fig. 2. Frequency distribution of the time occupied index for all cavities used by feral honey bee colonies identified by 1995 on the Welder Wildlife Refuge. Time occupied refers to the proportion of the time surveyed that a cavity was active (contained a colony).

Preferences for different nest site characteristics have been proposed for Africanized and European honey bees. Schmidt and Hurley (1995) reported that Africanized honey bees showed no preference for cavity sizes ranging from 13.5 to 30 liters, whereas European honey bees preferred larger cavity sizes. However, no differences were found in the structural and environmental attributes of cavities occupied by Africanized or European colonies in this study. Cavity volume could not be measured, so perhaps differences do exist in volume between cavities used by Africanized and European colonies in the study area. To date, selection for volume and shape (Schmidt and Thoenes 1992) are the only nest site characteristics that have been compared between European and Africanized colonies.

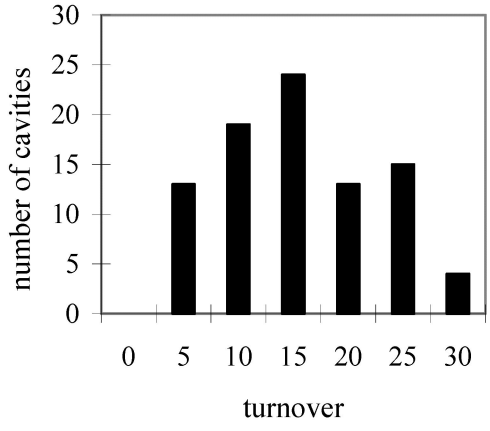

Fig. 3. Frequency distribution of the turnover index for all cavities used by feral honey bee colonies identified by 1995 on the Welder Wildlife Refuge. Turnover reflects the number of changes in cavity status from active (contained a colony) to inactive (did not contain a colony) or inactive to active on consecutive surveys.

With the exception of Taber (1979), no other published studies have examined cavity occupancy through time. The time occupied and turnover indices provide different information about the quality of a cavity. Cavities occupied a majority of the time, but with high turnover rates, may not be as suitable for feral colonies as cavities occupied for long periods of time with little or no turnover. Therefore, together these indices provide an estimate of overall cavity quality. However, none of the measured cavity attributes were correlated with the time occupied and turnover indices, so cavities do not seem to vary in their suitability for honey bees based on the measured structural and environmental attributes. Other studies have documented preferences for certain nest site characteristics. For example, colonies selected nest sites 5 m and 3 m off the ground over nest sites 1 m off the ground (Seeley and Morse 1978, Schmidt and Thoenes 1987). However, these preferences have seldom been related directly to corresponding data on colony survival, growth, and reproduction and are not comparable to this study in terms of the time occupied and turnover indices. It was not possible to measure cavity volume, which is perhaps the most important cavity attribute, or at least the best documented in terms of honey bee preferences.

Spatial and Temporal Patterns. The distribution of feral colonies was aggregated throughout the time period of this study, so spatial patterns do exist in cavity use by the feral colonies (Table 1). However, few studies have reported aggregations of *A. mellifera* (but see Oldroyd et al. 1995, 1997 for European colonies in Australia; McNally and Schneider 1996 for African colonies in Botswana, Africa). The lack of reported aggregations by *A. mellifera* suggests that swarms tend to disperse, suitable nest sites are not common, or few surveys have been conducted for feral colonies (Oldroyd et al. 1995).

Swarm dispersal distances range from a few hundred meters to 10,000 m (Schmidt and Thoenes 1990, Schneider 1995, Camazine et al. 1999), and Otis et al. (1981) estimated maximum flight distances based on engorgement and metabolic rates as 64 km for reproductive swarms and 131 km for absconding colonies. Some studies have reported swarms selecting nearby nest sites (Jaycox and Parise 1980, 1981), but others have reported swarms selecting more distant sites (Seeley and Morse 1977). These ambiguous results probably represent genetic differences between the colonies studied and/or local patterns of resource availability (Winston 1987). Colony aggregations also may result from the attraction of swarms to existing colonies not near the parent colony (Oldroyd et al. 1995). These different scenarios can be evaluated by examining the relatedness of colonies in aggregations. Related colonies would support the short dispersal distance scenario and unrelated colonies would support the attraction scenario. Oldroyd et al. (1995) found some completely unrelated colonies in aggregations in Wyperfeld National Park, northwestern Victoria, Australia, rejecting the explanation of short dispersal distances. The familial relationships among

