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Nesting sites, nest density and spatial distribution of *Melipona colimana* Ayala (Hymenoptera: Apidae: Meliponini) in two highland zones of western, Mexico

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Abstract

Melipona colimana Ayala is endemic to the temperate forests of western Mexico and may be in conservation risk due to forest exploitation. Differences between the density of nests, nesting sites and spatial distribution in two places with different levels of human disturbance were established. A preserved (P) and a disturbed area (D) were identified: the forest had not been exploited for more than 18 years in the P zone, while there had been recent forest exploitation of D zone in less than two years. It was determined that nesting sites, nest density and the number of potential nest sites were predominant in the P zone. In total, 27 of 30 colonies were found on oak trees *(Quercus laurina)* with a diameter at breast height of 183.4 ± 34.21 cm which shows a close relationship of this bee species with this type of tree. A positive correlation between the DBH of the nesting sites in relation to the trees with nests and the presence of cavities was found. The nests are distributed in the form of aggregates in P and D zones (R = 0.31 and 0.39) with a density of 0.17 ha^{-1} and 0.04 ha^{-1} colonies respectively. Forestry exploitation seems to be affecting wild populations since the trees that bees use as nesting sites are destroyed in D zone.

Introduction

Stingless bees or Meliponini is a group of bees with great biological and morphological diversity distributed mainly in the tropical and subtropical regions of the world (Michener, 2000) and 250 species have been described in South and Central America (Camargo & Pedro, 1992; Nogueira-Neto, 1997; Michener, 2000). In Mexico, 46 species of stingless bees have been identified; two of them are classified as endemic to the mountain ranges of western Mexico: *Melipona colimana* Ayala and *Melipona fasciata* Latreille (Ayala, 1999). *M. colimana* endemism is linked to the mesophyll mountain forest in elevations over 1000 meters above sea level, in the geographical zones that correspond to the Manantlan mountain range, the National Park Nevado of Colima and the Tigre mountain range (Ayala, 1999). Usually stingless bees use diverse lodgings to establish their nests, as gaps and cavities in trees, electricity poles, under house´s roofs and abandoned termites and wasps' nests. (Nogueira-Neto, 1997, Roubik, 2006). For that reason, it is hard to acknowledge which are the places *M. colimana* sets its nest, therefore there is a scientific interest in studying and get to know the places it uses for this purpose in its origin habitat, which will provide us new information about the nesting sites of stingless bees in temperate weather.

On the other hand, the conservation of stingless bees is important because they take part in the ecological interactions that contribute to the biodiversity maintenance, acting as pollinators of different plant species, and because they can act as indicators of environmental disturbances (Brown & Albrecht, 2001; Imperatriz-Fonseca, 2002; Slaa et al., 2006). Forest exploitation has negatively affected the presence of

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stingless bee nests and their density, often leading to their disappearance (Cannon et al., 1994; Brown & Albrecht, 2001; Venturieri, 2002; Eltz et al., 2003; Samejima et al., 2004). In this regard, *M. colimana* might be in conservation risk since the species original habitat is a zone where there is commercial exploitation of forest resources, especially oak trees (*Quercus* spp) and pines (*Pinus* spp*)* (Comision Nacional Forestal [CONAFOR], 2010). The purpose of this work is to know the type of tree that *M.colimana* prefers to nest, and evaluate nest density, nesting sites and spatial distribution in two zones with different level of human disturbance; to infer if deforestation could affect the presence of this species.

Material and Methods

Study site

The observations were carried out in the Halo mountain range in southern Jalisco, Mexico (18° 58' 00" N, 102° 59' 45" W, 1600 meters above sea level), in the surroundings of San Isidro village. The territory of this zone is 132, 644 km², in which 79,055 km² are used for forestry. Vegetation is mainly mountain mesophyll forest, made up of various useful timber-yielding species such as oak and pine trees and other species such as *Abies religiosa, Lysoma apaculcensis, Betula pendula, Cornus disciflora* and *Litzea glucesens* (Instituto Nacional de Estadistica Geografia e Informatica [INEGI]; 2005, Carranza, 2008).

The weather is characterized by sub-humid temperate with summer rain (Relative Humidity 69%). The average temperature in the last 10 years was 69.08 °F (min. 52.23 °F and max. 79.88 °F) with an average rainfall of 930mm (min. 120 mm and max. 1230 mm) (Comision Nacional del Agua [CONAGUA], 2011). *M. colimana* is a 9.5 mm long bee, with black integument, yellow marks and orange pubescence; it is a species morphologically close to *M. fasciata* with the difference that *M. colimana* has black terga and yellow apical segments (Ayala, 1999). Two wild populations of *M. colimana* colonies were found in two contiguous areas with different degree of human perturbation. The first area was a disturbed zone (D) there had been recent forest exploitation in less than two years. In the second area, the forest had not been exploited for more than 18 years (P).

