A Landscape Analysis to Understand Orientation of Honey Bee (Hymenoptera: Apidae) Drones in Puerto Rico

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ABSTRACT Honey bees [*Apis mellifera* L. (Apidae, Hymenoptera)] show spatial learning behavior or orientation, in which animals make use of structured home ranges for their daily activities. Worker (female) orientation has been studied more extensively than drone (male) orientation. Given the extensive and large flight range of drones as part of their reproductive biology, the study of drone orientation may provide new insight on landscape features important for orientation. We report the return rate and orientation of drones released at three distances (1, 2, and 4 km) and at the four cardinal points from an apiary located in Gurabo, Puerto Rico. We used high-resolution aerial photographs to describe landscape characteristics at the releasing sites and at the apiary. Analyses of variance were used to test significance among returning times from different distances and directions. A principal components analysis was used to describe the landscape at the releasing sites and generalized linear models were used to identify landscape characteristics that influenced the returning times of drones. Our results showed for the first time that drones are able to return from as far as 4 km from the colony. Distance to drone congregation area, orientation, and tree lines were the most important landscape characteristics influencing drone return rate. We discuss the role of landscape in drone orientation.

KEY WORDS cardinal point, drone congregation areas, orientation, landscape analysis

Honey bees [*Apis mellifera* L. (Apidae, Hymenoptera)] show spatial learning behavior as part of their orientation behavior, given they make use of structured home ranges for their daily activities. Because bees need to orient in space, they depend on spatial characteristics such as the sun incidence angle (Frisch 1967, Lindauer 1985), landmarks in the landscape (Frisch 1967, Cartwright and Collet 1982, Lindauer 1985, Wehner and Rossel 1985, Cheng et al. 1987, Collett 1992, Collet 2000, Menzel and Giurfa 2001, Fry and Wehner 2005, Wolf et al. 2014), and Earth's magnetism (Gould 1986, Hsu and Li 1994, Frier et al. 1996). Interestingly, all existing studies on orientation and navigation in honey bees have been conducted only with workers (females; Frisch 1967, Cartwright and Collet 1982, Lindauer 1985, Wehner and Rossel 1985, Cheng et al. 1987, Collet 1992, Capaldi and Dyer 1999, Capaldi et al. 2000, Collet 2000, Menzel and Giurfa 2001, Fry and Wehner 2005). The reason for using workers when studying bees' navigation is that females, in contrast to males (drones), can be trained prior to the experiments. Drones do not respond to training and they are more delicate and show higher rates of mortality when handled for experiments (Dinges et al. 2013, Giannoni-Guzmán et al. 2014). Both workers and drones need to orient when searching for food or new nesting places (females; Winston 1987), and when navigating to and from the drone congregation areas (DCAs) where drones and queens (reproductive females) mate. Workers take on average five orientation flights before beginning to forage (Capaldi and Dyer 1999, Capaldi et al. 2000), and perform orientation flights that may last up to 20 min before leaving the colony for the first time (Capaldi and Dyer 1999, Palikij et al. 2012).

Information on drone flight is limited, but we know that the first flights that drones take occur between 5 and 8 d after emergence (Ruttner 1966). Initial orientation flights are short, and after ∼10 d of emergence, drones fly to mate in the DCAs (Witherell 1971). Drones fly daily and repeatedly to DCAs where they seek virgin queens using vision and pheromones (Witherell 1971, Koeniger 1990). If they are successful in mating with a queen, they die immediately after copulating. If they are unsuccessful, drones conclude their mating flights in the early evening, and return to their maternal hive or to other hive to eat, given that they do not forage individually. Drones are accepted in any hive when the conditions are favorable, but they may be rejected by the workers for different reasons, including the lack of food in the hive and the advanced age of drones. Because spatial orientation is critical for the survival and reproduction of honey bees, evident behavioral (Sullivan et al. 2003) and anatomical adaptations are observed in these insects, such as magnetic sensitivity and magnetite nanoparticles in the bee brain, thorax, and abdomen (Hsu and Li 1994, Frier et al.

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1996, Hsu et al. 2007), and neurological adaptations (Fahrbach et al. 1998).

Landscape configuration influences bees' orientation. Foragers orient using characteristics that are spatially variable such as resource abundance, resource distribution, resource distribution, resource quality, and resource distance from the colony. Complex landscapes with high habitat diversity have been shown to provide a more continuous supply of nectar and pollen than simple landscapes (Beekman and Ratnieks 2000, Steffen-Dewenter et al. 2002). In contrast to foraging bees, drones, and queens leave the colony in search of mating opportunities, specifically in the search of a DCA (Zmarlicki and Morse 1963; Witherell 1971; Koeniger et al. 2005a,b; Galindo-Cardona et al. 2012). These DCAs may be found in a radius of 2 km from the colony (Ruttner 1985; Koeniger et al. 2005a,b; Galindo-Cardona et al. 2012), though a shorter distance would be better to avoid predation, to spend less energy and for easier orientation (Koeniger et al. 2005b). We know that DCAs are more commonly found in areas oriented to the south (aspect) and areas showing a high density of trails (Galindo-Cardona et al. 2012). We do not know which landscape characteristics are important for the drones to fly to and from DCAs. A landscape analysis can assist in evaluating which spatial landmarks are more important for drones at different scales during navigation. Studies examining landmarks used by honey bees have used descriptions based on direct or short distance observations of females (Riley et al. 2003) and this limits our ability to understand navigation at larger distances. Current tools in Geographic Information Systems (GIS) are powerful, and can help us improve our understanding of honey bee orientation by expanding the scope of our observations to a larger geographic scale.

