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The Life Histories of the "Uruçu Amarela" Males (*Melipona flavolineata*, Apidae, Meliponini)

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Abstract

Here we describe the life histories of adult males of the the Amazonian stingless bee *Melipona flavolineata* Friese, commonly known as "uruçu amarela". Males reach sexual maturity inside nests, presenting seminal vesicles full of sperm cells and becoming able to fly at a mean age of 10 and 15 days, respectively. They aggregate twice in their lives, once before leaving the nest, and another at external congregation sites, by using their capacity to reach congregation sites dependent on morphological attributes, such as large eyes and elongated thorax. Furthermore, we describe three atypical phenomena for Meliponini males: *M. flavolineata* males have dimorphic color pattern; they lose their genital capsules, even when they fail to copulate; and penisless (sterile) males can stay alive for up to two days. The life history strategies of Meliponini males have only just started to be told and provide many interesting questions for future studies.

Over the last 50 years, the life histories of male stingless bees (Apidae: Meliponini) have been documented in great detail (Nogueira-Neto, 1954; Kerr et al., 1962; Van Veen et al., 1997; Koffler et al., 2016; Schorkopf, 2016), showing that male behaviors and reproductive strategies are diverse and very different of Apini (Engels & Imperatriz-Fonseca, 1990; Paxton, 2005). Males leave the nests after they reach sexual maturity (Van Veen et al., 1997), before forming reproductive aggregations in specific sites (Paxton, 2005). During the copula, the male attaches his genital capsule inside the female, resulting in a sterile individual (Kerr et al., 1962; Engels & Imperatriz-Fonseca, 1990).

This short note combines several field observations and experiments that, together, help to understand the life histories of *Melipona flavolineata* Friese males. All observations took place at Embrapa Amazônia Oriental, Belém, Pará State, Brazil, between 2014 and 2017.

Inside the nests: intranidal behaviors, sexual maturity and the point of no return

To study sexual maturation in *M. flavolineata* males, we used the total number of sperm cells in seminal vesicles as a proxy of a male's sexual maturity, and evaluated its variation as a function of age (time since eclosure). Males were held in minicolonies under laboratory conditions and sampled at different ages – from 0 to 25 days after emergence (ESM1, Table 1). We also tested the flight ability of each male through light stimulus (details in ESM1). The sperm migration from the testicles to the seminal vesicles started between zero and five days, when we could observe a greater number of sperm bundles in the seminal vesicles, and a few free sperm cells. From 10 days, we could not find the bundles anymore, observing only the presence of individual sperm cells until 25 days (Fig 1a). We found that males reach sexual maturity

between 10 and 15 days after emergence, following which, total sperm cells was similar to subsequent age categories (KW-H (5;55) = 36.425; p < 0.001), and the increment in this number was followed by simultaneous improvement in flight ability (Fig 1a).

To investigate males' intranidal behaviors and the age that males would leave the nests we marked 160 individuals with numbered and colored tags (40 worker bees from four different colonies). We observed three main activities performed by males inside the nests: walking-standing, self-grooming and nectar dehydration, as previously observed in other *Melipona* species (Van Veen et al., 1997). We also observed males forming groups of at least three individuals with a mean age of 14 days. Groups were observed near inner nest entrances, with a persistence of no longer than 48 h. After this, these individuals were never observed again inside the nests. Outside of nests, we identified eight marked males gathering in aggregations, with a mean age of 20.5 days (± 1.41 S.D.).

Outside the nests: reaching the congregation sites

Melipona flavolineata reproductive aggregations can be formed at the entrance of conspecific nests, and sometimes are composed of hundreds of males (about 150 to 300 individuals; Fig 2a-b). Although male aggregations are only temporary, we observed that these aggregations remain present for several days or weeks between the months of July and December over three consecutive years (2013, 2014 and 2015). *M. flavolineata* seems to differ from other species of *Melipona*, whose males are known to aggregate distant from nests and in some cases

in groups of few individuals (Sommeijer & de Bruijn, 1995; Santos et al., 2014), but whether such pattern sustains out of a meliponary context, it still need to be confirmed.

