



Stingless bees in urban areas: low body size and high frequency of diploid males at mating congregations of *Nannotrigona perilampoides* (Hymenoptera: Meliponini) in Mérida, Yucatán, México

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Abstract – In temperate regions of the world dominated by intensive agriculture, cities harbor a rich diversity and abundance of bee species, often exceeding those of the rural environment. In less industrialized tropical countries, in contrast, stressful conditions may exist in cities for bees with perennial colonies such as stingless bees because of the lack of resources such as amenity green spaces (flowers) and appropriate nesting sites. Yet, we currently lack studies on the ecology of bees in tropical urban areas to test this idea. Here, we compared the body size (as a possible indicator of floral resource supply) and the frequency of diploid males (as a measure of inbreeding) of a widespread stingless bee, *Nannotrigona perilampoides*, at mating congregations within the city of Merida, Yucatán, Mexico, and in surrounding rural areas. We found that males in Merida were significantly smaller compared with those in rural areas. While diploid males were found in both in the city and rural congregations, their frequency was significantly higher in urban sites. Our results suggest that the performance of colonies of *N. perilampoides* is poorer in tropical urban compared with rural sites, possibly as a consequence of limited resources and mating among a small number of colonies. We discuss the implications of these findings for the survival and fitness of stingless bees in the tropics.

Urbanization / deforestation / microsatellite / heterozygosity / Meliponini / inbreeding

1. INTRODUCTION

Urbanization is necessarily associated with decreasing coverage and diversity of natural vegetation (Hernandez et al. 2009; Williams et al. 2009). Surprisingly, in temperate regions, urban landscapes often harbor higher bee biodiversity than adjacent rural areas (Ahmé et al. 2009; Williams et al. 2009; Fortel et al. 2014; Baldock et al. 2015,

2019; Theodorou et al. 2020). Thus, temperate urban areas are believed to be important bee reservoirs (Tommasi et al. 2004; Hernandez et al. 2009; Gardiner et al. 2013), with associated higher pollination service provision (Theodorou et al. 2017, 2020).

Studies to date have primarily focused on temperate cities in comparison with their highly managed and intensively farmed surrounding landscapes containing a depauperate bee biodiversity and low bee abundance (e.g., Baldock et al. 2015; Theodorou et al. 2020). The value of urban centers in tropical regions for bee populations is less

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well known and may differ from urban-rural comparisons made in temperate regions, either because tropical cities are more hostile for bees or because their surrounding rural regions are less intensively managed. In Mexico and other Latin American countries, urban green spaces are generally insufficient and unevenly distributed (Wright Wendel et al. 2012; Fernández-Álvarez 2017), while rural areas are not subject to the intensity of industrial-scale agriculture as in many temperate regions of the world (Haberl et al. 2007). Thus, more stressful conditions may exist for pollinators in tropical urban cities of less industrialized countries, but evidence in these environments is lacking.

The most diverse eusocial taxon in the Hymenoptera is the Meliponini or stingless bees (Michener 2007). Generally, these insects are susceptible to habitat disturbance and genetic impoverishment because individuals and colonies exhibit low dispersal capacity, and many species have a dependence on old trees for nesting (Quezada-Euán et al. 2012). Swarms establish in a nest site that is within flight distance of the maternal nest (philopatry) and physogastric queens cannot relocate due to their inability to fly (Engels and Imperatriz-Fonseca 1990). As a consequence, stingless bees usually have highly structured populations (May-Itzá et al. 2012; Quezada-Euán et al. 2012). Furthermore, males also seem to disperse across comparatively short distances in comparison to other bee taxa (Carvalho and Kerr 2000; Zimmermann et al. 2011).

The reduced diversity of floral resources and reduced connectivity in cities could affect several traits of individuals and colonies of eusocial bees (Couvillon et al. 2010). One important trait for individual fitness is adult body size (Davidowitz et al. 2003). In bees, body size is highly dependent on the quantity and quality of food provided for larval development (for a solitary bee: Bosch and Vicens 2002; for a stingless bee: Quezada-Euán et al. 2011). In the case of male stingless bees, body size can be crucial for survival, as they abandon their colonies and live solitarily after reaching sexual maturity. Larger males may have longer life-spans and produce more sperm (Roubik 1990; Pech-May et al. 2012). Whether body size in stingless bee males has changed under tropical urban conditions is unknown.