Table 3. Spatial and temporal patterns identified by a nearest neighbor analysis of cavities occupied by colonies with African mtDNA (A) and non-African mtDNA (E) on the Welder Wildlife Refuge

Sample	Sample size	Mean nnd ± SD	Mean rand	Mean disp	Nn index	Z	Distribution
1993A	1	na	na	na	na	na	na
1993E	24	95.97 ± 116.92	182.47	392.13	0.526	−4.443	Aggregated
1994A	3	na	na	na	na	na	na
1994E	59	78.28 ± 71.69	146.13	314.04	0.536	−6.823	Aggregated
1995A	14	130.24 ± 121.42	213.86	459.6	0.609	−2.799	Aggregated
1995E	67	77.02 ± 68.22	135.23	290.61	0.570	−6.740	Aggregated
1996A	16	171.97 ± 142.43	230.81	496.03	0.745	−1.951	Aggregated
1996E	16	187.21 ± 190.93	239.89	515.55	0.780	−1.681	Aggregated
1997A	22	165.11 ± 171.5	201.93	433.95	0.818	−1.636	Random
1997E	13	278.39 ± 148.03	263.98	567.31	1.055	0.377	Random
1998A	28	116.25 ± 143.72	182.41	392.01	0.637	−3.672	Aggregated
1998E	10	288.61 ± 151.37	247.23	531.31	1.167	1.013	Random
1999A	49	91.44 ± 104.49	158	339.56	0.579	−5.642	Aggregated
1999E	12	161.59 ± 118.24	168.23	361.54	0.961	−0.261	Random
2000A	61	84.42 ± 93.87	141.61	304.33	0.596	−6.035	Aggregated
2000E	15	192.57 ± 201.44	193.03	414.84	0.998	−0.018	Random

na, not applicable (sample size too small for analysis).

Abbreviations are as follows: nearest neighbor distance (nnd), nearest neighbor distance expected for a random distribution (rand), nearest neighbor distance expected for a dispersed distribution (disp), the nearest neighbor index (nn index); a Z statistic was used to test for significance; resulting distributions can be random (nn index values close to 1.0), dispersed (nn index values >1.0), or aggregated (nn index values <1.0).

colonies have not been examined on the Welder Wildlife Refuge. However, four different mitotypes have been identified (Pinto et al. 2004). The number of colonies with different mitotypes through time does not seem to support the short dispersal distance (related colonies) scenario, although low colony or swarm survival could conceal this pattern.

Jaycox and Parise (1980, 1981) and Seeley and Morse (1977) suggested that swarms select nearby cavities when cavity availability is high. Therefore, colony aggregations would be expected when cavities are abundant. Oldroyd et al. (1994) estimated up to 11,000 hollows per km^2 within the same study area used by Oldroyd et al. (1995), suggesting an abundance of cavities. They also reported that nectar and pollen sources are abundant. However, the area surveyed only formed a 100-m-wide swath of suitable habitat (Oldroyd et al. 1995), so cavities may be uncommon at a broader spatial scale (larger extent). The same conclusion could be drawn for the suitable habitat on the Welder Wildlife Refuge. Although suitable habitat is abundant within the refuge and several adjoining counties, live oak mottes, the main cavity source for feral honey bee colonies, are not abundant at a larger scale. Based on vegetation communities defined by McMahan et al. (1984), cavities probably are available in the Texas coastal bend (including the counties of Gonzales, Lavaca, Dewitt, Victoria, Jackson, Goliad, Calhoun, McMullen, Live Oak, Bee, Refugio, Aransas, San Patricio, Duval, Jim Wells, Nueces, Kleberg, Brooks, and Kenedy) in the mesquite–live oak–bluewood parks, live oak woods and parks, post oak woods, forest and grassland mosaic, and post-oak woods and forest. These habitats comprise 22% of the area, whereas the highly suitable habitat of mesquite–live oak–bluewood parks found on the Welder Wildlife Refuge make up only 2% of the Texas coastal bend region. Thus, the distribution of cavity sources is patchy and potentially rare at a broader spatial scale (large extent).