Aggregations are known to be a source of pre-copulatory selection in eusocial bees, selecting more competitive males in *Apis mellifera* L. and *Scaptotrigona depillis* (Moure) (Jaffé et al., 2010; Koffler et al., 2016). Based on this, we compared the body size of *M. flavolineata* males before and after the formation of congregation sites (corresponding to pre- and post-selection scenarios), as follows: (i) we first sampled males emerging as adults inside different nests (brood combs: pre-selection group; n = 90 obtained from ten nests); and (ii) after about 20 days of this first step, males were sampled from congregation sites (congregation: post-selection group; n = 90 collected at random in five different congregation sites); finally (iii) all bees were measured in order to compare head and thorax attributes (details in ESM1). We highlight that male origin was not controlled for the congregation group, which may have affected our results. However, we believe our sampling effort represented the variation in population once we obtained males from different congregation sites.

We found differences in body size among males sampled before and after congregation formations: males sampled from brood combs showed higher median values and variation of interocular distance and intertegular distance, and lower median values of thorax length in relation to males sampled from congregation sites (Fig 1b-e), despite not differing in other body size attributes such as head width, head length and tibia length (ESM1, Table 2). Our results
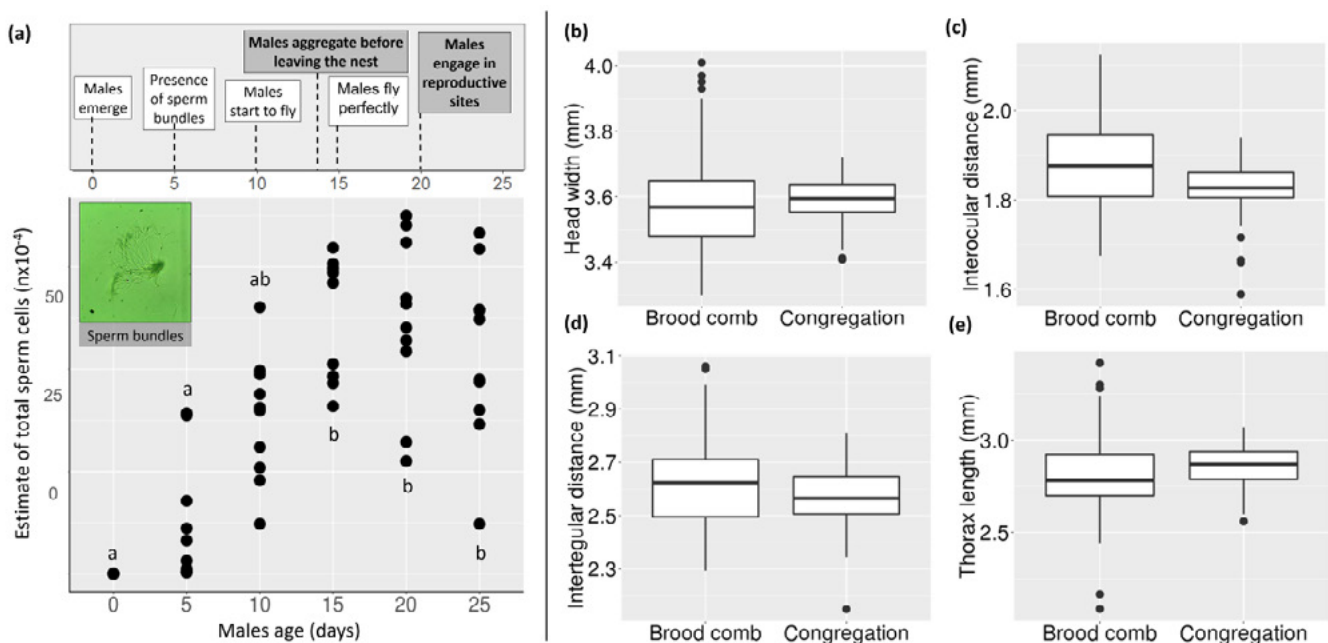