Habitat loss associated with the urban environment has also been associated with reduced gene flow and increased inbreeding in bees (Jha and Kremen 2013). In many haplodiploid Hymenoptera, fertilized eggs that are homozygous at the single complementary sex determination (*csd*) locus develop into diploid males (Cook and Crozier 1995), a potentially serious cause for reduced fitness because diploid males are mostly either inviable or sterile (Zayed 2009). In social Hymenoptera, they are produced instead of (female) workers, leading to a diminished colony workforce (Vollet-Neto et al. 2017). Thus, in the Hymenoptera, small population size and, for social species, a low number of colonies reduce allelic diversity at the *csd* locus, leading to a higher frequency of ‘matched matings’ (matings between partners carrying the same allele at the *csd* locus, and higher frequencies of diploid males (Zayed 2009). Assuming random mating, a high frequency of diploid males in a population can therefore be an indicator of low effective population size, with high diploid male frequencies also a potential cause of population decline (Zayed et al. 2004; Zayed and Packer 2005). Mating among siblings, which may occur as a consequence of reduced population size or low population density, also increases the frequency of matched matings and the production of diploid males (Cowan and Stahlhut 2004).

Few stingless bees thrive under human altered habitats as many species are bound to woodland, where mature trees provide nesting opportunities (Quezada-Euán 2018). One stingless bee species that is nevertheless widespread in urban areas is *Nannotrigona perilampoides*, showing eclectic nesting habits not only in trees but also in crevices of buildings (Quezada-Euán 2018). Workers and males of this species are small, about 4 mm in length, and their colonies can comprise up to 1500 individuals (Quezada-Euán 2005).

To assess how urban life may affect individual traits and inbreeding through reduced population size or density in stingless bees living in cities, we here compared *N. perilampoides* males at urban and surrounding rural sites of subtropical Merida, Mexico, in terms of body size and the frequency of diploid males. Though tropical cities may in general be hostile for stingless bees, we

hypothesized that *N. perilampoides*, because of its ability to nest in buildings, would not differ in body size and in the production of diploid males in city sites compared to adjacent rural sites.

2. MATERIALS AND METHODS

Our investigation was conducted in the city of Merida, Yucatan, in southeastern Mexico, the largest city in the Yucatan Peninsula, with ca. 900,000 inhabitants and an area of 20,975 ha. Merida has an estimate average of 6 m² of green cover per capita (Perez-Medina and López-Falfán 2015). This is below the minimum of 9 m² recommended by the World Health Organization (Sorensen et al. 1998) and 15 m² suggested by the United Nations for a healthy city (Perez-Medina and López-Falfán 2015). Rural areas surrounding Merida are characterized by highly anthropogenic (low intensity agricultural) land use rather than pristine forest (Krylov et al. 2018). However, the average green cover in rural localities around Mérida is above 10 m² per capita, increasing with distance from the city (Perez-Medina and López-Falfán 2015).

Mating congregations (MCs) of *N. perilampoides*, where males assemble in a swarm, presumably anticipating the emergence of a receptive queen from a nest in the vicinity, were located visually at different sites in and outside of the city. Males were collected at 10 MCs, 5 within the main urban area of downtown Merida and 5 in the rural areas in the outskirts of the city (Figure 1). Detected urban MCs were separated by at least 5 km from each other while the minimum distance between an urban and a rural MC was 10 km. Males within an MC were collected by netting individuals at the center of the swarm and transferring to ethanol for later analyses. Workers of the same species were also collected from flowers at each site in 100 m radius of each MC. Samples of rural and urban sites (see Figure 1) were all obtained between July and November 2010.

As estimators of male body size, we used head width, intertegular distance, and femur length which have been previously used in stingless bees (Quezada-Euán et al. 2007). The head, thorax, and hind leg of 50 males from each sampling site were

mounted on glass slides. Digital images of each structure were taken by means of a photographic camera (Leica MC 190 HD) adapted to a stereoscope (Leica S8AP0), and meristic traits were measured in micrometers by means of the software ImageJ (Rasband 1997–2012).

To analyze patterns of body size in urban and rural males, we first compared each trait between both groups by means of a linear mixed model (LMM) in which ‘urban/rural’ was the fixed factor and ‘site’ was the random factor. The residuals from the model were checked to verify that they were normally distributed, i.e., that the link function was an appropriate one to use. To support the statistical comparisons, we also built histograms of each trait presenting the distribution of their values in urban and rural males.