In addition to dispersal behavior and resource distributions, other proposed hypotheses to explain colony aggregations include predator defenses and mating efficiency (Seeley et al. 1982, Oldroyd et al. 1995). There is controversy over whether aggregations would serve to decrease or increase the probability of predation (Seeley et al. 1982, Oldroyd et al. 1995). However, aggregations may increase predator detection because colonies may become alerted when a nearby colony is disturbed (Seeley et al. 1982). Possible predators on honey bee colonies that are present on the Welder Wildlife Refuge include skunks, birds, opossums, shrews, armadillos, and invertebrates, such as wasps, ants, and moths (Winston 1987). However, these animals probably have a minimal impact on the feral colonies because most are located in tree cavities several meters off the ground with relatively small entrances. Therefore, the observed aggregated pattern probably does not result from predator defenses.

Last, aggregations may increase mating efficiency by decreasing the distance to drone congregation areas. In the case of unrelated aggregations, the probability of mating with brothers also would be decreased (Oldroyd et al. 1995). Mating with brothers results in diploid males and reduces brood viability (Page 1980). Therefore, mechanisms that decrease the probability of mating with brothers should be selected for, such as multiple matings and unrelated aggregations.

When examining the spatial and temporal patterns of Africanized and European colonies, we found that cavities used by both colony types were aggregated during 1995 and 1996, and randomly distributed during 1997 (Table 3). However, from 1998 through 2000, Africanized colonies were aggregated and European colonies were randomly distributed (Table 3). There-

fore, spatial patterns differ between Africanized and European colonies, and these patterns vary through time.

An analysis of spatial patterns of mtDNA follows the maternal ancestry of colonies through the invasion process, as Africanized honey bees arrived and became established in an area with an existing feral population of European honey bees. However, mtDNA does not reflect paternal contributions to the invasion process (Schneider et al. 2004), although the pattern of mtDNA is closely followed by the nuclear pattern within our study system (Pinto et al. 2005). After the initial 2 yr (1993 and 1994) when sample sizes of Africanized colonies were too small to evaluate spatially, Africanized honey bees were aggregated, with the exception of 1997. The 1997 sampling year seems to be a transition period, with the random distribution of Africanized and European colonies. After that time, European colonies remained randomly distributed, while Africanized colonies were aggregated. Therefore, the invasion of Africanized honey bees seem to have fragmented the existing European population, corresponding to a decrease in the overall number of European colonies in the study area.

Acknowledgments

We are grateful to J. S. Johnston for providing the facilities for the molecular analysis. A. Bunting, L. Ross, and M. Tchakerian provided valuable technical assistance throughout this project. We are grateful to A. Cavazos, R. Medrano, J. Teer, L. Drawe, T. Blankenship, and S. Glasscock for assistance in different phases of the project at the Welder Wildlife Refuge. Funding for this project was provided by the Welder Wildlife Foundation, the Beneficial Insects Research Unit, Honey Bee Group, USDA–ARS, and the Texas Legislative Initiative: Protection and Management of Honey Bees - Pollinators of Agricultural Crops, Orchards, and Natural Landscapes. K.A.B. was supported by a Welder Wildlife Foundation fellowship and the Texas Legislative Initiative: Protection and Management of Honey Bees - Pollinators of Agricultural Crops, Orchards, and Natural Landscapes. M.A.P. was supported by the Escola Superior Agrária de Bragança and the European Union program PRODEP II (Medida 5/Acção 5.3). This is contribution #638 of the Welder Wildlife Foundation.

References Cited

Avitabile, A., D. P. Stafstrom, and K. J. Donovan. 1978. Natural nest sites of honey bee colonies in trees in Connecticut, USA. J. Apic. Res. 17: 222–226.

Batschelet, E. 1965. Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. American Institute of Biological Sciences, Washington, DC.

Baum, K. A. 2003. Feral Africanized honey bee ecology in a coastal prairie landscape. Ph.D. dissertation, Texas A&M University, College Station.

Baum, K. A., W. L. Rubink, R. N. Coulson, and V. M. Bryant. 2004. Pollen selection by feral honey bee (Hymenoptera: Apidae) colonies in a coastal prairie landscape. Environ. Entomol. 33: 727–739.

Boreham, M. M., and D. W. Roubik. 1987. Population changes and control of Africanized honey bees (Hymenoptera: Apidae) in the Panama Canal area. Bull. Entomol. Soc. Am. 33: 34–39.

