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RESEARCH ARTICLE

Spatio-temporal dynamics of landscape use by the bumblebee *Bombus pauloensis* (Hymenoptera: Apidae) and its relationship with pollen provisioning

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Abstract

Understanding how bees use resources at a landscape scale is essential for developing meaningful management plans that sustain populations and the pollination services they provide. Bumblebees are important pollinators for many wild and cultivated plants, and have experienced steep population declines worldwide. Bee foraging behavior can be influenced by resource availability and bees' lifecycle stage. To better understand these relationships, we studied the habitat selection of *Bombus pauloensis* by tracking 17 queen bumblebees with radio telemetry in blueberry fields in Entre Ríos province, Argentina. To evaluate land use and floral resources used by bumblebees, we tracked bees before and after nest establishment and estimated home ranges using minimum convex polygons and kernel density methods. We also classified the pollen on their bodies to identify the floral resources they used from the floral species available at that time. We characterized land use for each bee as the relative proportion of GPS points inside of each land use. Bumblebees differed markedly in their movement behavior in relation to pre and post nest establishment. Bees moved over larger areas, and mostly within blueberry fields, before nest establishment. In contrast, after establishing the nest, the bees preferred the edges near forest plantations and they changed the nutritional resources to prefer wild floral species. Our study is the first to track queen bumblebee movements in an agricultural setting and relate movement changes across time and space with pollen resource availability. This study provides insight into the way bumblebee queens use different habitat elements at crucial periods in their lifecycle, showing the importance of mass flowering crops like blueberry in the first stages of queen's lifecycle, and how diversified landscapes help support bee populations as their needs changes during different phases of their lifecycle.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Animal assisted pollination is crucial for the reproduction of wild and domesticated plants, and worldwide, insects are the main provider of this service [1]. Insect pollinators help to maintain trophic networks in nature [2] and help improve both quality and quantity of crops for human consumption [3–5]. Approximately 35% of global food production, and approximately 70% of economically important crop species depend upon insect pollination (to different degrees) [6–7]. Bees are one of the most important insect pollinators, but both wild and managed bee populations are declining [8–12], decreasing their potential pollination service [13–15]. Land use intensification and fragmentation associated with agriculture have contributed to bee population declines [16–17]. Understanding how bees use the resources in agricultural landscapes is essential to develop meaningful farm-based land use management plans that sustain bee populations and maximize the potential pollination service they provide to farmers and ecosystems [18–20].

In these agricultural landscapes, bumblebees (*Bombus spp.*) are one of the most important groups of bee pollinators [21]. Even so, among insect pollinators, bumblebees have experienced some of the steepest population declines and range contractions [22–25]. *Bombus spp.* have a large foraging capacity and can fly in a wider range of ambient temperatures than many other bee species [26–27], present the characteristic “buzz-pollination” causes large amount of pollen to be released, making them efficient pollinators for a variety of crops (eg. blueberry) [28–33]. They have eusocial habits [34] with colonies that can reach up to 400 individuals with several queens [35]. Bumblebee colonies have an annual lifecycle and, unlike honeybees, they do not store large quantities of honey or pollen in their nest [36]. As such, the survival of the colony depends upon the availability of suitable food for the different stages of its life cycle within foraging distance of the nest, since their nutritional requirements differ pre- and post-establishment [34,37]. Environmental or habitat changes can negatively impact a colony’s success and chance of survival [38]. The forces that shape individual bumblebee flower or patch choice have been well studied [39–48]. Previous work has shown that *Bombus spp.* are guided by visual, olfactory and social cues as well as the quality and quantity of floral resources [38,49]. This last factor resources are subject to temporal and spatial changes, presenting marked differences with respect to the stage of the cycle where they are found and translating into changes in their availability within the landscape [34]. Understanding how bees use these patchy resources is essential for bee conservation within agricultural landscapes. Newly emergent queen bees, for instances, are known to first fly only short distances with periodic rests stops, before beginning their nest searching behavior and dispersal flights [50]. At other life stages, bumblebees develop efficient routes between their nests and floral resources, maximizing the food resources available from the landscape [51].

Historically, it has been difficult to track individual bee movements across landscapes [51]. The first studies of landscape use of bumblebees used harmonic radars [52,53]. These studies confirmed the general bee movement patterns, flight behavior and the first insight into resource selection in landscapes [54]. Harmonic radar studies require passive transponders (without a battery) to be fixed to insects and tracked using large radar [55]. Recently, newer, less expensive technologies have enabled biologists to use miniaturized radio telemetry transmitters on bees [55,56], and these micro-transmitters allow for real-time tracking of bees and the ability to link fine-scale habitat features with bee habitat selection and floral resource choice. This technology requires less infrastructure than harmonic radar and is smaller and more precise, though the detection range of radio telemetry is more limited [55]. Nonetheless, radio telemetry can allow for new insights into bee movements at the finer scale needed to make farm-based management plans for bees.

We studied habitat selection of one bumblebee species, *B. pauloensis*, using radio telemetry in an agroecosystem dominated by blueberries in the state of Entre Ríos, Argentina. Our objective was to determine how the queens of *B. pauloensis* modify their spatio-temporal use of the blueberry agroecosystem [57], and to provide new knowledge about how they change their flight behavior and landscape use during different lifecycle stages. We hypothesized that the *B. pauloensis* queens would use landscape resources differently, changing their foraging behavior (size and shape of the home range) and the preference for certain floral resources according to the pre- and post-nesting condition. To our knowledge, this is the first study of its kind to link spatial habitat selection of bees revealed by radio telemetry with floral pollen resources in a working agroecosystem landscape.