A second approach to analyze body size involved the use of a multivariate principal component analysis (PCA) in which the three meristic traits were combined to obtain a single measure of body size. When the coefficients obtained for the principal component 1 (PC1) are of positive sign, it is considered that the resulting individual scores are good indicators of body size (Wiley 1981). We plotted the individual scores of PC1 and PC2 and statistically compared them between urban and rural individuals by means of a generalized linear mixed model (GLMM) analysis assuming a Poisson distribution of the data. All statistical analyses were done with the software SPSS, Inc 2006).

For molecular comparisons, genomic DNA was extracted from the thorax of 50 males and 15 workers at each site. Six microsatellite loci for *N. testaceicornis* (Ntes-04, 34, 41, 46, 59 and 70) were amplified using the primers and amplification protocols of Oliveira et al. (2009). The resulting fragments were analyzed with an ABI 310 (Applied Biosciences) autosequencer. Microchecker was used to test for the presence of null alleles (Van Oosterhout et al. 2004). Allelic richness, linkage disequilibrium between pairs of loci, pairwise measures of *Fst* and Nei’s genetic distance between urban and rural populations were calculated using MSA version 4.05 (Dieringer and Schlötterer 2003). Rural and urban males were polymorphic for the six microsatellite loci studied, with no evidence of the presence of null alleles.

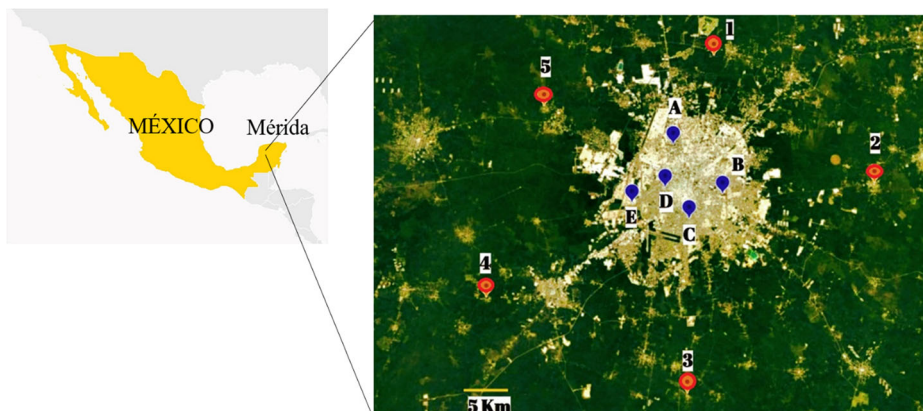


Figure 1. Map of the city of Mérida, in the Yucatán Peninsula, México, with the locations in which mating congregations of males of *Nannotrigona perilampoides* were sampled; in red with numbers, the five rural sites; in blue with letters, the five urban sites.

No pair of loci was significantly linked ($P > 0.05$). Males were considered diploid when they were heterozygous at one or more loci. The number of alleles per locus (N_a), allelic size range (ASR), observed heterozygosity (H_o), and expected heterozygosity (H_e) per microsatellite locus and per rural and urban sites as well a graph of the principal coordinates analysis of genotypes were calculated with the program GeneA1Ex 6.1 (Peakall and Smouse 2006). We tested for differences between urban and rural sites in genetic diversity (N_a , H_o , H_e) using LMMs. To compare the proportion of diploid males between urban and rural sites, we used a Kruskal-Wallis test of ranks (SPSS® v. 15, 2006). Finally, the software COLONY v1.2 (Wang 2004) was used to estimate the number of colonies (families) contributing drones to a given MC. To do so, we performed five replicate analyses with different seed numbers. Throughout, means are presented \pm the standard error.

3. RESULTS

For the three meristic traits measured in *N. perilampoides* males, individuals from urban sites were significantly smaller compared with those from rural sites (Figure 1; Table I). PC1 is regarded as an overall estimator of body size (Wiley 1981). As for the individual meristic characters, there were also significant differences in

PCA scores between sources of male; urban males had significantly smaller scores than rural ones (Figure 1; Table I).

