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The effects of land use on honey bee (*Apis mellifera*) population density and colony strength parameters in the Eastern Cape, South Africa

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Abstract Livestock farming in the Eastern Cape, South Africa is a common land use practice that has affected the biodiversity of plants and animals in the region negatively. Indigenous populations of wild honey bee (*Apis mellifera*) colonies also may suffer. Recently, farmers in the Eastern Cape have been converting their farms to game reserves as ecotourism attractions and nature conservation lands. Consequently, the goal of this research was to determine if land use habits (livestock farming and conversion to game reserves) in the Eastern Cape affect honey bee colony population density and colony strength parameters. A series of indices were developed to compare the relative population densities of colonies in two or more areas by counting the number of foraging bees and number of bee lines established at feeding stations. Wild colonies on farms and reserves were located and sampled to determine land use effects on colony strength parameters including total area of comb in the colony, the area of comb containing stored honey, pollen, and brood, adult bee population, weight per bee, and the colony nest cavity volume ratio. When viewed collectively, the data indicated that land use practices have affected honey bee nesting dynamics in the Eastern Cape. Trends in the data suggested that colonies nesting on the reserves may occur in greater densities than those nesting on livestock farms, though they do not appear to be healthier. Hopefully, this work will be continued since honey bee conservation in areas where they are native is crucial to the health of agriculture and whole ecosystems globally.

Keywords *Apis mellifera scutellata* · *Apis mellifera capensis* · Wild honey bee colonies · Nesting dynamics · Population ecology

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Introduction

The Eastern Cape Province, South Africa is home to two subspecies of Western honey bee: *Apis mellifera capensis* Escholtz (the Cape honey bee) and *Apis mellifera scutellata* Lepeletier (Hymenoptera: Apidae). The Cape honey bee's native distribution is restricted to the Western and Eastern Cape Provinces in South Africa (Hepburn et al. 1998). The majority of its habitat is located in the Cape Floristic Region (CFR). The rest of its distribution, that in the Eastern Cape, which includes the Albany Centre of Floristic Endemism (ACFE) (Victor and Dold 2003) of the Maputaland-Pondoland-Albany hotspot (MPA) is located in thicket, succulent, savanna, and afromontane forest biomes. A hybrid zone occurs on the northern boundary of the Cape honey bee's distribution where *A. m. capensis,* *A. m. scutellata,* and hybrids of the two are found (Hepburn et al. 1998). The distribution of *A. m. scutellata* occurs north of this transition zone throughout South Africa and into east/central Africa (Hepburn and Radloff 1998) (Fig. 1).

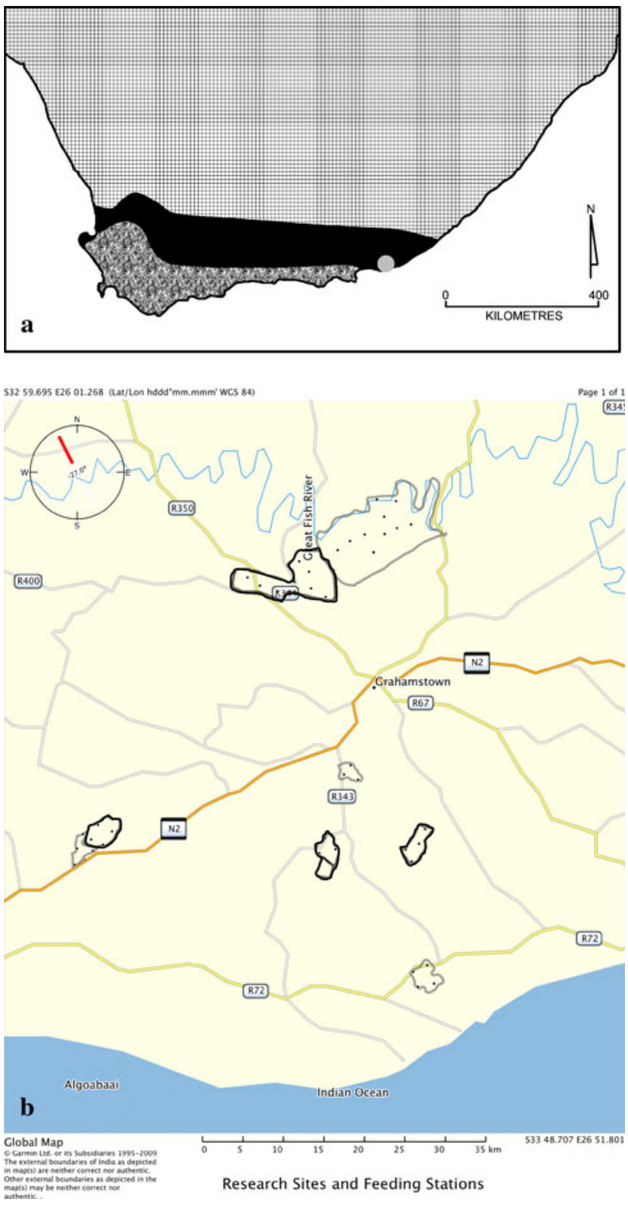

Fig. 1 Maps showing the research area and study sites. **a** Shows the natural distribution of the Cape honey bee in South Africa. The textured southernmost region represents the Cape Floristic Region and distribution of the Cape honey bee. The solid dark area represents the hybrid zone between the Cape bee and *A. m. scutellata*. The hashed northernmost region represents the natural distribution of *A. m. scutellata*. The dot represents the study region for this project. The figure is modified from Ellis (2008). **b** Shows the boundaries of the farm (*dark*) and reserve (*light*) study sites. Dots within each study site represent feeding station locations. The map generated using Garmin Base-Camp v. 3.1.4 (© Garmin Ltd. 2011)