Materials and methods

Study area

The study was carried out on large-scale commercial blueberry farms in Yuqueri station, Entre Ríos province, Argentina (31°22'22.4538" S / 58°07'23.7864" W) neighboring the National Institute of Agricultural Technology, Concordia Experimental Station. The agroecosystem is characterized by the presence of blueberry and citrus fields, and small-scale *eucalyptus* and *pine* plantations and windbreaks. This agro-forestry system is common and expanding in this region of northern Argentina. We conducted our study from the last week of July to the third of September 2015 when the blueberry bushes (*Vaccinium corymbosum* var. Emerald) are in peak bloom.

Bee capture and tracking

We opportunistically netted 24 *Bombus pauloensis* queens that were visiting blueberry bushes at the beginning of August and September 2015. Netted bees were transferred to small plastic tubes with fine gage cotton gauze on one end and foam plug on the other side. We then gently pressed the bee with the foam plug against the gauze so its abdomen was held flat against the gauze. Next we gently cut through the gauze and glued 0.2 g radio transmitter (ATS Series A2412) to the upper part of the abdomen with a combination of eyelash fixative (Striplash Adhesive DUO—240592) and cyanoacrylate (Fig 1A) (S1 Fig). Once the glue had dried and we confirmed the transmitter was active (approximately 15 minutes per bee), we released the bee at the point of capture.

The transmitter emits short radio pulses, allowing for real-time tracking on the ground by technicians using ATS receivers and yagi directional antennas (2.5 kHz, Advanced Telemetry Systems, Inc. R410 Reference User Manual - R06-11) (Fig 1B). We tracked the bumblebees through the agroecosystem daily from 8 am– 6 pm for 1–9 days. Once an individual bumblebee was relocated we recorded its GPS location. Prior to beginning the study, we tested the effective range of the transmitters by placing an active transmitter on a blueberry bush and listening to the signal's strength; we concluded we could relocate the transmitter within a radius of approximately 200 m.

All tagged bees took off a few minutes after release. We subsequently observed the same individuals flying through the agroecosystem and actively foraging for nectar and pollen on blueberry flowers, and we could not visually detect a difference between the flight of tagged bees compared to natural flight. We were unable to fully quantify how the transmitter weight affected flight performance in the field settings we worked, however. Bumblebees, though, are known to be strong flyers [55,56, 58] able to carry copious amounts of pollen; their ability to carry a 0.2 g radio-transmitter with little apparent difficulty is not surprising since bumblebees