The number of different alleles at a microsatellite locus ranged between 4 (e.g., locus Ntes-41) in the urban and rural sites to 8 (locus Ntes-46) in the rural sites and 8 (locus Ntes-04) in the urban sites (Table III). However, there were no differences ($P > 0.05$) among rural and urban sites in genetic diversity (males: N_a , H_e ; workers: N_a , H_o , H_e ; see Table II). For example, for the males, the mean number of alleles per locus per urban and rural site was, respectively, 4.4 ± 0.6 and 4.6 ± 0.6 . For the workers, the mean number of alleles per locus per urban and rural site was, respectively, 4.4 ± 0.4 and 4.4 ± 0.4 (Figure 2, Table II).

Nei's genetic distance between urban and rural sites was 0.005 for males and 0.006 for workers (Figure 3). There were no significant differences among urban MCs ($\theta = 0.024$) and among rural MCs ($\theta = 0.018$) in a pairwise analysis of population differentiation ($p > 0.05$). Moreover, there was no overall significant population differentiation between urban and rural MCs ($\theta = 0.034$; $p > 0.05$) (Figure 3). Sites seemed to be largely undifferentiated.

Diploid *N. perilampoides* males were found in both urban and rural MCs (Table III). However, there were significant differences in the number of diploid individuals found across types of environment. Overall proportions of diploid males were

Table I. Comparison of means for morphometric characters and scores for principal components (PC1 and PC2) of *N. perilampoides* males from five urban and five rural sites

Character	Landscape				<i>F</i> ($\alpha = 0.01$; <i>df</i> 5, 494)
	Urban (<i>n</i> = 500)		Rural (<i>n</i> = 500)		
	Mean	SE	Mean	SE	
Head width (mm)	1.713 ^a	0.003	1.758 ^b	0.003	115.62**
Intertegular distance (mm)	1.327 ^a	0.003	1.371 ^b	0.005	148.25**
Femur length (mm)	0.925 ^a	0.002	0.957 ^b	0.004	84.36**
PC1	0.564 ^a	0.047	- 0.521 ^b	0.055	185.29**
PC2	- 0.028	1.039	0.008	0.057	0.39 NS

**Significant at *P* < 0.01, NS denotes not significant at *P* = 0.05

Table II. Summary of genetic parameters of *N. perilampoides* males (♂) and workers (♀) from rural and urban sites in Mérida, including number of individuals analyzed (N), alleles per locus (Na), allelic size range (ASR), observed heterozygosity (Ho), and expected heterozygosity (He). Only haploid individuals were used to estimate the measures of diversity in males, and means of genetic diversity are per site

Zone		Locus						Mean ± SE per site
Male/ worker		NTES 59	NTES 34	NTES 70	NTES 41	NTES 46	NTES 04	
Urban								
<i>N</i>	♂	245	242	248	237	241	246	
	♀	150	149	150	150	150	150	
Na	♂	6	5	5	4	7	8	4.366 ± 0.623
	♀	4	4	4	4	6	6	4.400 ± 0.381
ASR	♂	200–218	210–218	200–232	182–188	122–136	144–162	
	♀	202–208	210–216	226–232	182–188	126–172	152–162	
Ho	♀	0.607	0.711	0.513	0.473	0.720	0.707	0.621 ± 0.189
He	♂	0.658	0.714	0.534	0.498	0.737	0.824	0.645 ± 0.148
	♀	0.744	0.726	0.652	0.603	0.761	0.796	0.665 ± 0.121
Rural								
<i>N</i>	♂	246	247	246	245	248	250	
	♀	150	150	150	150	149	150	
Na	♂	6	5	4	4	8	7	4.600 ± 0.643
	♀	4	4	4	4	5	7	4.433 ± 0.419
ASR	♂	200–218	200–218	226–232	180–186	122–136	144–162	
	♀	202–208	210–216	226–232	182–188	126–134	152–168	
Ho	♀	0.567	0.793	0.567	0.587	0.651	0.633	0.632 ± 0.213
He	♂	0.702	0.721	0.526	0.497	0.771	0.808	0.656 ± 0.162
	♀	0.729	0.733	0.645	0.598	0.726	0.825	0.652 ± 0.136