Where they are distributed naturally, honey bees are important pollinators in the ecosystems they inhabit, especially in South Africa. For example, Cape honey bees pollinate at least 27% of the herbs, 44% of the shrubs, and 28% of the trees present in the CFR (Hepburn and Radloff 1998). In the dry savanna climatic zone of Africa, which includes portions the MPA, honey bees in general pollinate at least 18% of the herbs, 29% of the shrubs, and 52% of the trees present (Hepburn and Radloff 1998). At least 21% of the plants in the MPA are endemic (Mittermeier et al. 2004).

Habitat loss may be a significant threat to honey bee populations in Africa as land is transformed for cities and agriculture (Dietemann et al. 2009) and through degradation of land by overgrazing (Fabricius and Burger 1996a, b, Mangnall and Crowe 2003, Witt and Samways 2004). The Eastern Cape provides one the opportunity to research honey bees that occur in their natural habitat and are free of and/or resistant to many pests, parasites, and diseases plaguing honey bees in the rest of the world (Dietemann et al. 2009, Murray et al. 2009). Furthermore, the Eastern Cape is a good place to study land use effects on honey bees as much of the original farmland is being preserved as ecotourism based private game reserves and converted back to pre-farming conditions (Langholz and Kerley 2006).

The effects of land use practices on biodiversity are well known in the fynbos, thicket, and succulent biomes where avian, arthropod, reptilian, and plant biodiversity have been affected negatively by agriculture (e.g. Fabricius and Burger 1996a, b, Mangnall and Crowe 2003, Witt and Samways 2004). Furthermore, agricultural practices have been a serious threat to rare plants found in Cape lowlands (Heydenrych et al. 1999) and the Eastern Cape region (Victor and Dold 2003). When farming livestock, much of the natural landscape is removed to accommodate pastureland for grazing livestock, which often leads to overgrazing (Kerley et al. 1995, Mills et al. 2005) and other land quality issues. When allowed to regenerate, natural vegetation and food webs could take an unpredictable amount of time to return to pristine condition, possibly being transformed forever through extinctions (Whitmore and Sayer 1992, Novacek and Cleland 2001). Furthermore, poor land use habits may cause and/or expedite soil erosion (Le Roux et al. 2008), which can reduce the productivity of an area.

Though livestock farming is common in the Eastern Cape, farmers in the area are converting their farms to game reserves due to the economic benefits associated with ecotourism in South Africa (Langholz and Kerley 2006). These reclaimed lands can regenerate, though overgrazing can still occur as livestock is replaced with other large herbivores. However, vegetative structural diversity is greater on reserves due to the varying sizes and feeding strategies of wild herbivores (Fabricius et al. 1996a, Lechmere-Oertel et al. 2005), which if managed properly, are not as destructive as large numbers of livestock (Fabricius et al. 1996b).

The effects of reduced native vegetation and diminished food webs on an ecosystem's stability have not been researched for wild honey bees in the Eastern Cape, South Africa. Some have proposed that honey bees are healthier in areas where there is no artificial selection of bees, lower proportion of cropland, lower use of pesticides, less beekeeping, and less developed land (Naug 2009, Vandame and Palacio 2010). Among the topics of special concern to native pollinators are population densities of wild pollinators, resource availability, land use effects, habitat loss and fragmentation, and the potential loss of nesting resources (Dietemann et al. 2009, Murray et al. 2009).

In this project, we investigated the effects of land use practices (livestock farming and game reserves) on honey bee colony population density and colony strength parameters, hypothesizing that livestock farming would affect colony density and health negatively while game reserves would optimize colony density and strength parameters. We predicted this because livestock farming may reduce the availability of resources in the environment, especially if bee-important plant communities have been removed or damaged by grazing. If this were the case, colony food stores (honey and pollen), adult bee population, and total brood may be affected. To test our hypothesis, we located wild colonies of honey bees on livestock farms and game reserves adjacent to each other in the Eastern Cape and measured various colony strength parameters to determine how land use practices affect colony health.

Methods and materials

Study area

Four reserves and four livestock farms located near Grahamstown, South Africa were used as study sites in this research (Fig. 1). Grahamstown and its surrounding vicinity are located in the Albany region of the MPA hotspot, which includes the ACFE (Victor and Dold 2003). Fynbos, thicket, xeric succulent thicket, and grasslands can be found intermixed and within close proximity to each other in this area (Victor and Dold 2003). Grahamstown and its vicinity are considered to be in the hybrid zone of the Cape honey bee and *A. m. scutellata* (Hepburn and Radloff 1998). We sampled honey bees from colonies in the region and had them identified at an independent lab at the University of São Paulo, Brazil. Using morphometric tests, the bees were confirmed to be hybrids between *A. m. capensis* and *A.m. scutellata*.