Nesting sites and nest density

In order to find wild nests, fourteen 1000-meter-long transects were established with a distance of 200m between each other. A 100 x 100 meter quadrant was traced around every 100 meters of each transect; in this area, wild colonies were located and recorded. The geographical position of each nest and the scientific name of the tree, where the nest was located, were recorded. The total study area and the nesting density per hectare were calculated with the geographical position data and the ArcView 8.3 software. In order to obtain numerical data from the oak trees, which the bees used as a nesting site, a 17.84-meter-long string-stick was placed every 100 meters along the transects. The string was spun around in circles making a circumference that covered a measuring area of 1000 m².

One hectare per measured transect was covered with this method (Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias [INIFAP], 2006). The number of oak trees, the diameter at breast height (DBH), and the number of oak trees with holes were counted at each measuring station. Afterwards, there was a comparison of this data in both areas P and D by using a t-test for the diameter at breast height (DBH) of the nesting sites; and a Chi-square goodness-of-fit test (X^2) for the number of nests, the trees with cavities (potential nest sites) and trees without cavities. The relationship between the DBH of trees with nests and the ones with cavities was evaluated with a correlation. To make a comparison with other tree types that exist in the region and the ones *M. colimana* prefers, a record of other tree species was made for every 100 oak trees found in each zone. The statistical software Statgraphics Plus® (1999) was also used for this statistical analysis.

Spatial distribution

The nearest neighbour distance (Clark & Evans, 1954) was used in order to determine the distribution pattern in the nesting sites. The comparison of average distances between the closest neighbour nest that was observed (XrA) and the average distances in the closest neighbour nest that was expected (Xre), which would be obtained if the nests were placed randomly. Clark and Evan's index is calculated as follows (Krebs, 1998): R= XrA/XrE. If the R value >1, the spatial distribution pattern is regular or uniform, if R<1 the organisms are forming aggregations, and if R=1 the distribution is random.

Results

The total study area was 594 hectares from which 280 were registered in the search of nests. Thirty wild nests were located: 24 in the P area and six in the D area. 27 nests were found in oak trees *Q. laurina;* the other three nests were found in other tree species: *L. apaculcensis, C. disciflora* and *L. glucesens* in the P area. The average diameter at breast height of trees with nests was 169.20 ± 42.74 in the P area and 197.16 ± 56.27 in the D area. There were statistical differences between the two zones in the number of nests ($X^2 = 10.8$; $p<0.05$, DF= 1, 29; 0.05 = 3.84), the number of *Q. laurina* trees ($X^2 = 568$; $p<0.05$, DF= 1, 1286; 0.05 = 3.84), the oak trees DBH (T=35.14, DF=1, 1285, $p<0.05$) and the number of oak trees with cavities ($X^2 = 23.52$; $p<0.05$, DF= 1, 67; 0.05 = 3.84).The P area had the highest values in almost

Table 1. Number of oak trees without nests, diameter at breast height, number of trees with cavities and density of the nests in the zone preserved (P) and disturbed (D).

Study zone	Number of oak trees	Diameter at breast height (cm)	Number of trees with cavities	Density of nests (ha^{-1})
Zone P	216 a	116.22 ± 40.91 a	54 a	0.17 a
Zone D	1071 b	26.24 ± 32.83 b	14 b	0.04 b

Different letters denote statistic differences $P<0.05$

all parameters, except that in the D area the number of oak trees was higher, but with a DBH lower than the P area. The number of oak trees without nests, their diameter at breast height, the number of trees with cavities and the density of nests in every zone are shown in table 1.

For every 100 trees of *Q. laurina* there is an average 8.5 of other trees in the P area. In the D area there were 2.6 other trees for every 100 *Q. laurina* that had no cavities in their trunks. In both areas there is a relationship between the nesting sites DBH (oak trees), the nests in trees and tree holes availability in them. (Ji^2= 62.244, DF=1, 58 $P<0.01$ y Ji^2= 252.456, DF=1, 282 $P<0.01$). Nests presented aggregated distribution in both areas (P= 0.39 y D=0.31). The number of nests and their spatial distribution can be seen in Fig. 1.