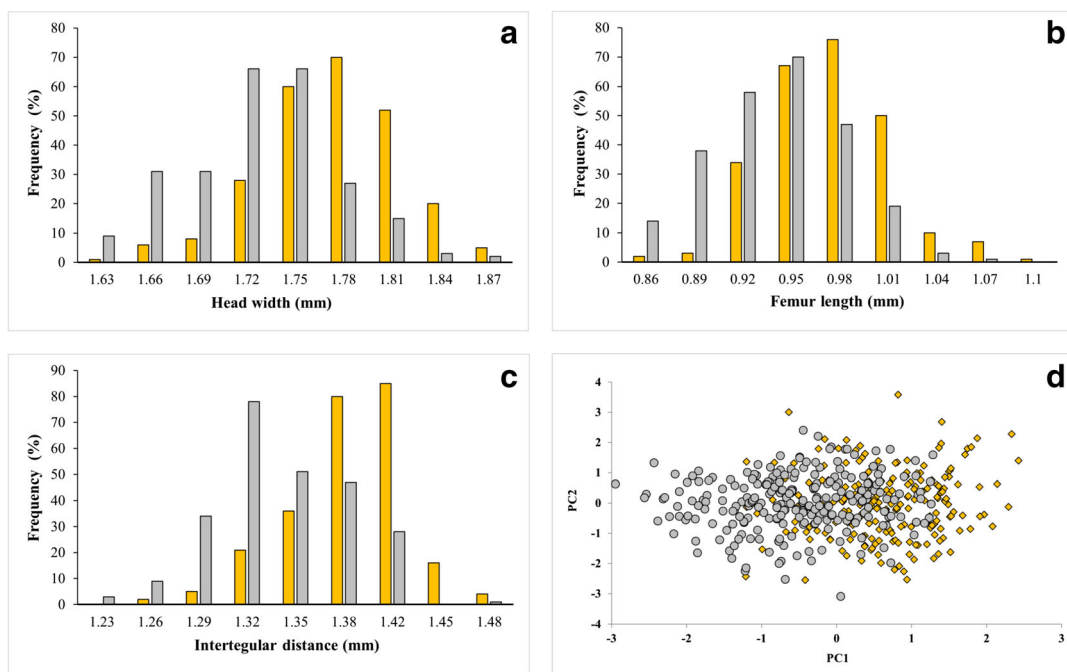


Figure 2. Frequency distribution of male *Nannotrigona perilampoides* for three meristic morphometric traits: **a–c** 250 rural males in orange bars and 500 urban males in grey bars. In **d**, the distribution of scores of males from urban (gray circles) and rural sites (orange diamonds) against PC1 (x-axis) and PC2 (y-axis).

7.2% (± 2.3) in urban and 1.7% (± 2.4) in rural areas ($H = 4.55$, $df = 1$, $p = 0.032$) (Table III). Using COLONY software, we found that males from urban sites originated on average from 8 colonies and that males at rural sites originated on average from 16 colonies.

4. DISCUSSION

We found a significant reduction in the body size of *N. perilampoides* males and a higher frequency of diploid males at urban sites of Merida compared with rural sites; thus, we are forced to reject our initial hypotheses that body size and diploid male frequency would not differ across the urban-rural transition. Our results indicate that *N. perilampoides*, despite its ability to nest in the urban environment, could suffer from reduced resource availability and quality, potentially leading to lower population size or density, as evidenced by greater diploid male production.

Bee diversity and abundance in urban environments are most strongly affected by the availability and diversity of floral resources and nesting

sites (Kearns and Oliveras 2009; Bates et al. 2011; Hicks et al. 2016). However, less work has been conducted to evaluate effect of urbanization on other components of bee biology and fitness (Banaszak-Cibicka et al. 2018). In the case of stingless bees, the evidence is controversial. One study on the Australian stingless bee *Tetragonula carbonaria* revealed that colonies were fitter in urban environments, although worker body size was conserved across different environments (Kaluza et al. 2018). In contrast, workers of the stingless bee *Heterotrigona* were smaller in cities of Malaysia compared with rural areas (Hamid et al. 2016). Effects of urbanization on (eusocial) bumble bee body size have been found to vary across *Bombus* species and are possibly driven by the degree of habitat fragmentation within the urban environment (Theodorou et al. 2021). Here, we found that males collected in MCs were smaller in the city of Merida compared with rural sites. Differences among study conclusions probably derive from variation across studies in the abundance, diversity, and distribution of floral resources across cities. It seems that, in diverse