The four reserves used in this project were Amakhala Game Reserve/Carnavron Dale, Crown River Safari and Wildlife Reserve, Emlanjeni Game Reserve, and Kwandwe Game Reserve. Each reserve contained land that was converted from cattle and other livestock farms to reserves within the preceding 5–14 years at the time of study (Table 1). The four livestock farms used were Assegaai Trails, Brentwood, Theo Harris's and Ezra Schoonbee's cattle farms (shared borders), and Hounslow (Table 1). All had been livestock farms for at least 15 years, with the farmers following farming practices typical of those in the area.

Population density

Ten feeding stations were used to attract foraging honey bees (Fig. 2). The stations consisted of a 2 m tall iron rod on which a 10 × 10 cm iron plate was welded to the top. The rod was angled at the bottom to facilitate insertion into the ground. A plastic container (~11.5 × 17 × 4 cm, L × W × H) was nailed through the center onto a 25 × 25 cm wooden plate. This wooden plate, which could be removed or replaced, was affixed to the top of the stand after insertion into the ground (Fig. 2).

Each station was baited with a 1:3:3 mixture of honey:water:sugar, respectively, and by volume. Pure honey was not used because of its expense and lack of availability in the study area. However, the honey used was from a single source to control for any differences in the attractiveness of honeys from various sources. It also was irradiated to remove all pathogens and reduce the risk of disease spread to wild colonies. The plastic container on each feeding station was filled with ~600 mL of bait into which sticks and twigs were placed to provide bees a platform from which the bees could drink without drowning (Fig. 3). Once honey bees were attracted to the station, they continued to return up to 7 days after the station was emptied (personal observation).

The stations were placed ~2 km away from each other throughout a study site, with a maximum of ten stations being used at any one site (Fig. 1). African races of honey bees typically forage within 1 km of the nest, though they can forage further distances (Schneider 1989, Schneider and Hall 1997; Hepburn, personal communication). Consequently, the stations may attract honey bee colonies nesting within a 1 km radius, minimizing the possibility that one colony will be attracted to two stations.

The locations for each station were selected before visiting a site to prevent sampling bias. Station placement sites were selected using Google Earth v.5.1 (Google Inc. 2009). Since the different research sites were different sizes, the number of feeding stations placed in each varied. A total of 21 feeding stations were placed on reserves and

Table 1 Reserve and farm study site summaries

Name	Location	Type	Year of conversion	Dominant vegetation biome	Large mammals present	No. feeding stations
Amakhala Game Reserve/ Carnavron Dale	33°29′45.07″S, 26°7′24.05″E	Reserve	1999/2005	Thicket, savanna	Cheetah, buffalo, elephant, giraffe rhinoceros, wildebeest, zebra, various bovines	4
Crown River Safari and Wildlife Reserve	33°24′33.49″S, 26°29′20.81″E	Reserve	1996	Thicket	Zebra, various bovines	3
Emlanjeni Game Reserve	33°38′23.15″S, 26°35′22.51″E	Reserve	~1999	Thicket	Buffalo, giraffe rhinoceros, zebra, various bovines	5
Kwandwe Game Reserve	33° 8′38.63″S, 26°32′19.00″E	Reserve	1999	Thicket, fynbos, savanna, succulent	Cheetah, buffalo, elephant, giraffe, lion, rhinoceros, wildebeest, zebra, various bovines	10
Assegaai Trails	33°29′23.97″S, 26°35′2.85″E	Farm	–	Thicket, savanna	Cattle	4
Brentwood	33°28′25.78″S, 26°9′28.21″E	Farm	–	Thicket, savanna	Cattle	4
Hounslow	33°11′49.01″S, 26°25′20.86″E	Farm	–	Thicket, fynbos, succulent	Sheep, goat	8
Theo Harris/Ezra Schoonbee	33°29′49.34″S, 26°27′44.82″E	Farm	–	Thicket, afromontane forest, savanna	Cattle	3