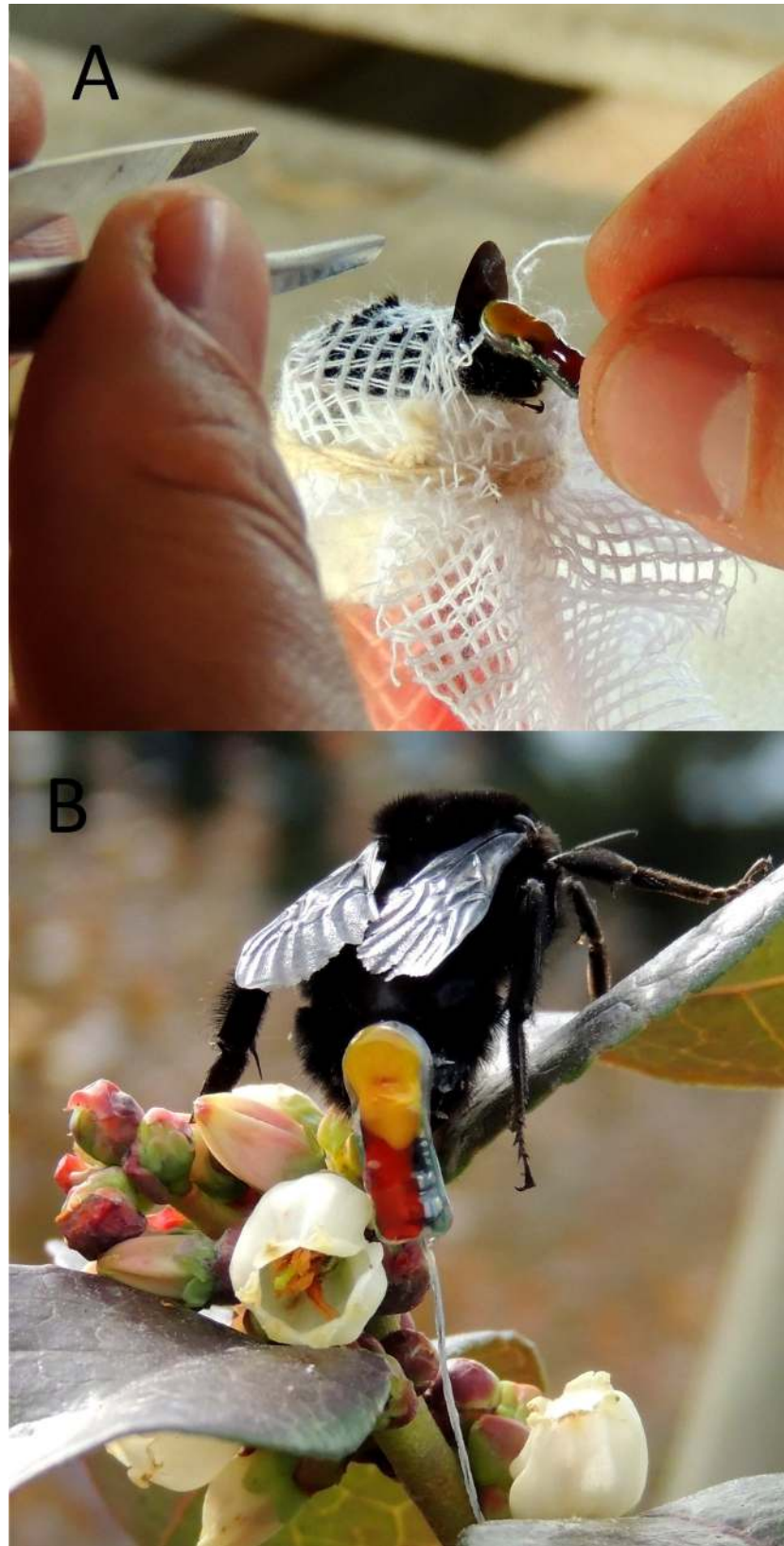


Fig 1. Fixing of the radio transmitter. A) Immobilization of the individual to be tracked in a soft rubber tube with a foam plunger; transmitter was attached with fast-acting glue. B) *Bombus pauloensis* queen with transmitter foraging on blueberry flowers. Photo credits: P. Cavigliasso.

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have been documented to carry nectar loads of up to 90% their body weight [36] (see S1-S3 Video in [S1 File](#)).

After releasing the tagged bees, we tracked the on foot and from a truck throughout the blueberry fields and the surrounding landscapes. The search-and-locate procedure was carried out systematically for all the individuals studied. At the time of release, queen bees were followed for as long as possible, recording their location only when we could visually see the bee. We continued to track the bees and record their location when it was > 5 m from the previous position, or in the case of static periods (eg: inside the nest or "resting"), once every 5–10 minutes. When a bee's radio signal was lost, we then scanned the area using the directional yagi antennas and the "Scan" mode of the ATS receiver for the nearest bee. Once an individual was located the procedure was repeated. When searching for a signal from the tagged bees, we began at the outer margin of the fields and worked our way towards the center, with the intention of capturing both internal and external locations of the bees. When searching for a bee the following day, we would return to the area where we last recorded its position and began the search-and-locate procedure again.

This procedure was carried out in two different time periods of the bees' life cycle: 1) during the nest searching location that immediately follows emergence from hibernation when the queens seek suitable a site to rear a colony; and 2) after nest establishment, when the queen has established its nest and is rearing the first cohort of workers. The nest searching period coincided with the beginning of the blueberry crop's flowering (July 28 to August 7). The post-nest establishment period occurred during the end of the blueberry bloom and the beginning of the blooming of most native plants (August 31 to September 22) (Abrahamovich, personal observation). When a nest location was confirmed, we also recorded that location and notes its substrate.

The majority of this research was completed at the Concordia Research Station, part of the National Agricultural Technology Institute (INTA). The station is located at Ruta Nacional 14 Km 259 (Concordia, Entre Ríos, Argentina). A minority of the research took place on private agricultural land immediately adjacent to the INTA station that was planted with blueberries. This occurred when the radio-tagged bee flew to the blooming blueberry fields. Before following the bees, we secured the permission of the land owners. This non-lethal research involved netting the bees, which are not endangered, and gently restraining them while attaching the tag, which fell off naturally over time.

Land use classification

We classified the study area vegetation that cover 3,141.5 km² using five land uses categories (LUC hereafter). The LUCs were grouped into: 1) *Blueberry*, the area occupied by blueberry field; 2) *Forest plantations*, comprised of planted blocks of *Pinus* and *Eucalyptus* spp. and windbreak of *Casuarina* spp.; 3) *Semi-natural area*, including pastures, abandoned lots, areas in recovery and road margins; 4) *Other fruits*, primarily citrus; and 5) *Developed*, representing human-constructions such as houses, barns and roads. The classification was done using the "Google Satellite" option of the "OpenLayers plugin" tool of QGIS (version Essen 2.14.3, available at <https://www.qgis.org/es/site/>), with a WGS / Pseudo Mercator projection (EPSG: 3857). We then calculated the proportional use of each land cover type based on the observed GPS locations, giving each observed point a class (e.g., blueberry or semi-natural) and quantifying

the relative frequency of occurrence for each bee individual, allowing us to compare habitat use before and after nesting. These LUCs were then used in further analysis (described below).

Bee home ranges and habitat selection

To estimate the home range and habitat selection of the queen bumblebees, we used two methods: Minimum Convex Polygon (MCP) and kernel density (KD). These two methods show complementary information on home range and habitat use, with MCPs representing the furthest ranging territory of the bees and the KD demonstrating which habitats the bees were more likely to use [59–61]. These metrics thus show us where the queens can fly and what LUC they use more intensely and thus prioritize [62].