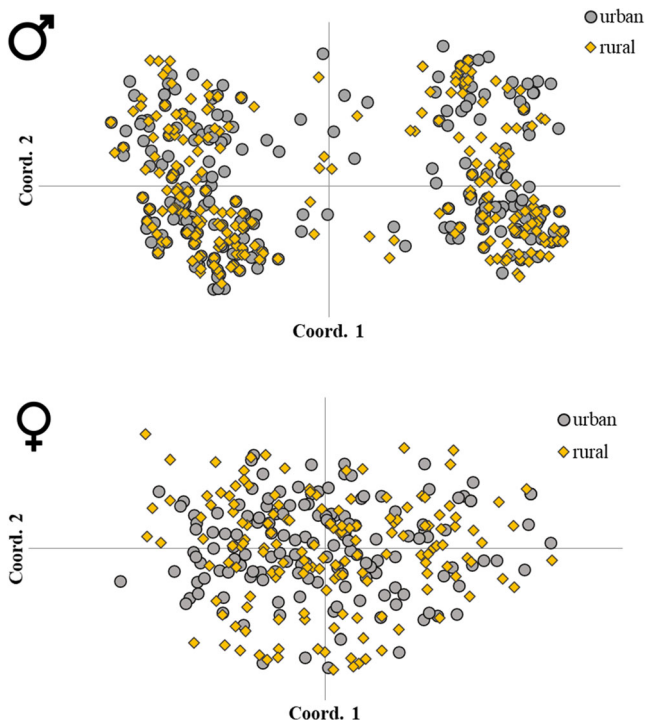


Figure 3. Principal coordinate analysis showing the distribution of urban (red circles) and rural (blue triangles) *Nannotrigona perilampoides* male haplotypes (♂) and worker genotypes (♀).

Table III. Number of *N. perilampoides* diploid males found at each site (mating congregation) within urban and rural environments in Yucatan, México. The numbers and letters in brackets correspond to sites in Figure 1

Zone	Diploid males (N)	%
Urban		
Chubumá (A)	4	8
Fraccionamiento del Parque (B)	5	10
Santa Rosa (C)	2	4
Facultad de Medicina (D)	4	8
Fraccionamiento Juan Pablo (E)	3	6
Total	18	7.2
Rural		
Dzibichaltún (1)	1	2
Tixpeual (2)	2	4
Yaxnic (3)	2	4
Oxholon (4)	0	0
Yaxché (5)	1	2
Total	6	1.7

habitats, highly eusocial and perennial colonies of stingless bees have better opportunities to collect and store food that support continuous growth (Kaluza et al. 2018). In urban environments with limited floral resources, in contrast, stingless bee colonies may face more stressful conditions; Merida and Malay cities may represent such city environments for stingless bees.

Limited food reserves (honey and pollen pots) in stingless bee colonies affect both the number of individuals produced and also their physiological condition (Moo-Valle et al. 2001; Quezada-Euán et al. 2011; Pech-May et al. 2012). As the body size of *N. perilampoides* workers is affected by the quantity and quality of the food provisioned to larvae (Quezada-Euán et al. 2011), colonies in floral resource-poor urban environments may have smaller food reserves and produce smaller adults. Males of this species are reared in cells similar in size to those of workers and within the same comb, and males may therefore be affected by a restriction in nutrition, just as are workers.

Food limitation can have a strong impact on the fitness of male stingless bees. Males of *Melipona beecheii* bees reared under a shortage of colony pollen reserves were smaller, produced less sperm, and had delayed sexual maturity compared with males reared in colonies with larger food reserves (Pech-May et al. 2012). It therefore seems likely that males reared under food limitation are at a disadvantage with their fitter counterparts. Body size may also be crucial in the case of stingless bees because, after reaching sexual maturity, males live a solitary life during which they join MCs in search of mates (Roubik 1990), and male size may correlate with adult longevity. In the case of the honey bee, heavier drones had a longer life-span compared with light-weight drones (Czekońska et al. 2019). Yet, in the case of the stingless bee *Scaptotrigona depilis*, smaller males resided for longer at a MC (though sperm quality was not related to individual body size; see Koffler et al. 2016). The size-longevity relationship in male stingless bees may therefore vary across species. Another related aspect, but not yet studied, is the ability of stingless bee males to disperse in relation to body size. If reduced

body size compromises male dispersal, it may further contribute to reducing male and also colony fitness.