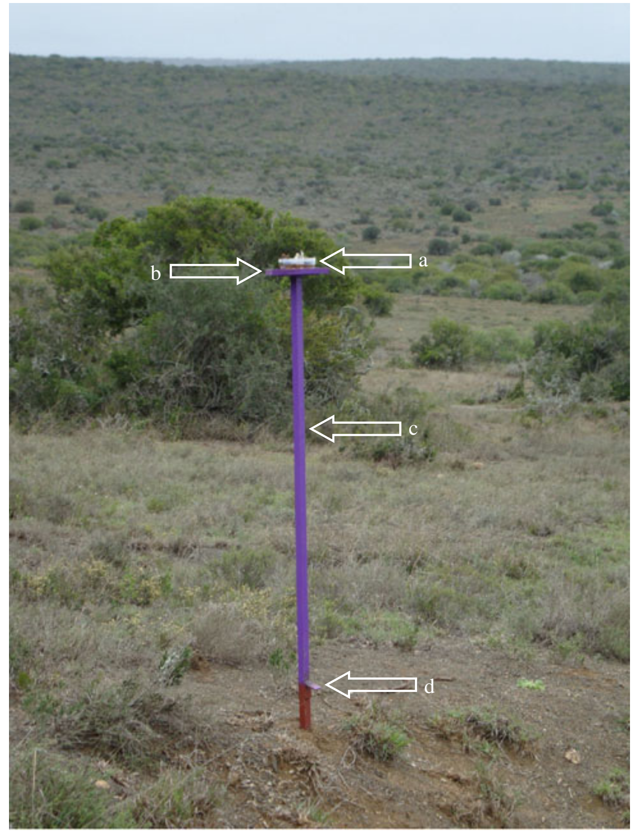

Fig. 2 A honey bee feeding station. *Arrow a* shows the container partially filled with bait. *Arrow b* shows the removable feeding plate. *Arrow c* shows the main iron rod driven into the ground. *Arrow d* shows the crosspiece used to hammer the station into the ground

19 were placed on farms. We attempted to maximize the number of stations placed in each study area so that the bee attraction radii of the stations covered as much of the study sites as possible (Fig. 1). Stations were placed close to fences only if the landscape was the same at a considerable distance from the fence into the adjacent property and/or the adjacent property was managed identically to that on which the station was placed. All feeding stations were entered as waypoints into a Garmin GPSmap 60CSx (Garmin International Inc., Olathe, KS) so that they could be placed in the exact predetermined locations. Stations were placed and baited at the pre-selected sites about 24 h before monitoring.

All population index data collection occurred from 8 April to 1 May 2010. Data collection lasted no loner than 60 min during one visit at each station and was made by the same observer. Data were collected only during sunny weather with little to no wind and between the hours of 0900 and 1500 (peak foraging conditions). Feeding stations within a site were made in the order that the stations were established the preceding day so that data could be collected as close to 24 h after baiting as possible. If bees

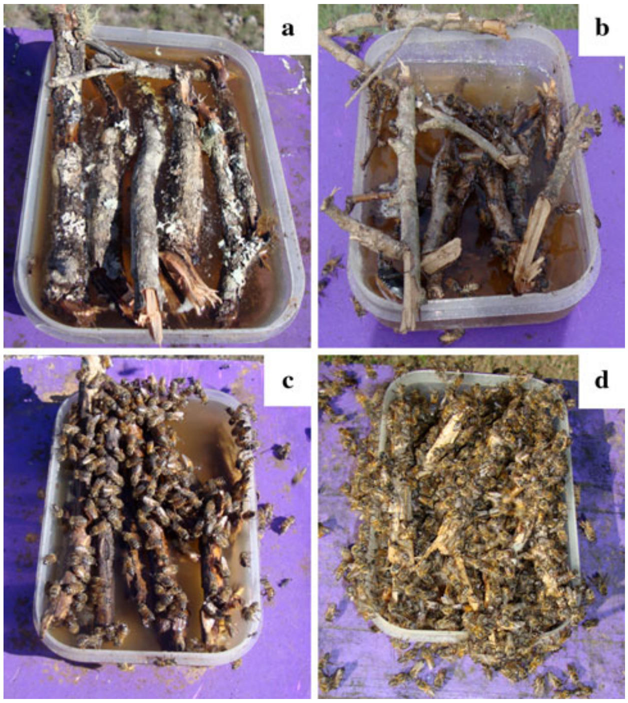

Fig. 3 Population density index field and photograph ratings. **a** Rating 0: 0 foraging bees. **b** Rating 1: 1–50 foraging bees. **c** Rating 2: 51–200 bees. **d** Rating 3: ≥200 foraging bees. **b–d** Represent the approximate maximum amount of foraging bees for their respective ratings

had removed the bait from the stations prior to data collection, the stations were refilled, attracting bees almost immediately.

The number of bee lines established at each feeding station was counted and was considered the first population index. The maximum number of bee lines counted at any feeding station was four and the minimum was zero when no foraging bees were observed. A bee line is the flight path taken by honey bees to and from a food source and the colony. The number of bee lines suggests a possible number of colonies within the attraction radius of the feeding station. Bee lines were counted by observing the directions that the bees fly when leaving the food, which was more direct than when they landed. Bee lines were viewed best when standing level or below the station and watching the flight direction of the bees against the sky as they left the station. Each station was monitored until all established bee lines were recorded.

A second set of data was collected at each feeding station. Each station was rated on a scale of 0 (no bees foraging) to 3 ("many" bees foraging) based on the intensity of honey bee foragers visiting a station. This was a qualitative scale and is called the "field rating", thus serving as a second population index. For the third and final population index, each feeding station was photographed during the estimated peak bee foraging activity. One photograph per feeding station was analyzed on a 15″ MacBook Pro 4,1 (Apple Inc., Cupertino, CA) using iPhoto'08 v.7.1.5. (Apple Inc. 2008). Photographs were rated on a scale from 0 to 3. This rating is a qualitative one and was called the "photograph rating". The ratings were assigned as follows: (0) = zero foraging bees, (1) = 1–50 foraging bees, (2) = 51–200 foraging bees, (3) = ≥ 200 foraging bees (Fig. 3).