MCP were calculated from the connected perimeter of the 5 most external recorded GPS locations taken for each individual. This method generates a polygon with an area equivalent to the minimum portion of the landscape used by each individual. From the MCP, we made inferences on the way they move, maximum flight distances, and preferences for any land use present within the landscape (land uses categories, described below). As the maximum flight distance, for each individual we used the most distant two vertices of the MCP [56]. We also characterized the shape of the polygon using two parameters: Coefficient of Compactness (K_c) and Circularity Ratio (R_{ci}). K_c is defined as the relationship between the perimeter of a polygon and the perimeter of an area circumference equivalent to that of the polygon to be evaluated (Formula A), and is a continuous variable between 1 and 3; high values indicate very elongated areas and low values indicate more circular areas. R_{ci} is the quotient between the areas of the polygon and that of a circle whose circumference is equivalent to its perimeter (Formula B, range from 0–1 with 1 being totally circular areas for the unit value, square for the value 0.785 and irregular and elongated for values lower than 0.20). This coefficient is used in a complementary way for the interpretation of K_c since they describe similar parameters. These geometric parameters are widely used to classify the two-dimensional areas on maps [63–65]. These indices, although not previously used to characterize movement in animals to our knowledge, can be easily calculated and provide an accurate approximation of the non-uniform two-dimensional movement areas.

$$A) K_c = \frac{P}{2(\pi A)^{0.5}} = 0,282 * P/A^{0.5}$$

$$B) R_{ci} = 4\pi A/P^2$$

Where: K_c - Coefficient of Compactness; R_{ci} - Circularity Ratio. Symbols, P - perimeter of the MPC; A - MPC area; D - diameter of a circle with the same surface as the MCP.

We calculated the KDs for *B. pauloensis* queens for both time periods. For this, we used the "Heatmap plugin" tool of QGIS, to create a raster layer through the density of points observed in each stage studied. For this calculation, we use the kernel function "Quartic (triponderated)" that resembles a circular kernel with a fixed radius to 60 layer units, which defines the direct distance from the estimated point and specifies the influence of the kernel [66]. It has been shown that this procedure is suitable for this purpose [67]. The estimators of the Kernel functions calculated for both stages are presented in S1 Table. The MCPs and KDs were calculated on all bees that were relocated five or more locations.

Use of the floral resource around the agroecosystem

To evaluate changes in the use of floral resources before and after nest establishment, we collected queen bees each week to analyze pollen loads on their bodies, and we collected pollen

from all available flowering plants in the landscape to make a pollen reference library. Additional *B. pauloensis* bees (not used in telemetry study) were captured using an entomological vacuum while walking a random transect for 10 min in the same fields where we tracked the bees. Collected bumblebees were stored individually in Falcon tubes with 10 ml of 70% alcohol. We then collected the pollen that was adhered to bumblebee bodies by gently agitating the tube, resulting in a homogenized solution of pollen. From this solution, we extracted 10 μ l, stained the pollen with Alexander's stain, and used a Neubauer's chamber to count the relative abundance and identity of the first 100 pollen grains observed under an optical microscope (Boeco BM-300/I/SP). Pollen found on the bumblebees was compared in three time periods following blueberry flowering and the date of capture: Early flower (4th week of July and 1st week of August); Peak flowering (2nd and 3rd weeks of August); and Post-peak (4th week of August to 2nd of September). The pollen library floral specimens were collected from blooming plants in the study area. Pollen samples were dried in an oven for 4 hours at 65° C to and we took a microphotograph of the pollen from each species (adaptation from Gui et al. 2014 [68]) (S2 Fig).

Data analysis

First, we compared foraging metrics within the condition (before and after) of nest establishment. We considered as responses variable the MCP area, maximum flight distances and shape parameters (Kc and Rci) and used a Kruskal-Wallis test.

The observed GPS locations were compared it to a simulated random habitat use. To simulate random habitat use, we chose MCP area of each of the marked queens and randomly located the same number of points registered within this areas. We performed this procedure and calculated the proportional availability of landcover types by intersecting the random point locations with the GIS landcover types. We used a Chi²-test (including the frequencies of all four landcover types) to test whether observed frequencies of habitat use of the radio-tracked bumblebee significantly deviate from the mean simulated random habitat use of landcover types. For this, we used the "Random points tool" of QGIS 2.14.3 Essen, to create a vector layer which contains a random points series at the boundaries of the "MCP" layer of "n" points according to the n—waypoints taken for each queen captured.

The relative frequency of waypoints observed in each LU during the pre- and post-nest life stages we compared through generalized linear mixed models (GLMM). For this analysis, the relative value of waypoints present in each LU within the Minimum Convex Polygon (MCP) was a response variable (RV) (negative binomial distribution) and the stage (before and after establishing a nest) was a fixed effect. We took into account the effect of the different individuals including this variable as a random factor (Function: $RV \sim \text{Stage} + (1|\text{Ident})$). The analyzes were done with the statistical software R 3.5.1 (R Development Core Team, 2013). We used the *glmer* and *glmer.nb* function of the "lme4" package version 1.1–12 for the GLMM.

Finally, the number of plant species and the proportion of the pollen species best represented as indicated by pollen on bees (response variable) on every *B. pauloensis* queen for the three blueberry flowering time stage (early, peak and post) was compared to explore how bumblebee queens use floral resources over time. Because of the non-normal nature of these data, were completed the pollen analysis using Kruskal-Wallis test.