The main goal of this study was to examine the importance of spatial landmarks and distance in the orientation of the understudied honey bee drones at the landscape scale and to show how GIS and remote sensing can improve our understanding of spatial orientation. We studied the effects of landscape variables on the returning time of drones released at four distances and at four cardinal points from the colony. The null hypothesis was that more drones would return from nearer releasing sites and that no differences would be observed among cardinal points. Identifying the most significant spatial cues for drone orientation will contribute not only to theoretical aspects of the evolutionary and ecological study of this species but also to applied aspects, such as selection of apiaries location to improve their performance.

Materials and Methods

Study Area and Experiment. We conducted the observations and experiments at the Agricultural Experimental Station of the University of Puerto Rico at Gurabo, Puerto Rico (latitude 18°15′, longitude 65°59′; Fig. 1). Since 2001, we have an apiary at this location consisting of 40 to 80 gentle Africanized honey bee colonies (see Galindo-Cardona et al. 2013). We captured and marked 1,013 drones from 30 hives in the apiary to conduct a mark–recapture experiment during the mating season of 2009 in Puerto Rico (May–October). These data would help us examine their return rate and identify important landmarks for their orientation (e.g., Lindauer 1985, Wehner and Rossel 1985, Giurfa and Capaldi 1999, Menzel and Giurfa 2001). The selected drones were at least 10 d old, meaning that they all had previous flight experience. After marking the drones with a color on the thorax, they were released at three distances from their hives (1, 2, and 4 km), and from the four cardinal points around the apiary (Fig. 1) in groups of ∼40 drones, respectively. When released, the drones made short circular upward flights and then flew off in the direction of the apiary (precise vanishing bearings were not measured). We recorded the number of drones that returned to the original hive after being released and their time of arrival. At each distance, drones were released at 2:30 p.m. from each cardinal point at the same time. The releasing dates were different (separated by ∼60 d) for each distance due to logistic limitations. The hive entrance was observed between 2:30 p.m. and 5:30 p.m. and returning drones were recorded. These time periods were determined to be drone flight time at this location in previous observations (Galindo-Cardona et al. 2012). The experiment was run twice for each distance, on sunny and dry days. The males were killed right after their returning to the hive to avoid re-counting of the same individuals. Because the experiment was repeated under similar meteorological conditions and the days of release were close in time during the mating period, we combined the data of both replicates for some of the subsequent analyses. In addition, we recorded drones returning to the original hive (within the same day and the following 2 d) and to other hives in the apiary (examined only the following day). We focused on drones returning to the original hive for subsequent analyses because these drones provided more precise information on time from release to recapture.

Post hoc Experiment, Drone Release at 50 m From the Hive. Because the return rates were low at distances of ≥1 km, a post hoc experiment with release of displaced drones from a near distance was attempted. Similar to the original experiment, 20 drones were released from each cardinal point at a distance of 50 m from the hive in two trials, using different colonies each time. The number of drones returning indicates that drones do have some knowledge of the immediate surroundings of their hive.