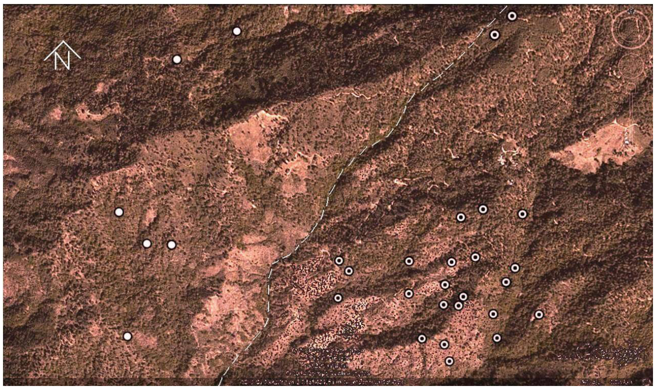

Fig 1. The spatial distribution of *Melipona colimana* wild nests in the study area. Every O mark indicates the position of a nest in the disturbed (D) area (n=6) and the Θ mark indicates a nest in preserved (P) area (n=24).

Discussion

Many stingless bee species are opportunistic and take advantage of several trees' cavities for nesting (Hubbell & Johnson 1977; Roubik, 1989). *M. colimana,* should be closely related to the *Q. laurina* trees with cavities, since it is one of the main species in this region and it is very common to have natural trunk cavities in old trees (Cuevas et al., 2004). Meliponini species have a natural tendency of taking advantage of the arboreal species predominant in their place of origin, such as *Melipona subnitida* Ducke*, Melipona asilvai* Moure, and *Melipona quadrifasciata* Lepeletier in Brazil, which nesting in *Commiphora lepthophoeos*, *Caesalpinia pyramidalis* and *Caryocar brasiliense* trees (Martins et al., 2001; Antonini & Martins, 2003). If there is a strong preference for a predominant tree to nesting in the distribution area, it would leave the bees in a risky situation as the absence of these tree species would directly affect bee populations. Unfortunately, for *M. colimana*, the oldest *Q. laurina* trees having higher biomass are the most useful ones for companies dedicated to vegetal coal production (Reyes, 2012), so the activities arising from this industry may be decreasing the nesting site availability. The activity of the forest exploitation of zone D seems to be also affecting the presence of different trees to *Q. laurina*, given that the low number of trees that are not oak trees, reflects the negative impact that has been held in the zone.

It is observed that the P zone was the one with the highest number of nests, DBH and *Q. laurina* trees with cavities that might be a consequence of having no recent forest exploitation in the zone, which has allowed the subsistence of thicker oak trees. In forests habitats stingless bees have been found making their nests mainly in trees over 60 cm in diameter (Brown & Albrecht 2001; Antonini & Martins 2003; Eltz et al., 2003; Fierro et al., 2012) thus, a higher DBH and quantity of oak trees with available holes might have been a factor for the P zone to have a higher number of nests of *M. colimana*. Despite the higher number of oak trees found in D zone compared to the P zone, these are not useful for bees, since they are young trees which do not have any cavities in their structure. The number of nests found per hectare in both zones was low compared to what was reported by other authors. Roubik (2006) estimates that the usual colonies of *Melipona* and *Trigona* quantity per hectare is from 2 to 6, while in works that were carried out in rainy and broken up forest areas, the general density of stingless bees wild nests was 8.4 and 6.7 nests per ha^{-1} (Batista et al., 2001; Eltz et al., 2002). As the same way, Antonini & Martins (2003), found a density of 3 nests per ha^{-1} of *M. quadrifasciata* in a study conducted in a Brazilian savanna, which is a high nest density compared with the density nests found in *M. colimana.*

Although this low nest density found in *M. colimana* is the first report about stingless bees in a temperate climate, the results suggest that wild populations of this species might be endangered by forest exploitation. The *M. colimana* nests were found forming aggregations in both study zones, which coincides with the report about other stingless bees species such as *Scaptotrigona. pectoralis* Dalla Torre*, Frieseomelitta nigra* Cresson*, Trigona. fulviventris* Guérin, *Tetragonisca angustula* Latreille and *Nannotrigona testaceicornis* Lepeletier (Slaa, 2002; Santos, 2006). Since stingless bees nest on tree's hollow spaces, its density and spatial distribution depends on the presence and distribution of the trees that are used as nesting site (Batista et al, 2001; Santos, 2006), in this case the nest distribution of *M. colimana* depended on the distribution of *Q. laurina* trees.

Despite not having replicas of these observations, this information can give us sign that forest exploitation could be affecting wild nesting of *M. colimana*, resulting in a lower number of trees which bees can use as nesting sites. It has been found that the anthropogenic activity, directly or indirectly, affects density, diversity and spatial distribution of stingless bee communities (Brown & Albrecht, 2001, Hsiang et al., 2001; Moreno & Cardoso 2002; Samejima et al., 2004), therefore, it would be important to do another study with more replicas to get data that show us in a conclusive way that the forest exploitation of *Q. laurina* can put in risk the presence of *M. colimana* nests in its original habitat, which is why it is important to emphasize its protection.

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