Results

In total, during both study periods, we captured and tracked 24 bumblebee queens but only 17 were regularly relocated (more than 5 GPS locations) and only these individuals were used for data analysis, per the criteria of the MCP and KD method, we recorded 473 waypoints, of

which 166 were obtained before bees established their nests and 307 were post-establishment. We recorded at the beginning of the bloom, 24 ± 11 (mean \pm SD) location were recorded. In contrast with bees at the end of bloom that added 31 ± 20 location per queen. Nine of the ten post-establishment bees were associated with a confirmed nest location (Table 1).

Foraging metrics

Bombus pauloensis were found to visit different foraging areas behavior before and after nest establishment. Before selecting a nest, queens foraged over larger areas based upon MCPs (84% larger before vs. after. $H = 6.94$, $p = 0.0068$) (Table 2), with a tendency to forage within an oval shape ($H = 1.87$, $p = 0.0702$), whereas after establishing a nest bumblebees queens foraged in smaller and more elongated areas. The average maximum flight distance was 642.58 ± 396.89 m (mean \pm SD), not finding significant differences between stages ($H = 2.44$, $p = 0.1331$) (Fig 2).

Use of the landscape and floral resource around the agroecosystem

The quantitative habitat analysis of 17 *B. pauloensis* queens showed that the proportional habitat use of 15 of the 17 bumblebees deviated significantly from the mean simulated random habitat use of landcover types in the study area (S2 Table). Before selecting a nest, queen bees focused on blueberry fields that were just beginning to flower. After nest establishment, queens tended to forage in the periphery of the blueberry, often near *semi-natural* habitats and *other*

Table 1. Complementary information of each queen of *B. pauloensis* studied. The summary measures of each estimated movement area are presented.

Stage	ID ^a	N° waypoint	Tracking time			Nest ^b	Nest location ^c	MCP (ha) ^d	Max. homing distance (m) ^e
			Start date	End date	N° Day				
Before nest	oo5	8	30/07/2015	04/08/2015	6	No	-	93.1	1573.5
	o85	30	02/08/2015	07/08/2015	6	No	-	11.2	593.82
	105	20	29/07/2015	06/08/2015	9	No	-	20.9	870.58
	124	41	28/07/2015	05/08/2015	9	No	-	2.84	296.18
	144	32	30/07/2015	07/08/2015	9	No	-	17.4	1088.02
	304	17	28/07/2015	01/08/2015	5	No	-	7.46	766.11
	304.2	18	05/08/2015	07/08/2015	3	No	-	6.1	508.52
After nest	164	38	17/09/2015	22/09/2015	6	Yes	Blueberry	0.96	187.4
	185.3	65	16/09/2015	22/09/2015	7	Yes	<i>Casuarina sp.</i> Windbreaks	13.5	1167.29
	244	15	02/09/2015	07/09/2015	5	Yes	<i>Eucalyptus</i> plantation	7.88	771.56
	244.2	18	09/09/2015	10/09/2015	2	Yes	Savanna	0.55	256.77
	244.3	7	10/09/2015	11/09/2015	2	Yes	<i>Eucalyptus</i> plantation	0.95	853.13
	264	46	16/09/2015	22/09/2015	7	Yes	Young <i>Eucalyptus</i> plantation	2.68	277.03
	264.1	11	16/09/2015	16/09/2015	1	Yes	Young <i>Eucalyptus</i> plantation	5.13	827.42
	364.2	20	19/09/2015	19/09/2015	1	No	-	0.96	289.18
	364	34	31/08/2015	01/09/2015	2	Yes	Citrus	1.56	184.15
	385	53	18/09/2015	22/09/2015	5	Yes	Old Blueberry	1.35	413.19

^a Identification code of each individual studied corresponding to the frequency of the transmitter fixed in his body. All queens were different and it was only possible to follow them during one stage. When possible, at the end of the monitoring period, transmitters were removed from the queens.

^b Presence or absence of nests established.

^c Description of the LUC where the nests are located.

^d Area of the Minimum Convex Polygon expressed in hectares.

^e Included among the most extreme waypoints.

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Table 2. Parameters of size and form of MCP in both stages of the home ranges of radio tracked *B. pauloensis* queens.

Establishment Nest	Before	After
MCP-Area (ha) *	22.7 (\pm 31.69)	3.56 (\pm 4.20)
MCP-Kc ^a	1.35 (\pm 0.18)	1.99 (\pm 1.08)
MCP-Rci ^b	0.56 (\pm 0.13)	0.37 (\pm 0.21)
Maximum flight distance (m)	813.82 (\pm 421.77)	522.71 (\pm 350.25)

Comparison by non-parametric variance analysis Kruskal-Wallis. Mean values (\pm Standard Deviation).

“*” parameters show significant differences ($p = <0.05$).

^a Coefficient of Compactness

^b Circularity Ratio

<https://doi.org/10.1371/journal.pone.0216190.t002>

fruit LUC with blooming wild and domesticated plants (i.e., citrus plantations) (Fig 3). After nest establishment, queen bumblebees' home ranges appear to shrink.

The proportional use of different habitats differed in accordance with nest establishment. For instance, they increased their foraging in forested areas once they established a nest (GLMM. Negative Binomial. $F = 4.90$, $p = 0.0428$). Bees increased by nearly 68.14% their use of plantations once they have a nest (Table 3). It should be noted that 56% of the nests observed were located on the edge ($\sim 3\text{--}5$ m) of *Eucalyptus grandis* plantations or forest windbreaks of *Casuarina* sp., both of which are part of the plantation LUC (S3 Table).