Camazine, S., P. K. Visscher, J. Finley, and R. S. Vetter. 1999. House-hunting by honey bee swarms: collective decisions and individual behaviors. Insect Soc. 46: 348–360.

Clark, P. J., and F. C. Evans. 1954. Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology 35: 445–453.

Clarke, K. E., T. E. Rinderer, P. Franck, J. G. Quezada-Euán, and 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. C., S. Koulianos, and R. H. Crozier. 1991. An improved test for Africanized honey bee mitochondrial DNA. Experientia 47: 968–969.

Gambino, P., K. Hoelmer, and H. V. Daly. 1990. Nest sites of feral honey bees in California, USA. Apidologie 21: 35–45.

Hunter, L. A., J. A. Jackman, and E. A. Sugden. 1993. Detection records of Africanized honey bees in Texas during 1990, 1991 and 1992. Southwest. Entomol. 18: 79–89.

Jaycox, E. R., and S. G. Parise. 1980. Homesite selection by Italian honey bee swarms *Apis mellifera ligustica* (Hymenoptera: Apidae). J. Kans. Entomol. Soc. 53: 171–178.

Jaycox, E. R., and S. G. Parise. 1981. Homesite selection by swarms of black-bodied honeybees, *Apis mellifera caucasica* and *A. m. carnica* (Hymenoptera: Apidae). J. Kans. Entomol. Soc. 54: 697–703.

Kerr, W. E. 1971. Contribuição à ecogenética de algumas espécies de abelhas. Ciência Cultura 23 (suppl): 89–90.

Levine, N. 2002. CrimeStat II: a spatial statistics program for the analysis of crime incident locations (version 2.0). Ned Levine & Associates, Houston, Texas, USA and the National Institute of Justice, Washington, D.C.

McMahan, C. A., R. G. Frye, and K. L. Brown. 1984. The vegetation types of Texas. Texas Parks and Wildlife Department, Austin, TX.

McNally, L. C., and S. S. Schneider. 1996. Spatial distribution and nesting biology of colonies of the African honey bee *Apis mellifera scutellata* (Hymenoptera: Apidae) in Botswana, Africa. Environ. Entomol. 25: 643–652.

Morse, R. A., S. Camazine, M. Ferracane, P. Minacci, R. Nowogrodzki, F.L.W. Ratnieks, J. Spielholz, and B. A. Underwood. 1990. The population density of feral colonies of honey bees (Hymenoptera: Apidae) in a city in upstate New York. J. Econ. Entomol. 83: 81–83.

Oldroyd, B. P., S. H. Lawler, and R. H. Crozier. 1994. Do feral honey bees (*Apis mellifera*) and regent parrots (*Polytelis anthopeplus*) compete for nest sites? Aust. J. Ecol. 19: 444–450.

Oldroyd, B., A. Smolenski, S. Lawler, A. Estoup, and R. Crozier. 1995. Colony aggregations in *Apis mellifera* L. Apidologie 26: 119–130.

Oldroyd, B. P., E. G. Thexton, S. H. Lawler, and R. H. Crozier. 1997. Population demography of Australian feral bees (*Apis mellifera*). Oecologia (Berl.) 111: 381–387.

Otis, G. W., M. L. Winston, and O. R. Taylor, Jr. 1981. Engorgement and dispersal of Africanized honeybee swarms. J. Apic. Res. 20: 3–12.

Page, R. E. 1980. The evolution of multiple mating behavior by honey bee queens (*Apis mellifera* L.). Genetics 96: 263–273.

Pinto, M. A., J. S. Johnston, W. L. Rubink, R. N. Coulson, J. C. Patton, and W. S. Sheppard. 2003. Identification of Africanized honey bee (Hymenoptera: Apidae) mitochondrial DNA: validation of a rapid PCR-based assay. Ann. Entomol. Soc. Am. 96: 679–684.

Pinto, M. A., W. L. Rubink, R. N. Coulson, J. C. Patton, and J. S. Johnston. 2004. Temporal pattern of Africanization in a feral honeybee population from Texas inferred from mitochondrial DNA. Evolution 58: 1047–1055.

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 honey bees (*Apis mellifera* L.) by an African hybrid swarm. Genetics (in press).

Ratnieks, F.L.W., and J. Nowakowski. 1989. Honey bee swarms accept bait hives contaminated with American foulbrood. Ecol. Entomol. 14: 475–478.