Landscape Description. The landscape around our apiary, where the mark–recapture experiment was conducted, was highly heterogeneous, showing no evident gradients of spatial variables (Fig. 1). This landscape included different land covers and land uses, such as forest, urban cover, and rivers and different elevations, slope, and other geophysical characteristics. The landscape variables of the releasing sites ($n = 12$) were described using a high-definition digital aerial photograph (resolution: 0.33 m) of Gurabo. The
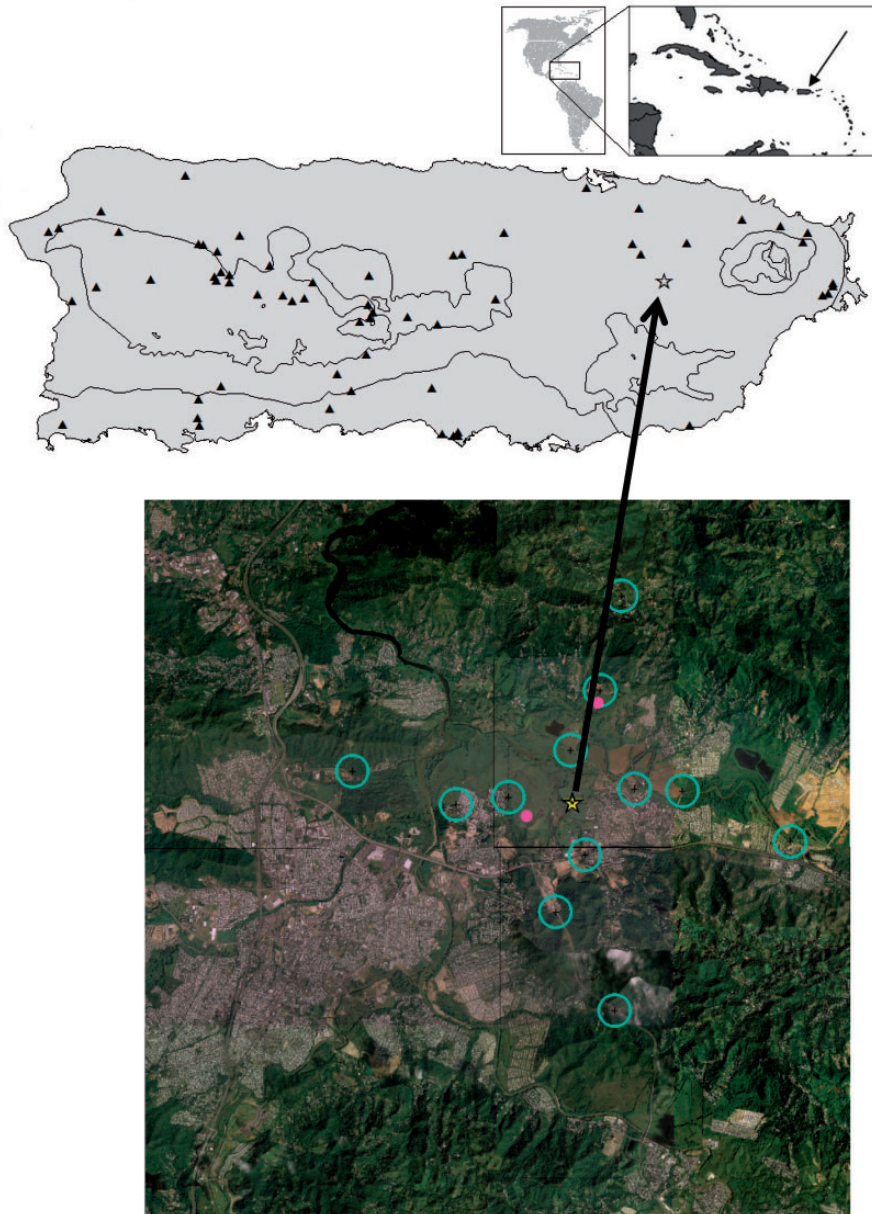
Fig. 1. Map of Puerto Rico and its location in the Caribbean region (inset) showing the apiary at the Agricultural Experimental Station of the University of Puerto Rico at Gurabo (star). Enlarged below, the satellite image shows the area of the experiment. The crosses are the drones releasing sites at three distances and four cardinal points from the apiary. The circles around each cross are the 400 m buffers inside which landscape was characterized. The two pink dots show known DCA.

analysis was done using GIS software (ArcGIS v 9.3) to map and retrieve the geographical data from shapeifles. We delineated circular concentric buffer areas centered on each releasing point with radii of 400 m (Fig. 1). Capaldi et al. (2000) and Menzel et al. (2000) found that 300 m is the distance used by workers during orientation flights when they first depart from the colony. In a previous study, we found that an area of 400-m radius around DCAs had distinctive spatial

characteristics that separated it from smaller and larger areas (Galindo-Cardona et al. 2012). The buffer was the unit area that ArcGIS used to analyze different layers of spatial information. Inside each 400-m buffer, we used the following land cover types: pasture, urban, and crops obtained from Gould et al. (2008). In addition, we characterized the following landscape variables given their potential use as landmarks by drones: aspect (terrain orientation) and slope, tree lines, tree groups,

Table 1. Description of variables used in the landscape analysis to examine landscape effects on the orientation of drones

Name	Description	Units	Range
ASP.M	Mean aspect	Radians	0–3
ASP.SD	SD of aspect	Radians	0–3
SLO.M	Mean slope	Radians	0–1.5
SLO.SD	SD of slope	Radians	0–1.5
Urban	Proportion of urban cover	–	0–1
Tree lines	Proportion of tree lines cover	–	0–1
Tree Groups	Proportion of tree groups cover	–	0–1
Single trees	Proportion of single trees cover	–	0–1
Interm Grass	Proportion of intermedia grass and pastures cover	–	0–1
Tall grass	Proportion of tall grasslands and pastures cover	–	0–1
Highways	Proportion of highways cover	–	0–1
Canal	Proportion of rivers and canals cover	–	0–1
Distance DCA	Distance from each releasing point to a known Drone Congregation Area	Meters	0–4000
Tracks.M	Mean density of tracks and trails	Square meters	0–0.0649
Tracks.SD	SD of tracks and trails density	Square meters	0–0.0649

Name, description, units, and range values are shown.

single trees, short grass, tall grass, highways, and canals (Table 1). We used a digital elevation model, which is a computer representation of the earth's surface, and as such, provides a database from which topographic parameters can be digitally generated. The highest resolution digital elevation model available for Puerto Rico (5 by 5 m) was used to calculate the slope and the aspect within each buffer. Aspect is a topographic variable that refers to the direction from high to low of the land area with respect to a reference point.

Data Analysis. To test for differences in drone returning rates, we used a two-way analysis of variance in JMP 8 (SAS) with both distance and cardinal points as factors. Data from the two sampling days were pooled for the analyses.