The pollen present on *B. pauloensis* queens ($n = 44$) captured inside the blueberry fields during the whole flowering of the var. Emerald, was from 54 plant species and did not differ across the time of the blueberry flowering ($H = 3.58$, $p = 0.165$). During the peak flowering of blueberry fields, the bumblebees focused their foraging on this mass flowering resource, but by the end of the blueberry flowering, other floral species increased their importance as resources for the bees. Plant species *Conium maculatum* L., *Buddleja stachyoides* Cham. & Schtdl. and *Nothoscordum arenarium* become more important and are collected more by queens of *B.*



Fig 2. Location of the MCPs observed in both monitoring stages. The different foraging areas of the *B. pauloensis* queens before (before) and post- (after) the establishment of their nests are detailed. **References:** Land uses, LU categories included in the landscape use analysis; *Before and After nest*, in each case, the code used for identified each individual corresponding to the radio frequency of ATS Series A2412 transmitter.

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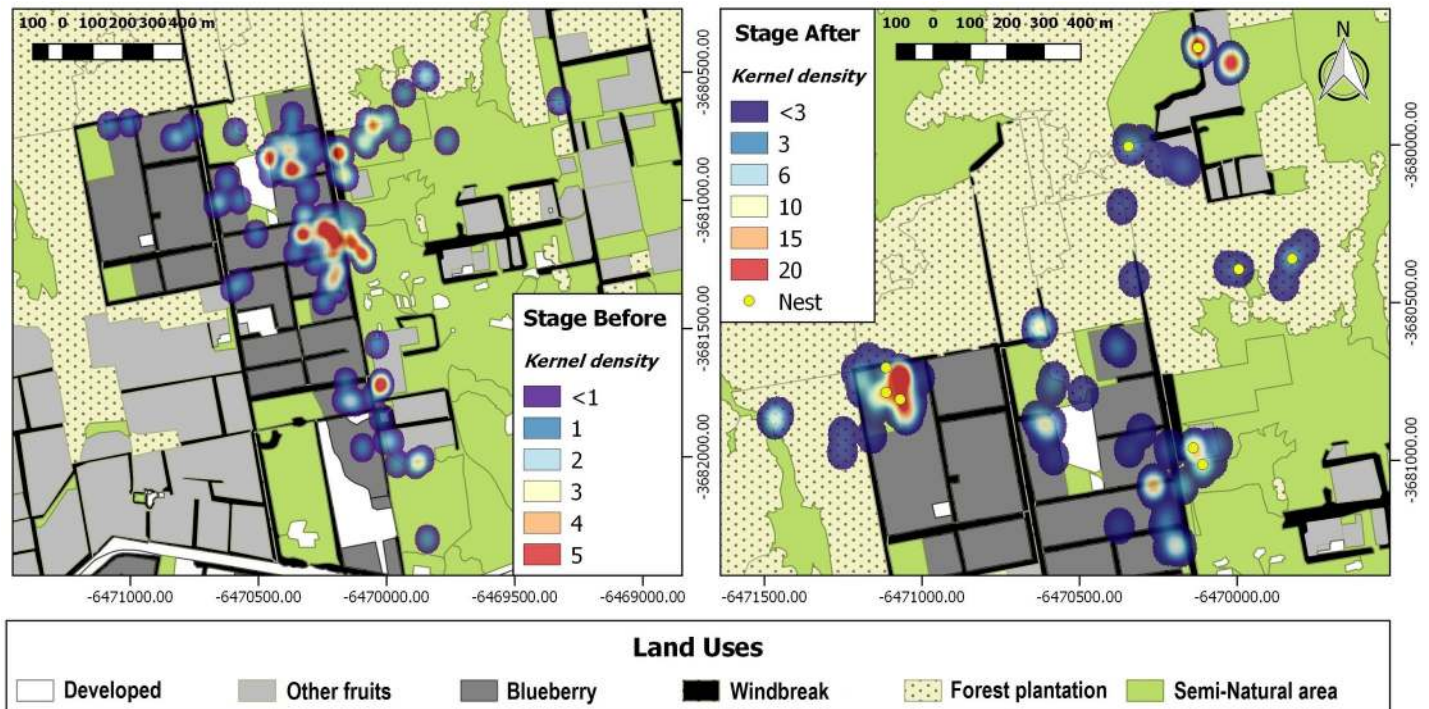


Fig 3. Kernel density maps of tracking bumblebees’ queens before and after setting a nest. Red values (warm colors) indicate high probability presence while cool colors (blue) tend to low probability of using the space. The maps were made from the pooled data for all the queens followed, differentiated before and after setting a nest.

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pauloensis in the post-peak period. These analyzes also show an increase in the botanical diversity of pollen present on *B. pauloensis* of ~ 38% more species between the peak of flowering and the post-peak (Table 4) (S4 Table).

Discussion

We investigated bumblebee habitat selection, flight distance, and home range to better understand how *B. pauloensis* selects floral resources in a complex and intensively used agricultural landscape. In real-time, we observed variation in the size and shape of their foraging areas, flight distances, and habitat preferences related to food and nesting. Queen *B. pauloensis* appear to decrease their foraging areas and flight distances once they establish nests, using mostly the

Table 3. Proportional occupation of the different land uses (LUs) quantified in both stages of monitoring. Estimates statistics calculated using GLMM are presented.