Colony strength

Wild colonies of honey bees were located on the study sites already identified. The following parameters were determined for each experimental colony: number of bees, weight per bee, total comb area, cm^2 honey, cm^2 brood, cm^2 pollen, cm^2 filled comb, cm^2 empty comb, brood pattern, the number of bees per nest volume, and proportion of comb used for brood, honey, and pollen.

All colonies were located between September 2009 and March 2010. Most of the colonies were found using a bee-lining method modified from the one described originally by Edgell (1949) and subsequently by others (Seeley and Morse 1976, Seeley et al. 1982, Seeley 1983, Visscher and Seeley 1989; Seeley, personal communication). Additional colonies were found by interviewing local landowners and reserve employees. To establish bee lines to wild colonies, baited feeding stations as described above were employed. To reduce bias, feeding stations were placed throughout each study site so that some were close to cliffs, valleys, trees and open areas. Once the feeding stations were deployed, bee lines were observed at each station and then single bee lines were followed away from the station to the wild nest.

Thirty-three colonies (17 from reserves and 16 from farms) were extracted from the cavity in which they nested and dissected to conduct colony strength readings. Though more than 33 colonies were found, up to five colonies from each study site were selected randomly for extraction. If the colonies were difficult to access, another colony was selected in its place. All colonies were extracted between 7 May 2010 and 7 June 2010 to ensure that colony life cycles were similar for all colonies at the time of removal. This controlled seasonal differences that would occur in colony brood rearing, honey production and storage, wax production, and pollen storage and to precede the swarming season between August and December (Hepburn and Radloff 1998).

Before all removals, smoke was pumped into the colony entrance. Removal of ground-nesting colonies was accomplished by opening the entrance to the colony using a chisel or pick if necessary and efforts were taken to not allow the earth to collapse onto the colony during the removal process. The comb was removed from the cavity ceiling. Colonies nesting in cliffs were extracted by removing the propolis covering over the nest entrance, then cutting and removing the comb from the cavity ceiling. Colonies nesting in trees often were extracted using a machete, handsaw, and/or chisel to gain access to the cavity. This allowed us to remove the comb without damaging it. The combs were stacked by colony side by side and right side up in 20 L buckets to prevent damage and limit honey leakage. The buckets were sealed and placed in a freezer (−10°C) until ready to be measured.

Once the combs were removed from the cavity, we attempted to collect the bees to determine the number of bees in the nesting colonies. Once dissected, the bees from the extracted colony would form a cluster similar to that of a swarming colony either inside the nest cavity or in a nearby bush or tree. Such clusters were collected from 19 of the extracted colonies (10 from reserves and 9 from farms) and placed into a cardboard box. We collected as many clusters as possible, but not all clusters were obtainable.

Once as many of the bees were collected in the box as possible, the box was weighed (g) in the field on a triple beam scale. A small sample, 30–200 worker bees, was collected randomly from the cluster in a pre-weighed air-tight plastic jar, then stored in a freezer (−10°C) until measurements were made. The sample of bees collected from each colony cluster was weighed and the number of bees counted. This permitted the determination of an average weight per bee (g). To estimate the number of bees in each colony, cluster weight was divided by the average weight per bee for each colony. The colony population was divided by the cavity volume to determine the number of bees/L nest cavity.

Nest cavity volumes were estimated by observing the general shape of each nest cavity, measuring various dimensions to the nearest centimeter, and using that information to calculate the cavity volume (L). Only the volume occupied by the comb was calculated for colonies that were located in open nests.

A transparent 500 cm^2 grid (vertical and horizontal lines every 1 cm) was used to estimate the total surface area of comb (cm^2), cm^2 honey, cm^2 pollen, and cm^2 brood (eggs, larvae, and pupae) for each comb from a nest. The amount of filled comb was calculated by adding the amount of comb containing honey, brood, and pollen. The amount of empty comb was calculated by subtracting the amount of filled comb from the total comb area. The data were used to determine the proportion of comb that contained honey, brood, or pollen or was otherwise empty.

The brood pattern (roughly, the percent of brood comb that contained brood) of each extracted colony was determined using a three point scale. A rating of 1 indicated a very poor brood pattern, with many empty cells (>50% empty), and/or indications of disease. A rating of 2 was assigned to colonies having a somewhat spotty brood pattern (20–50% empty cells). A rating of 3 was assigned to combs with a solid brood pattern (<20% empty cells), even distribution of eggs, larvae, and pupae, and no visible diseases (Fig. 4).

Statistical analyses

Population density index

Land use (reserve or livestock farm) effects on the average number of bee lines per feeding station per study site were analyzed using a weighted one-way ANOVA, thus allowing us to give more consideration to study sites having the most feeding stations. For the field and photograph indices, Pearson's χ^2 analyses were used to determine if there was a difference in the distribution of ratings (both field and photograph) between feeding station ratings on the two land use types. All analyses were conducted using the statistical software package JMP v 8.0 (SAS Institute 2009).