To characterize the landscape in the releasing sites, we used an ordination method, principal components analysis (PCA). Ordinations are used as graphical representations of multivariate data in a low-dimensional space, and they aim to organize data into a new set of coordinates in order of decreasing variance (Borcard et al. 2011). PCA was an appropriate method to examine a heterogeneous landscape such as our study area, given that no evident gradient was observed in the spatial variables that structured the landscape. This contrasts with other situations where spatial gradients are easily observed and multivariate methods like canonical correspondence analysis would be more appropriate (McCune et al. 2002). The PCA was run using the package *vegan* (Oksanen et al. 2011) in *R* (R Development Core Team 2011, Vienna, Austria).

PCA is an unconstrained ordination that, in contrast to constrained ordinations, are descriptive, given that no statistical test is used to assess the significance of the structures detected (Borcard et al. 2011). We performed a PCA to examine a matrix of sites (rows) by landscape variables (columns; Table 2). Because distance and orientation from the releasing sites to the apiary were predetermined and were separately analyzed, these variables were not included in the matrix but were part of the site description (e.g., D1N represents the releasing site at 1 km north to the apiary). In a PCA, the first axis accounts for most of the variation

in the ordination and the subsequent axes account for the remaining variation in a decreasing fashion. Because data were measured in different units, we used the built-in standardization option in *vegan* and calculated a correlation matrix to run the PCA. The scaling used (scaling two in *vegan*) emphasized the correlations among variables, represented by the angles between the arrows in the ordination plot. In the plot, sites are represented by their labels and landscape variables are represented by arrows (vectors). Vectors pointing in the same direction are positively correlated, vectors pointing in opposite directions are negatively correlated, and vectors at right angles are not correlated. Projecting a site at right angle on a vector is an approximation of the position of the site along that landscape variable.

To identify the landscape variables that contributed most to changes in the return rate of drones, we ran a stepwise generalized linear model selection in *R*. Data from the two releasing dates were not combined in this case. Generalized linear models are useful when transformations are not effective in making errors normal. In our case, landscape variables followed different distributions (Table 1). The residuals of the relation between proportion of returning drones and distance were analyzed as the response variable and landscape metrics were predictor variables. After eliminating correlated landscape variables, the predictor variables were mean aspect, aspect SD, mean slope, slope SD, proportion of urban cover, proportion of tree lines cover, proportion of tree groups cover, proportion of solitary trees cover, proportion of intermediate grass cover, proportion of tall grass cover, proportion of highways cover, proportion of canals and rivers cover, distance to DCA, mean tracks density, and tracks density SD (Table 2). The predictor variables were examined both as additive and interacting factors. We used an identity link function and treated the residuals of return rate as a Gaussian-distributed variable. In *R*, we used the *glm* (family = Gaussian) function and the automatic function *step* to fit and select the best model, respectively. The function *step* conducts a stepwise selection procedure starting with an arbitrary model,

Table 2. Landscape variables by site used in the PCA for the analysis of landscape characteristics

	ASP.M	ASP.SD	SLO.M	SLO.SD	Urban	Tree lines	Tree groups	Single Trees	Interm grass	Tall grass	Highways	Canals	Distance DCA	Tracks.M	Tracks.SD
D1N	3.07	1.56	0.01	0.03	0.00	0.23	0.00	0.36	0.35	0.00	0.03	0.04	1034.64	1134.50	809.92
D1S	2.79	2.31	0.04	0.04	0.72	0.12	0.10	0.00	0.03	0.00	0.04	0.00	1320.78	2029.11	582.36
D1E	2.95	2.08	0.03	0.04	0.68	0.04	0.00	0.05	0.10	0.06	0.03	0.04	1771.89	1830.74	667.23
D1W	2.87	2.07	0.02	0.02	0.31	0.06	0.01	0.00	0.62	0.00	0.00	0.00	500.07	1996.73	1078.18
D2N	2.88	1.45	0.11	0.07	0.12	0.11	0.22	0.00	0.33	0.20	0.03	0.00	241.32	1159.37	955.69
D2S	2.81	1.93	0.14	0.07	0.16	0.00	0.72	0.00	0.00	0.00	0.12	0.00	1885.85	1096.06	994.65
D2E	3.04	1.79	0.02	0.03	0.00	0.02	0.00	0.00	0.54	0.38	0.04	0.02	2309.42	1560.00	1191.57
D2W	3.02	2.27	0.06	0.07	0.63	0.00	0.04	0.00	0.34	0.00	0.00	0.00	1351.32	1940.18	1181.34
D4N	3.12	1.58	0.17	0.07	0.15	0.01	0.31	0.03	0.08	0.38	0.04	0.00	2074.93	1782.17	873.37
D4S	3.01	1.65	0.10	0.06	0.03	0.00	0.91	0.00	0.01	0.00	0.05	0.00	4033.78	1314.38	1035.26
D4E	2.86	2.16	0.03	0.04	0.53	0.09	0.00	0.30	0.00	0.01	0.00	0.07	4483.06	2328.09	1203.56
D4W	3.03	0.74	0.07	0.06	0.16	0.00	0.80	0.00	0.02	0.02	0.00	0.00	3399.06	1365.22	1051.96