LU ^a	Nest ^b		Wald-Test	
	Before	After	F	p
Blueberry	55.42 (± 17.86) A	24.55 (± 6.88) A	3.64	0.076
Other fruits	2.1 E-03 (± 0.01)A	2.4 E-03 (± 0.01) A	2.1 E-03	0.964
Plantation	7.37 (± 2.99) B	23.04 (± 7.39) A	4.9	0.043
Semi-natural área	10.80 (± 7.07) A	5.08 (± 3.00) A	0.75	0.400

^a Land use groups compared between the different monitoring stages. Mean (± standard deviation)

^b Different stages of monitoring depending on the presence of nests. **Before:** pre-nesting; **After:** post-nesting.

Degree of significance. ns: not significant; p: < 0.05 significant. Means with a common letter are not significantly different (p > 0.05)

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Table 4. Pollen diversity and proportion of the pollen content of the most represented species on *B. pauloensis* at each time of flowering.

	Stage			Kruskal-Wallis test	
	Early flower	Peak flowering	Post-peak	H	p
n ^a	19	12	13		
N° Pollen spp. ^b	10.95 (± 4.92)	8.50 (± 7.00)	13.62 (± 6.16)	3.58	ns
<i>Vaccinium corymbosum</i>	40.11 (± 30.65) B	61.75 (± 43.37) B	5.31 (± 10.01) A	14.59	0.0007
<i>Justicia tweediana</i>	4.05 (± 5.86)	4.08 (± 5.85)	3.54 (± 4.22)	0.03	ns
<i>Nuttallanthus canadensis</i>	5.58 (± 7.06)	6.50 (± 10.59)	3.15 (± 4.06)	0.59	ns
<i>Nothoscordum arenarium</i>	4.89 (± 5.95) AB	2.67 (± 4.70) A	8.15 (± 6.85) B	6.73	0.0298
<i>Echium plantagineum</i>	3.53 (± 3.69)	3.00 (± 4.43)	10.08 (± 11.16)	1.93	ns
<i>Solanum sisymbriifolium</i>	1.47 (± 2.59)	2.00 (± 3.16)	4.31 (± 6.09)	2.47	ns
<i>Conium maculatum</i>	0.21 (± 0.63) A	0.08 (± 0.29) A	17.23 (± 29.52) B	8.18	0.0008
<i>Cuphea glutinosa</i>	10.58 (± 24.15)	7.58 (± 22.36)	1.38 (± 2.81)	0.86	ns
<i>Buddleia stachyoides</i>	4.21 (± 16.70) A	0.25 (± 0.62) A	12.69 (± 22.44) B	5.32	0.0200
Others ^c	25.37 (± 20.08) B	12.08 (± 14.61) A	34.15 (± 20.17) B	10.00	0.0067

^a Number of *B. pauloensis* individuals analyzed. Mean (± standard deviation)

^b Number of floral species represented in the palynological characterization on *B. pauloensis* queens.

^c The category "Other" is composed of 45 species and morpho-species pollen.

Degree of significance. ns: not significant; p: < 0.05 significant. Means with a common letter are not significantly different (p > 0.05)

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edges of the forest plantations to establish their colonies. During this stage, they prefer land uses with greater floral diversity to supply their growing worker colony (e.g. *Semi-Natural*). Overall, our results show the importance of a diversified habitat within agricultural areas to help sustain bumblebee's colonies that provide pollination service to both blueberry and native plants within this region.

These results suggest two different patterns of movement for queen bumblebees during different periods in their life cycle. During the pre-nesting period, queen bumblebees flew within relatively large and circular-oval home ranges. During this life stage, queen bees often conduct reconnaissance flights of the environment in search of suitable nesting sites [27–69]. This period coincided with the beginning of the blueberry flowering, and this massive bloom likely serves as an important source of energetic resources that sustains what are likely energetically expensive nest-searching flights (Table 4). Relative to some bees, bumblebees have only a modest ability to excavate a nest cavity [33]. For this reason, features correlated with variation in soil density and accumulation of leaf litter such as hedgerows, fence lines and forest edges have been found to have higher densities of bumblebee nests compared to such features as closed woods or grassland [70]. Here, we found that queens selected nest sites in habitats with a greater amount of leaf litter accumulated on the soil (i.e. windbreak and edges of plantations of *Eucalyptus* sp. and *Pinus* sp., *personal observation*), selecting sites adjacent to land uses with a diversity of suitable food sources and within their range of flight [71]

After *B. pauloensis* queens had established their nests, they were found to visit different areas and the Minimum Convex Polygons grew to be more elongated areas. In this later period, the flight behavior was more likely to be oriented with the predominant winds of spring (NW and SW), and in our landscapes this period coincides with the end of the blueberry bloom and the beginning of the other flowering plants. When experienced with the landscape and its resources, bumblebees tend to exploit well-defined foraging areas within which they use stable routes to efficiently exploit known profitable feeding sites [72,73]. This "trap-line" behavior is a means to minimize the total distance between floral patches by optimizing their flight distances. It is therefore likely that, at this later stage, queens intensely foraged in

restricted yet highly familiar area to collect pollen in mass to feed the growing worker bee population that would soon emerge.