Colony strength

The effects of land use (reserves or livestock farms) on colony strength parameters was determined using a one way ANOVA recognizing land use or nest site as the main effects and the following parameters as dependent variables: weight per bee (g); # bees/colony; # bees/L cavity volume; cm^2 honey, brood, pollen, empty (nothing in cells), or filled (contained anything in cells); total comb area; and the proportion of comb that contained honey, brood, pollen, nothing, or was filled. Proportion data were arcsin $\sqrt{x}$ transformed prior to analysis though untransformed means are reported in this manuscript. The distribution of brood pattern ratings was compared within land use types using Pearson's χ^2 test. A single queenless colony was omitted from brood analyses. All analyses were conducted using the statistical software package JMP v 8.0 (SAS Institute 2009).

Results

Population density index

The average number of bee lines observed per feeding station was not significantly different between livestock farms and game reserves (Table 2). The feeding stations on reserves exhibited a skewed distribution toward the highest rating of foraging bees (3) in the field rating and toward the highest two ratings of foraging bees (2, 3) in the

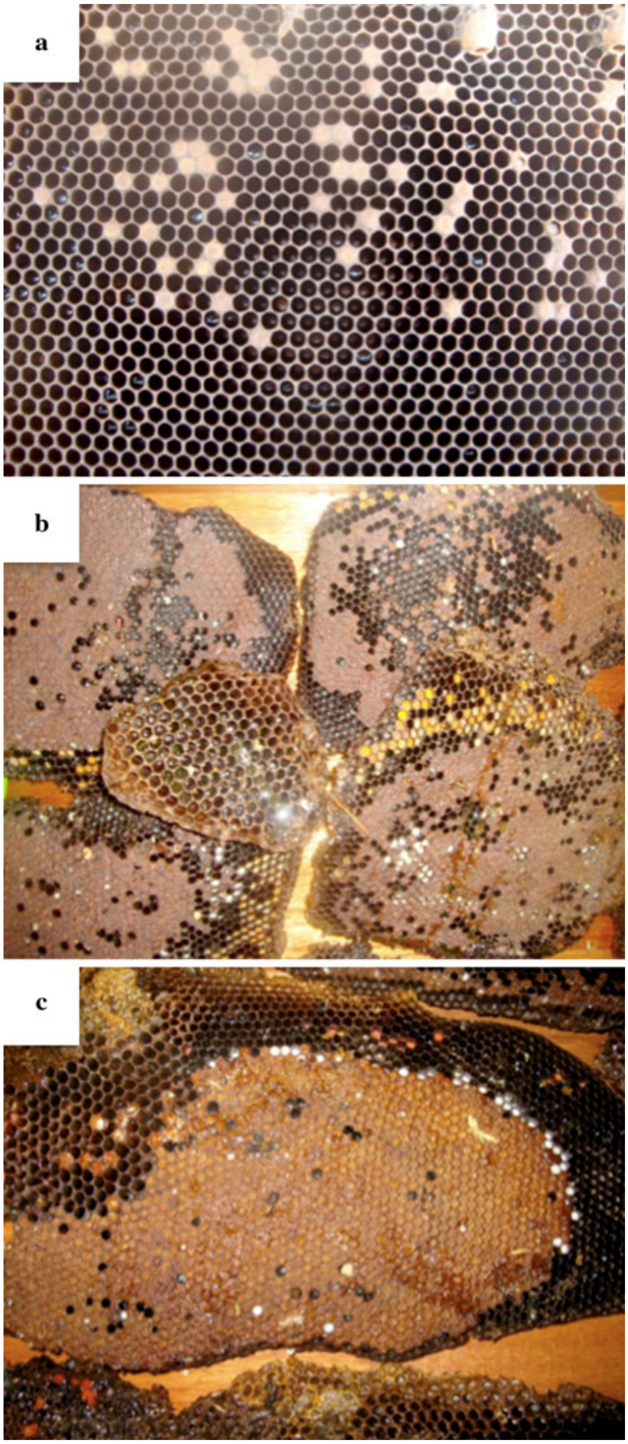

Fig. 4 Brood pattern ratings. **a** Represents brood pattern #1 (very spotty brood; >50% empty cells). **b** Represents brood pattern #2 (somewhat spotty brood; 20–50% empty cells). **c** Represents brood pattern #3 (solid pattern; <20% empty cells)

photograph rating (Table 2). Photograph and field ratings were distributed evenly across all ratings for stations on farms (Table 2).

Colony strength

Land use (reserve or livestock farm) did not affect any measured strength parameter significantly (Table 3). Brood pattern ratings were generally higher in colonies nesting on reserves than those nesting on farms (Table 3).

Discussion

Population density index

The impact of land use on honey bee population density in the Eastern Cape, South Africa differed based on the index used to measure population density. Using the bee line counting method, we observed no significant differences between honey bee population densities on game reserves and livestock farms. On the other hand, the field and photograph rating indices indicated that there were more bees visiting feeding stations on reserves than on farms. Consequently, we conclude that there was a measureable impact of land use on honey bee population density.

The increase in population density on reserves suggests that converting farms to game reserves does benefit honey bees. This benefit may be even greater over the long term than what we found considering that the reserves were converted from livestock farms within the preceding 15 years. The accuracy of this index should be confirmed by comparing the results from the index (# bee line and field/photograph ratings) to the actual population density of honey bee colonies and to predications made using the other estimation methods (Baum et al. 2005; Oldroyd et al. 1997; Moritz et al. 2008). With this information in hand, the index methodology could be modified further to increase its predictive power. For example, one could adjust the distances between feeding stations, both shortening and lengthening that distance. All observed bee lines could be followed to their respective colonies to create a colony density map for an entire area. One could optimize feeding station placement to avoid stations locating the same colonies while still sampling most colonies through the study site.