Ratnieks, F.L.W., M. A. Piery, and I. Cuadriello. 1991. The natural nest and nest density of the Africanized honey bee (Hymenoptera, Apidae) near Tapachula, Chiapas, Mexico. Can. Entomol. 123: 353–359.

Rubink, W. L., P. Luévano-Martinez, E. A. Sugden, W. T. Wilson, and A. M. Collins. 1996. Subtropical *Apis mellifera* (Hymenoptera: Apidae) swarming dynamics and Africanization rates in northeastern Mexico and southern Texas. Ann. Entomol. Soc. Am. 89: 243–251.

SAS Institute. 1998. StatView: StatView reference, 2nd ed. SAS Institute, Cary, NC.

Schmidt, J. O., and R. Hurley. 1995. Selection of nest cavities by Africanized and European honey bees. Apidologie 26: 467–475.

Schmidt, J. O., and S. C. Thoenes. 1987. Swarm traps for survey and control of Africanized honey bees. Bull. Entomol. Soc. Am. 33: 155–158.

Schmidt, J. O., and S. C. Thoenes. 1990. The efficiency of swarm traps: what percent of swarms are captured and at what distance from the hive? Am. Bee J. 130: 811–812.

Schmidt, J. O., and S. C. Thoenes. 1992. Criteria for nest site selection in honey bees (Hymenoptera: Apidae): preferences between pheromone attractants and cavity shapes. Environ. Entomol. 21: 1130–1133.

Schneider, S., and R. Blyther. 1988. The habitat and nesting biology of the African honey bee *Apis mellifera scutellata* in the Okavango River Delta, Botswana, Africa. Insect Soc. 35: 167–181.

Schneider, S. S. 1990. Nest characteristics and recruitment behavior of absconding colonies of the African honey bee, *Apis mellifera scutellata*, in Africa. J. Insect Behav. 3: 225–240.

Schneider, S. S. 1995. Swarm movement patterns inferred from waggle dance activity of the neotropical African honey bee in Costa Rica. Apidologie. 26: 395–406.

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

Seeley, T. D. 1977. Measurement of nest cavity volume by the honey bee *Apis mellifera.* Behav. Ecol. Sociobiol. 2: 201–227.

Seeley, T. D. 1985. Honeybee ecology. Princeton University Press, Princeton, NJ.

Seeley, T. D., and R. A. Morse. 1976. The nest of the honey bee (*Apis mellifera* L.). Insect Soc. 23: 495–512.

Seeley, T. D., and R. A. Morse. 1977. Dispersal behavior of honey bee swarms. Psyche 83: 199–209.

Seeley, T. D., and R. A. Morse. 1978. Nest site selection by the honey bee, *Apis mellifera.* Insect Soc. 25: 323–337.

Seeley, T. D., R. H. Seeley, and P. Akratanakul. 1982. Colony defense strategies of the honeybees in Thailand. Ecol. Monogr. 52: 43–63.

Sheppard, W. S. 1989a. A history of the introduction of honey bee races in the United States: part I of a two-part series. Am. Bee J. 129: 617–619.

Sheppard, W. S. 1989b. A history of the introduction of honey bee races in the United States: part II of a two-part series. Am. Bee J. 129: 664–667.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd ed. W. H. Freeman and Company, New York

Szabo, T. I. 1983. Effects of various entrances and hive direction on outdoor wintering of honey bee colonies. Am. Bee J. 123: 47–49.

Taber, S., III. 1979. A population of feral honey bee colonies. Am. Bee J. 118: 842–847.

Visscher, P. K., and T. D. Seeley. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63: 1790–1801.

Wenner, A. M. 1989. "Bee-lining" and ecological research on Santa Cruz Island. Am. Bee J. 129: 808–809.

Winston, M. L. 1987. The biology of the honey bee. Harvard University Press, Cambridge, MA.

Winston, M. L., J. A. Dropkin, and O. R. Taylor. 1981. Demography and life history characteristics of two honey bee races (*Apis mellifera*). Oecologia (Berl.) 48: 407–413.

Winston, M. L., O. R. Taylor, and G. W. Otis. 1983. Some differences between temperate European and tropical African and South American honeybees. Bee World 64: 12–21.

Received for publication 25 November 2004; accepted 11 February 2005.