In the same way that the requirements of the species of floral visitors are modified during their life cycle, the supply of nutritional resources that the environment provides generally changes, forcing the bees to have an adaptive behavior relating to pollen and nectar availability [74]. This study is a snapshot in time of how *B. pauloensis* queens modified their interactions with the habitat before and after the formation of nest. During the nest-searching period the queens intensely used the blueberry fields since the flowers provide rich and abundant nectar and pollen. Following nest establishment, queens care for their emerging worker bees and reduced their travel outside the nest [33]. At this stage of their life cycle, the nutritional requirements for the queen and the colony change. The future worker bees require protein-rich food for its development [74]. Consequently, the bees' movements shifted to include the land use categories with greater pollen heterogeneity [75,76] despite continued, albeit reduced, availability of blueberry flowers. Results from Kraus et al. (2019) [77], who studied *Bombus* diets in captivity, also suggest that protein levels are critical for larval development, and these protein levels may be sustained from the more diverse plants.

Bombus pauloensis movements are similar to those reported for other bumblebees from Europe (see S5 Table). Few studies have studied the flight behavior in *Bombus* queens, finding results similar to those obtained by Walther-Hellwig & Frankl (2000) [78] by the capture-recapture method for *B. terrestris* and ~ 50% less than those observed by Hagen et al. (2011) [56] using telemetry technology in queens of *B. hortorum*. Likewise, more studies of movements in this bumblebee caste are lacking to be able to specify a flight pattern and generalized foraging behavior for these stages of its life cycle.

The results obtained from our study of *B. pauloensis* queens around the blueberry agroecosystem demonstrate how they change the size and shape of their home ranges, but also the use of land use categories as their dietary needs change. Although the relative presence of bumblebees in land use groups in general does not show significant differences, after the establishment of a nest, forest plantations emerge as an important habitat feature, increasing their use by more than 65% and housing 56% of nests observed. This observation suggests that these small-scale plantations can represent a valuable resource for this species providing shelter and possible nutrients [79,80]. The plantations may also serve as guides in foraging flights since bumblebees are more likely to perform straight flights when flying along windbreak compared to when they are flying in open fields, suggesting that they may follow linear landscape features [81]. In addition, these actors are actively pollinating within the fields at a time when there are not many other species of native pollinators, giving them an intrinsic value in this agroecosystem

The analysis of body pollen reinforces our telemetry experiment by showing that between the periods of blueberry bloom there was a variation in the pollen proportion of floral species collected from the bumblebees. In the post-peak blueberry period there was an increase of 30+ % in the diversity of pollinic morphotypes present on the bumblebees. This result suggests that they looked for food in the other land use categories to meet the changing nutritional needs of the workers. It should be noted that, the Emerald variety of blueberry planted in the fields is the first to bloom in the region and conventional blueberry production systems may combine batches of different varieties with subsequent or sequential flowering curves. This observation supports our hypothesis that the *B. pauloensis* queens change how they use the available landscapes based upon the resource availability and perform a cost-benefit evaluation according to the nutritional needs required by the stage of their life cycle [82–86]. This is likely one of the most sensitive stages of the bumblebee's life cycle, aggravated when there is a shortage of resources for foraging, which could cause the death of the young queen and her colony [34]. In

this context, the massive bloom of blueberry fields emerges as an important source of nectar and pollen in this period, supporting the establishment of new colonies.

Final considerations

This is among the first studies to link flight behavior with floral and nesting resources in a productive mosaic agroecosystem, and demonstrates how the resource needs of bumblebee queens' changes over time and relies on semi-natural areas surrounding agricultural fields as foraging habitat. Heterogeneous landscapes can provide diverse resources that are needed by *B. pauloensis* queens at different moments of their life cycle. Blueberry fields appear to be an important resource at the beginning of their life cycle until the moment of nesting. At the same time, the edges of forest plantations seem to offer nesting habitat for native bees when they are adjacent to pollen-rich fields, and the semi-natural areas are harnessed for the larvae' protein-rich diet [77]. We emphasize that we did not directly observe the bees using the bare soil or the land uses developed during our study.

Bees provide vital ecosystem services as pollinators and we need to work to sustain these wild pollinators. The management and conservation of these semi-natural land use categories is an important part of achieving sustainability of agro-ecological systems because they help supplement bee nutritional needs with diverse pollen sources [87] and nesting sites. Semi-natural habitats provide essential resources for the formation and survival of the worker caste that, when upon emerging, will take the lead in supplying the colony with pollen, and thus providing for the next season's queens [88].

Our work contributes to the growing understanding of how bumblebees use the environment, and provides valuable information for conservation planning and sustainable management of the land at a crucial moment in its life cycle. We suggest that land owners and managers of agricultural lands should consider the full life cycle of bees from nest formation to the worker bee emergence, and this longer-term perspective can help maintain native bees in farmlands from year after year, maximizing the pollination service they provide.

Supporting information

S1 File.

(DOCX)

S1 Fig. Technical specifications avian glue-on transmitter (model A2412).

(DOCX)

S2 Fig. Support information on the palynological characterization of pollen present in the *Bombus pauloensis* queens.

(DOCX)

S1 Table. Complementary information of the kernel maps.

(DOCX)

S2 Table. Complementary information of the Chi²-test between the observed locations and the random points.

(DOCX)

S3 Table. Complementary information of the proportional use in each LU.

(DOCX)

S4 Table. Complementary information of the proportional use of the floral resources by *Bombus pauloensis* queens (n = 44).

(DOCX)

S5 Table. Review of published works that estimate the homing distances for *Bombus* species.

(DOCX)

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