Using the number of bee lines to a feeding station may not serve as an accurate index of colony population densities, making it a less reliable index than that offered by the field and photograph ratings. For example, our bee line index suggests a population density in the Eastern Cape considerably lower (<1 colony/km^2, if a beeline reflects a single colony and assuming a colony flight distance of 1 km) than those shown by other authors studying honey bees in Texas (12.5 colonies/km^2—Baum et al. 2005), Australia (40–150 colonies/km^2—Oldroyd et al. 1997), and the Kalahari (10.6 colonies/km^2—Moritz et al. 2008).

Table 2 Honey bee population density index parameters compared between colonies nesting on reserves and livestock farms in the Eastern Cape, South Africa

Parameter	Statistical test	Reserves	Livestock farms
Average # of bee lines per feeding station	ANOVA (weighted) $F_{1,6} = 4.4$; $P = 0.08$	2 ± 0.2 (4)	1.3 ± 0.3 (4)
Field rating	Pearson's χ^2	0: 2	0: 3
		1: 1	1: 2
		2: 5	2: 8
		3: 13	3: 6
		χ^2(3, N = 21) = 16.9; $P < 0.01$	χ^2(3, N = 19) = 4.8; $P = 0.19$
Photograph rating	Pearson's χ^2	0: 2	0: 3
		1: 1	1: 4
		2: 8	2: 7
		3: 10	3: 5
		χ^2(3, N = 21) = 11.2; $P = 0.01$	χ^2(3, N = 19) = 16.9; $P = 0.61$

Data in row one are mean ± s.e. (N) number of bee lines. χ^2 data are number of feeding stations assigned to a given category. Field rating categories: 0 (no bees), 1 (few foraging bees), 2 (some foraging bees), and 3 (large number of foraging bees). Photograph rating categories: 0 (no bees), 1 (1–50 bees), and 2 (51–200 bees), and 3 (>200 bees)

Table 3 Strength parameters for honey bee colonies extracted from livestock farms and reserves

Strength parameter	Reserves	Livestock farms	ANOVA
Weight per bee (g)	0.09 ± 0.003 (10)	0.1 ± 0.011 (9)	$F_{1,17} = 3.1$; $P = 0.1$
# Bees/colony	12,700 ± 2,057 (9)	11,420 ± 1,445 (9)	$F_{1,16} = 0.3$; $P = 0.62$
# Bees/L cavity volume	631 ± 127 (9)	337 ± 76 (9)	$F_{1,16} = 3.9$; $P = 0.06$
cm^2 Brood	1,672 ± 243 (16)	1,733 ± 270 (16)	$F_{1,30} = 0.03$; $P = 0.87$
cm^2 Honey	4,057 ± 726 (17)	3,215 ± 425 (16)	$F_{1,31} = 1$; $P = 0.33$
cm^2 Pollen	417 ± 83 (17)	372 ± 90 (16)	$F_{1,31} = 0.1$; $P = 0.72$
cm^2 Empty	2,514 ± 457 (17)	3,932 ± 704 (16)	$F_{1,31} = 2.9$; $P = 0.1$
cm^2 Total comb	8,562 ± 1,110 (17)	9,252 ± 968 (16)	$F_{1,31} = 0.5$; $P = 0.49$
Proportion of comb containing brood	0.25 ± 0.04 (16)	0.18 ± 0.02 (16)	$F_{1,31} = 0.2$; $P = 0.64$
Proportion of comb containing honey	0.42 ± 0.05 (17)	0.36 ± 0.05 (16)	$F_{1,30} = 1.2$; $P = 0.28$
Proportion of comb containing pollen	0.05 ± 0.01 (17)	0.04 ± 0.01 (16)	$F_{1,31} = 0.6$; $P = 0.46$
Proportion of comb empty	0.3 ± 0.04 (17)	0.41 ± 0.05 (16)	$F_{1,31} = 0.7$; $P = 0.42$
Brood pattern rating	1: 0*	1: 1	
	2: 1	2: 7	
	3: 15	3: 8	
	Pearson's χ^2(1, N = 16) = 12.3; $P < 0.01$	Pearson's χ^2(1, N = 16) = 12.3; $P < 0.01$	

Data are mean ± s.e. (N number of colonies) for ANOVA tests and # colonies assigned a given brood pattern rating for χ^2 analyses. For χ^2 tests, P values >0.05 indicate a random distribution of brood pattern ratings among colonies. Data labeled with an asterisk were excluded from analysis because χ^2 tests do not recognize "0". For brood pattern ratings: 1 = very spotty brood (>50% empty cells), 2 = somewhat spotty brood (20–50% empty cells) and 3 = solid pattern (<20% empty cells)

There are possible reasons for this. First, if two colonies exist in close proximity to one another, their respective bee lines away from the feeding station may overlap too much to be distinguished separately, meaning a single beeline can represent >1 colony. Second, the feeding stations may sample colonies within a much smaller radius than hypothesized earlier. When using the feeding stations to locate colonies, the colonies were rarely >500 m from the stations, possibly suggesting an increased population density. Regardless, the method developed should be used as a comparative index of colony population density rather than an estimator of an actual colony number per unit area.