See Table 1 for the description of explanatory variables. DCA: Drones Congregation Areas.
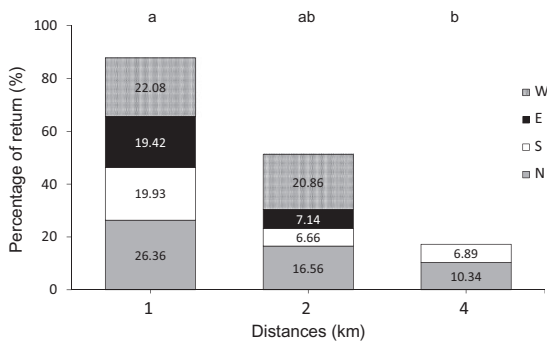
Fig. 2. Percentage (%) of drones returning to the apiary from each distance (in kilometers). Numbers inside the bars indicate the percentage of drones returning from each cardinal point (N: north, S: south, E: east, and W: west). Upper letters indicate significant (a, b), or not significant differences (ab) among distances.

adding or removing a term from the model that most reduces the Akaike's information criterion (AIC) and stops when no step decreases the AIC (Chambers and Hastie 1993). The AIC identifies the best model taking into account both the sample size and the number of predictors (Quinn and Keough 2002); models with the lowest AIC are the best fit, more parsimonious models.

Results

The drones released at the three distances (1, 2, and 4 km) were able to orient properly and return to their hives. Those released closer to their hives (1 and 2 km) exhibited a higher return rate ($F = 4.2213$; df = 2; $P = 0.01$; Fig. 2). Drones returned from all four cardinal points ($F = 0.7910$; df = 3; $P = 0.49$; Fig. 2) but those released from east and west did not return from 4 km. The return rate was higher between 3:30 and 4:30 pm (120–150 min after release), which was the observed peak for daily drone flights (Fig. 3A). The percentage of drones that returned to their original colony when released at 1 km was 10%, and those drifted to other hives, captured 1 d after release was 30%. In

the post hoc experiment, when drones were released at 50 m from the hive, 80% ($n = 80$) returned to the colony within 20 min. Marked–released drones returned the same day, and on subsequent days when released at 1 and 2 km from the apiary; in contrast, they returned at least after 1 d when released at 4 km (Fig. 3B).

PCA of Landscape Variables. The measure of the variance of each PCA axis is represented by the eigenvalue. Axis 1 explained 32.1% and axis 2 explained 17.1% of the variation in the landscape variables (Table 3; Fig. 4), and cumulatively axes 1–4 explained 77% of this variation.

Axis 1 separated sites at 1 km (right part of axis 1) from sites at 2 and 4 km (left part of axis 1). Sites at 1 km showed a higher proportion of canals and rivers cover, higher mean track density, and higher proportion of tree lines cover; in contrast, sites at 2 and 4 km showed higher proportion of tree groups cover, higher mean slope, and higher SD of slope (scores more than ± 0.56). Axis 2 separated sites with orientation N and W (upper part of axis 2) from sites with orientation S and E (lower part of axis 2). Sites at the N and W of the apiary showed higher proportion of intermediate grass cover and higher mean aspect, while sites at the S and E showed higher proportion of urban cover.

Generalized Linear Model. The best generalized linear model (lowest AIC) was as follows: residuals of percent return versus distance = distance DCA + orientation + proportion of tree lines cover + orientation × proportion of tree lines cover + orientation × distance DCA (Table 4). The AIC was 44.49 and the model did not show over-dispersion (residual deviance: 5.0053 on 18 degrees of freedom), which was an indicative that the Gaussian error distribution was appropriate.