Though we did not find significant genetic differentiation between urban and rural populations and no difference in genetic diversity in urban versus rural sites, we found more diploid males in urban Merida sites compared with rural sites. Our genetic data therefore suggest that sites were largely interconnected by gene flow and that populations in urban sites were not markedly genetically depauperate. Diploid males in the urban populations of our study could alternatively be the result of elevated sibling mating, possibly due to lower colony density, rather than a reduction of the diversity of *csd* alleles (Zayed and Packer 2005; Zayed 2009). Indeed, we found that the males at MCs in urban areas originated from approximately half the number of colonies than calculated for the rural MCs. This suggests higher proportions of closely related males in urban MCs, leading to higher frequencies of chance sib mating.

In the eusocial Hymenoptera, diploid male production reduces both population and colony growth rates (Cook and Crozier 1995). Diploid males in Hymenoptera have two possible fates, elimination as immature or alternatively develop to adulthood. An example of the first route is the well-known case of *Apis mellifera* in which diploid male larvae are killed by the workers (Woyke 1967), possibly detected by their odd pattern of cuticular hydrocarbons (Santomauro et al. 2004). In contrast, adult diploid males can be frequent in the primitively social bees *Lasioglossum zephyrum* (Kukuk and May 1990), *Halictus poeyi* (Zayed and Packer 2001), and *Andrena scotica* (Paxton et al. 2000). Similarly, in the bumble bees *Bombus atratus* (Plowright and Pallet 1979) and *B. terrestris* (Duchateau and Mariën 1995), diploid males, albeit rare, are viable and develop to adulthood. Interestingly, the evidence in stingless bees suggests that the fate of diploid males varies across species. In some species, the rate of diploid males in the brood may sometimes exceed 10% (Tavares et al. 2010; Alves et al. 2011; Francini et al. 2012). However, in other species, diploid males seem surprisingly rare at MCs (Kraus et al. 2008; dos Santos et al.

2013). Notably, there are also species in which diploid males survive to adulthood and can join MCs (dos Santos et al. 2013; Vollet-Neto et al. 2015, 2018). Our study species *N. perilampoides* falls into the latter category.

Colonies of *Nannotrigona* stingless bees are widespread in urban areas (Aidar et al. 2013). Therefore, the long-term impact of diploid males on these apparently “urban-adapted” species is still an open question. It has been argued that a loss of genetic variability may not be disruptive for all stingless bee species and may result in less severe consequences than previously suspected in this taxon (Nogueira-Neto 2002). Several traits may help stingless bees to avoid the genetic load imposed by diploid males. Firstly, in some species, queens that produce diploid males are rapidly killed and replaced by the workers (Camargo 1979; Vollet-Neto et al. 2017). Mechanisms of sex determination at the genic level may be different to single locus *csd* and/or diploid males may not be sterile, thus not representing a genetic load to the population (Cowan and Stahlhut 2004; Paxton 2005).

One corollary from our results is a precautionary word for stingless beekeeping in urban areas. In Mexico, urban meliponiculture projects are on the rise, frequently using species not naturally found in cities (Quezada-Euán 2018). It is important to consider that the urban environment may not be equally (temporarily and spatially) rich in resources (Lau et al. 2019). Suboptimal conditions may impede colony growth. Before developing such urban stingless beekeeping projects, it is important to carefully analyze the potential of specific urban areas for sustainable meliponiculture. More generally, our results suggest that in the tropics, in contrast to temperate environments, the urban environment may not represent a haven for bees.

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AUTHOR’S CONTRIBUTION

WJMJ, JGQE, and RJP conceived this research; CAF LMM HMV performed experiments and interpreted the data; WJMI, JGQE, RJP wrote the paper. All authors read and approved the final manuscript. Funding

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The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

DECLARATIONS

Ethics approval This is an observational study. The Internal Research Committee at the Universidad Autónoma de Yucatán has confirmed that no ethical approval is required.

Conflict of interest The authors declare no competing interests.

Abeilles sans dard dans les zones urbaines: faible taille corporelle et fréquence élevée de mâles diploïdes dans les congrégations d'accouplement de *Nannotrigona perilampoides* (Hymenoptera: Meliponini) à Mérida, Yucatán, Mexique.

urbanisation / déforestation / dérive génétique / Meliponini / consanguinité.

Stachellose Bienen in Stadtgebieten: Geringeren Körpergrösse und ein höherer Anteil an diploiden Männchen in Merida, Yucatan, Mexiko.

Verstädterung / Entwaldung / genetische Drift / Meliponini / Inzucht.

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