Knowing the population density of honey bee colonies in an area is important. Being able to predict population densities of honey bees accurately would permit one to determine how land use practices, global climate trends, and other large-scale environmental issues affect wild populations of honey bees. For example, Oldroyd et al. (1997) suggested that population densities of honey bees may be reduced in drought conditions. This could be verified if methodology is developed to track fluctuations in population density over time. Then, scientists can use tools such as GIS technology, climate change data, etc. to determine how honey bee populations are affected. Ultimately, such information could be used to develop conservation practices for wild honey bees, a worthy endeavor considering the plight of honey bees globally.

Colony strength

Converting land to agricultural use can cause the destruction of habitat and natural resources. Agriculture can affect population structures, wildlife diversity, plant communities, abiotic material, and encourage the spread of disease and use of toxic substances (e.g. pesticides) (Matson et al. 1997, Williams et al. 2002). The possibility of species extinction and climate change is magnified through deforestation which often accompanies agriculture (Whitmore and Sayer 1992). In the Eastern Cape, South Africa specifically, livestock farming can affect wildlife populations and plant structure and diversity negatively for small animals such as lizards and arthropods (Kerley et al. 1995; Fabricius and Burger 1996a, b; Fabricius et al. 1996a, b; Mills et al. 2005; Lechmere-Oertel et al. 2005). Its effects on honey bee colony strength parameters in the Eastern Cape was a subject of the research presented herein.

Overall, the data suggest that land use practices did not affect the colony strength parameters measured in this study. Because colony strength could reflect the health of surrounding ecosystems, the lack of significant effects of land use on nesting colonies suggest that livestock farming has minimal impact on individual honey bee colony strength parameters in the Eastern Cape. That said, most trends in strength parameters favored colonies nesting on reserves over those nesting on farms, with the exception of the total amount of brood in colonies and the weight per bee. As such, the data present a dichotomy: no *significant* effects of land use on colony strength, but a number of trends that suggest there may be an impact of land use on colonies. We discuss the findings considering both possibilities.

There are a number of possible reasons that land use would not affect the strength of the observed honey bee colonies in this study. First, the game reserves and farms tested in the study may not have differed significantly in land composition. The game reserves used in the study were converted from livestock farms within the previous 15 years. Therefore, it is possible that the land on game reserves did not have enough time to recover or affect nesting bee colonies significantly. To address the possible impacts of land transformation and conservation, one should continue the research on undisturbed and natural habitat (as much as this is possible), thus giving one baseline data of the honey bee dynamics studied for comparison. Owners of conserved land need to educate rangers about the value of honey bees to ecosystems, especially since poaching of honey bee colonies on the test reserves was frequent.

Second, though the health status of honey bee colonies was not affected by land use, the colony density was as described above. As such, livestock farms may have fewer resources available to nesting honey bees, thus affecting nest density per unit area. Because nest density was lower on livestock farms, the limited resources available to colonies there were sufficient to support the reduced number of colonies nesting on the farms. This would result in colonies similar in strength to those nesting on reserves, but just fewer of them.

The third possible reason that the strength of honey bee colonies was unaffected by land use is that the farms tested in this study bordered reserves and/or had patches of unaltered land on the farm (especially in valleys where livestock would not graze). Consequently, the livestock farms were not truly homogenous and equally resource-poor but rather heterogeneous, with many habit compositions available. Honey bees may have been accessing these areas in order to obtain their resources. To determine this further, one would have to employ GIS technology to see if colony strength can be predicted accurately by land use practices rather than simply recognizing “farm” and “game reserves” as all-encompassing terms.

On the other hand, there is some evidence that land use practices did affect colony strength parameters, at least numerically. All but one of the colonies nesting on reserves were assigned a brood pattern rating of 3 while the pattern ratings were distributed more evenly on colonies nesting on livestock farms. This may have resulted from poor resource availability on farms, lower availability of non-related drones, or other similar factors. For example, pesticides can affect honey bee brood, possibly resulting in spotty brood patterns (Toth and Ellis, unpublished data). Pesticides for cattle and crops were used on the test farms while not on the reserves, and honey bees have been observed in at least one of the study areas collecting water from dipping troughs where Amitraz is used to control ticks on livestock (Cambray pers. Com.). Furthermore, inbreeding is a significant contributor to spotty brood patterns (Moritz 1984). With lower colony densities on farms than on game reserves, one would expect inbreeding to be more frequent on farms. Despite the differences in brood pattern ratings between colonies nesting on farms and game reserves, the average brood rating across all colonies was 2.7, suggesting that wild bee colonies generally had healthy queens, no visible brood diseases, etc.

In conclusion, livestock farming appeared to affect nesting parameters of wild honey bees in the Eastern Cape, South Africa, population density significantly but various health parameters only numerically. The data contributed to a better understanding of honey bee ecology and provided background information on *A. m. capensis*/*A. m. scutellata* hybrids useful for promoting their health and conservation.

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