Residuals were positively related to distance to DCA; residuals increased from −1.82 at ∼1,000 m away from the DCA to 1.91 at ∼4,000 m away from the DCA (Fig. 5). Residuals were negatively related to orientation meaning that they decreased from N and W to S and E. Last, residuals were negatively related to the proportion of tree line cover; though variability was high, residuals decreased from between −1 and 2 at 0 tree line cover to between −1.7 and 0.08 at >0.20 tree line cover.
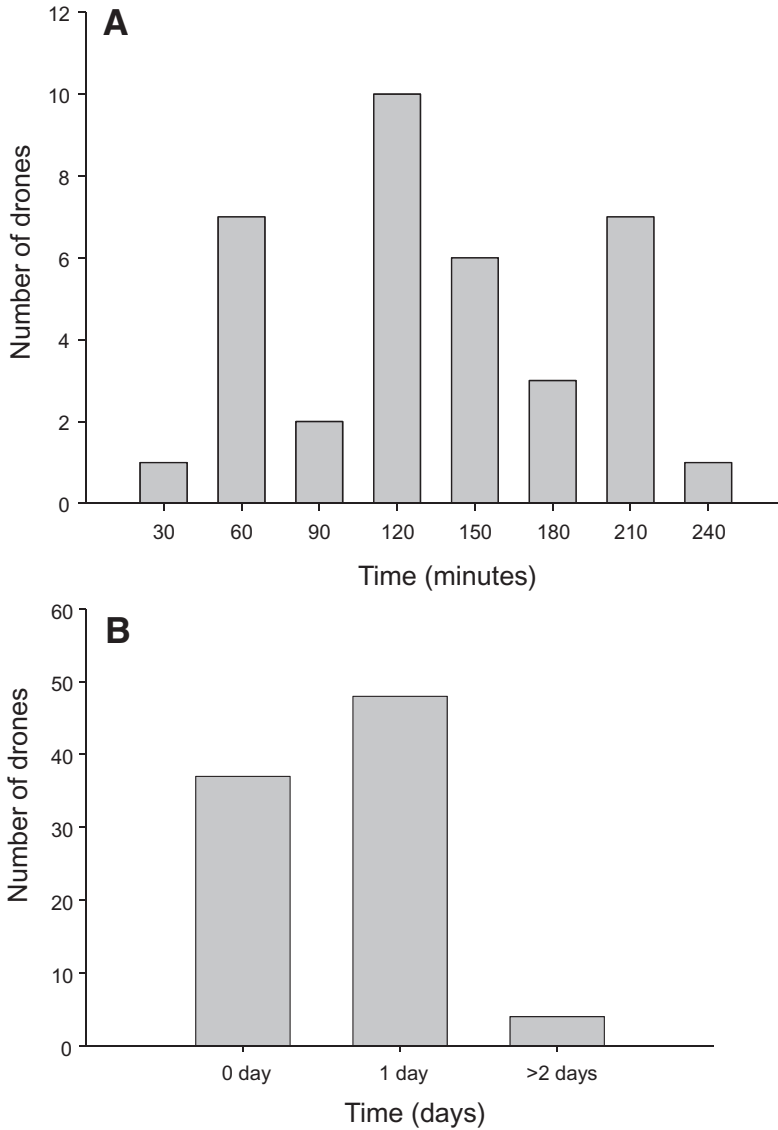
Fig. 3. Total number of returning drones through time. (A) Shows the four hours of observation in detail (from 2 PM to 6 PM). (B) Shows the observations per day in a three-day period.

Table 3. Results of PCA of landscape variables, and preliminary and final RDA of landscape variables effect on drone returning rate to the apiary

Analysis	Ordination axes			
	Axis 1	Axis 2	Axis 3	Axis 4
PCA of landscape variables				
Eigenvalues	4.8101	2.5569	2.2214	1.9357
Proportion explained	0.3207	0.1705	0.1481	0.1290
Cumulative proportion	0.3207	0.4911	0.6392	0.7683

Discussion

We examined the role of landscape on drone navigation in a mark–recapture experiment in Puerto Rico. Although the experiment with drones resulted in high mortality, because of their fragility, we showed for the first time that drones could return when released at 4 km from the apiary. More drones returned from closer than farther distances from the apiary, and they returned from all four cardinal points, as expected. Though direction alone did not significantly affect the orientation of drones, there was an interaction between distance to DCA and direction that resulted in no drones returning from the east and west at 4 km. The landscape at the north and south could have been familiar for the released drones, facilitating their return to the apiary. Alternatively, the absence of urban cover on north and south (see Fig. 1) could have facilitated drones to orient better and return to their hives. Urban cover has been shown to have divergent effects on bees of different taxonomical groups and this effect likely
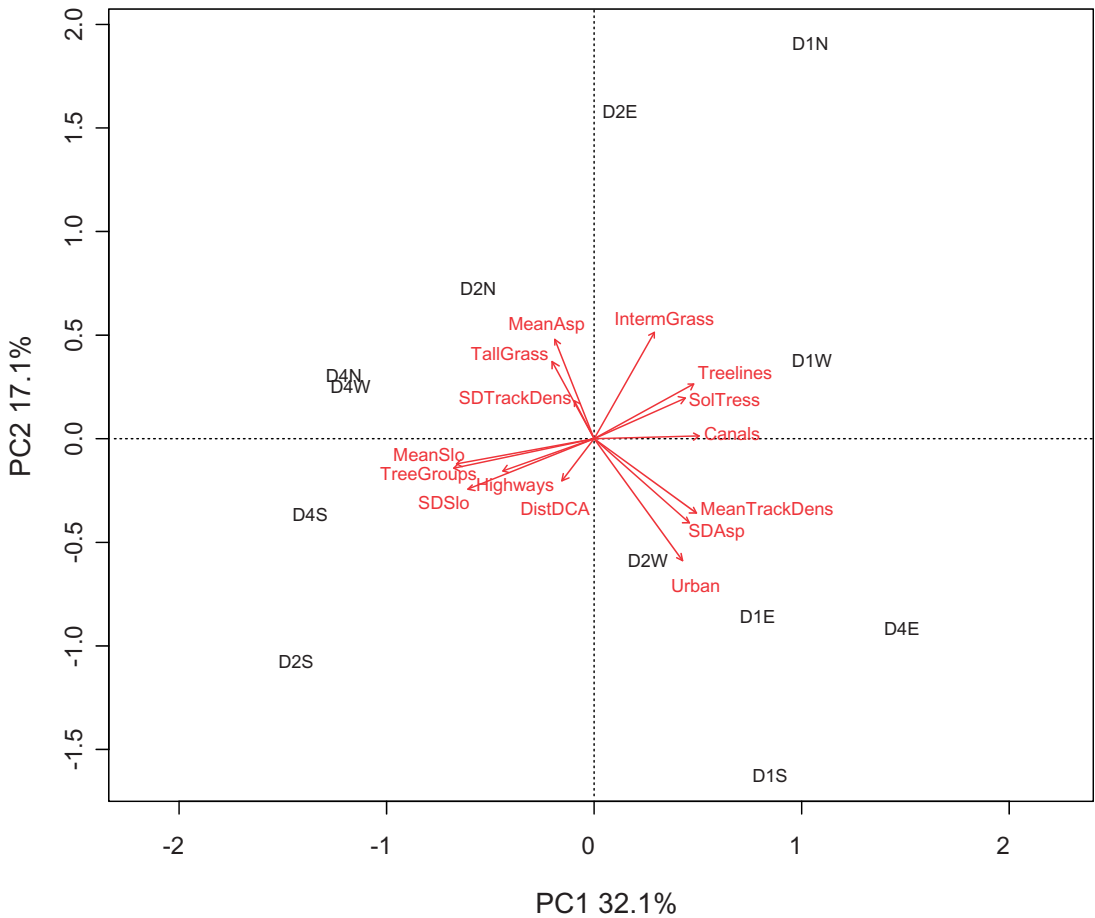
Fig. 4.   Principal components analysis of the 12 drone releasing sites based on landscape variables (Table 2). The sites are labeled according to distance from the apiary (D1-: 1 km, D2-: 2 km, and D4-: 4 km), and cardinal point (N: north, S: south, E: east, and W: west). The variables are represented by vectors which length indicates their importance in building each axis. The scaling used for the PCA emphasized the correlations among variables, represented by the angles between the arrows in the plot. Projecting a site at right angle on an arrow is an approximation of the position of the site along that landscape variable.

Table 4. Coefficients resulting from the automatic generalized linear model selection using *step* in *R*. Significance codes: 0 '*',
0.01 '*' 0.05 '.'**

	Estimate	Std. Error	*t* value	Pr(>|t|)
(Intercept)	1.292e + 00	7.013e − 01	1.843	0.081
Distance to DCA	4.272e − 04	3.184e − 04	1.342	0.196
Orientation	−6.998e − 01	1.596e − 01	−4.384	0.000***
Tree line cover	−9.463e + 00	3.664e + 00	−2.582	0.019*
Orientation × tree line cover	1.711e + 00	1.157e + 00	1.479	0.156
Orientation × distance to DCA	8.787e − 05	6.648e − 05	1.322	0.203

depends on the quantity, quality, and distribution of floral resources (Goulson et al. 2002, Wojcik and McBride 2012). Our data suggest that drones may better orient in seminatural than in urban environments.

Distance from the hive has previously been studied in relation to orientation, and it has been suggested that bees have spatial memories for navigation that operate at two scales, one specialized route memory and other general landscape memory (Menzel et al. 2000, 2005; Palikij et al. 2012). Both memories likely

had an influence in our experiment. In our pos hoc experiment, we released drones 50 m away from the maternal hive and 80% of the marked drones returned to their maternal hive in <20 min. Including these data, we tested the linear and nonlinear relationships between distance from the hive and percent of returning drones and found that the percent of returning drones decreased at a rate slower than predicted by a linear relationship at the farthest distance. Only the fit to the log transformed distance data was significant
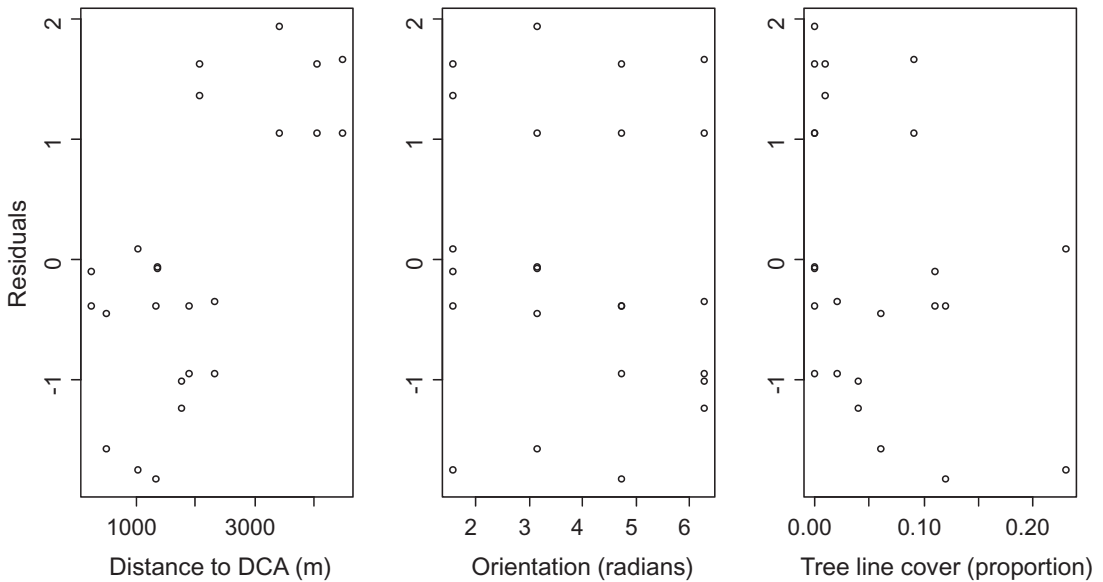
Fig. 5. Landscape variables included by the automatic generalized linear model selection in *R*, where the best model was: Residuals of percent return vs distance = Distance DCA + Orientation + Proportion of tree lines cover + Orientation * Proportion of tree lines cover + Orientation * Distance DCA (see Table 4 for model coefficients).

suggesting that landscape features at large distances had been memorized by drones before and are being used as coarse-grained landmarks to navigate back home. Because their geographical knowledge is probably fine-grained close to home and coarse-grained further away, we recommend to combine local (e.g., vegetation structure) and landscape analysis to understand what spatial characteristics are fundamental for navigating drones at different scales.

The number of drones returning to the hive through the day showed the highest peak of drone return 2 h after the release time, which corresponded with the highest peak of drone flight activity in the hives (Figs. 3A and 4). It took a few drones as long as 2 d to return when they were released at 1 and 4 km. It appears that returning to the general colony location (the apiary in this experiment) was more important than returning to the original colony for drones (30% found in other colonies, and 10% in the original colony). This lack of precision suggests that drones that did not return to the colony could have drifted to other colonies (see Giray and Robinson 1996). The small distance of ~1–2 m between hives in the same orientation in our apiary, and 40 hives in one apiary could explain the low return rate to the original hive. Currie and Jay (1991) demonstrated that 50–60% of the drones drifted at 15 d of age when distances between colonies were <50 m. In addition, predation by birds, successful matings (and death as a result), orientation mistakes, and drones resting overnight in flowers (Ackerman James 2009; personal communication) could contribute to low overall return rate of mark–release drones. These factors would be similar across different release points, and we only compared the drones that successfully returned to the original colony.

The landscape characteristics varied among releasing sites and this was related to drones return rates. Worker honey bee navigation is influenced by both a sun compass and landscape learning (Frisch 1967, Towne and Moscrip 2008). In the PCA, sites at 1 km were more similar among them in one dimension, showing higher proportion of canals and rivers cover, higher mean track density, and higher proportion of tree lines cover than sites at other distances. Tree line cover, at the same time, was one of the three selected variables in the generalized linear model that explained variability in drone return rates when distance was controlled for. The relative homogeneity of landscape variables at 1 km may represent a familiar landscape for drones, though a high variability was observed in return rates. At 2 and 4 km, the landscape was more heterogeneous, and this corresponded to a decrease in the return rate variability. It has been reported that bees orient better in heterogeneous than in homogeneous landscapes because the first offer more spatial cues (Averill 2011). In the other dimension, sites with orientation N and W were different in their landscape from sites with orientation S and E. Sites at the N and W of the apiary showed higher proportion of intermediate grass cover and higher mean aspect, while sites at the S and E showed higher proportion of urban cover. We previously found that aspect (direction of slope) was a landscape characteristic that distinguished the locations were DCAs were present (Galindo-Cardona et al. 2012). At the same time, drones returned more frequently but with a higher variability from the N and W releasing points. The generalized linear model showed that distance to DCA and orientation are interacting and reduce the variance explained in the return rate of drones. Drones have been previously reported as

returning from nearer DCAs from the home apiary than from DCA farther apart (Koeniger et al. 2005a). We confirm that these variables, in combination with orientation and tree lines, are strongly influencing the return rate of drones independently of grass cover and aspect. Tree lines were previously reported as important refuges for honey bees (Oleksa et al. 2013). There may be other important variables that may explain the remaining variation. These may include variables that we did not account for, such as wind direction, or density of floral resources.

There is evidence showing the connection among magnetic sensitivity, circadian rhythm, and light sensitivity in insects (Yoshii et al. 2009, Muhein et al. 2014). Drone flight could be a good model to study this interaction because drone flight shows a strong circadian pattern and involves important navigational abilities. Especially at the greater distances (≥ 2 km), on an unfamiliar terrain, magnetic sensitivity could provide cues for the orientation of bees in all directions (Hsu et al. 2007). In Puerto Rico, drones take mating flights only in the afternoon, 1430 to 1730 hours (Galindo-Cardona et al. 2012); this time is precisely when the Earth's magnetic field is highest. Future studies could include magnetism at a fine scale as an additional landscape variable, taking into account that it varies depending on location, landscape features, and time of day and year.

This study is the most comprehensive application of landscape study to drone flight behavior. We revealed the drones are able to navigate home from as far as 4 km, and we discussed the relevance of three important factors influencing bee orientation, i.e., distance to DCA, orientation and tree lines. Future studies that would include additional parameters such as wind direction and examining different scales from hundreds of meters to kilometers would result in better understanding of honey bee navigation and the potential problems we may be creating in urban and agricultural landscapes for our pollinators (Huang and Giray 2012, Delgado et al. 2012).

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