Fig 1. Development of sexual maturity of the "Uruçu Amarela" males, *Melipona flavolineata*. Here we show the phases of males' life history and the variation of estimated number of total sperm cells in seminal vesicles as a function of males' age in days; rank comparisons are indicated with letters (a); we also show the comparison of four body size attributes of *Melipona flavolineata* males sampled from brood combs (n = 90) and congregation sites (n = 90), showing similarities in head width (b), and differences in interocular distance (c), thorax length (d) and intertegular distance (e).

show that although all males had similar head sizes, the males sampled from congregation sites had shorter interocular distances, meaning they have larger eyes; they also had shorter intertegular distances and more elongate thoraxes than males sampled from brood combs, suggesting their body shape may be more aerodynamic. We also suggest that extremes were negatively selected as variation in body size was much lower in congregations. Even though the brood comb males were not compared to males from the same colonies that successfully reached an aggregation, which would be ideal, our results corroborate with previous findings that Meliponini males undergo pre-copulatory selection (Koffler et al., 2016).

Atypical phenomena: color dimorphism and penisless males

Males of *M. flavolineata* exhibit dimorphic color-patterning, including both light and dark morphs (Fig 2). Light morphs can be diagnosed by their yellow metasoma, yellow-ferruginous legs with blackish marks, and vertex and mesosoma with corn yellow setae. The decumbent setae on third to fifth metasomal tergum are bright yellow. All these patterns being similar to those of worker bees. The dark morph differs from light morphs in having black legs and metasoma, vertex and mesosoma with brownish-yellow setae. The decumbent setae on third to fifth metasomal tergum are brownish-yellow. We observed that the two morphs can emerge in the same colony. From a sample of twenty-one brood combs with pre-emergent pupae from twelve colonies, we observed of the 1,364 males emerging as adults, 78.15% were light morph and 21.85% were dark morph. The proportion of light and dark phenotypes produced in each brood comb varied and did not follow a pattern (ESM1, Table 3). According to Pereboom and Biesmeijer (2003) abdominal coloration in stingless bees might be involved in the regulation of body temperature in extreme thermal conditions. This suggests that, it is possible
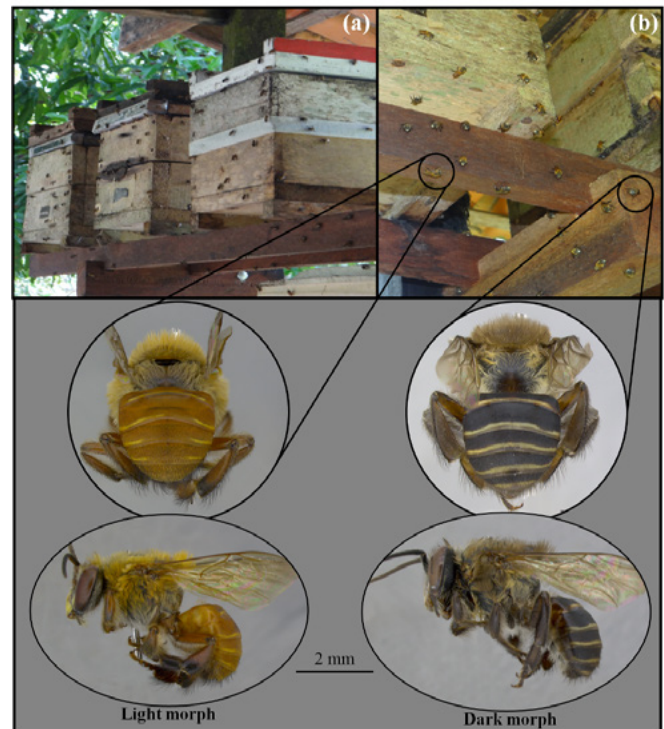

Fig 2. *Melipona flavolineata* reproductive aggregation placed at a congregation site near conspecific nests (a). Detail of the aggregation showing males with different body color-patterns (b). *Melipona flavolineata* dimorphic males, the light morph (left) and the dark morph (right).

that variation in color-patterns of males of *M. flavolineata* has also a role in thermoregulation. To our knowledge, this is the first report of color dimorphism in male stingless bees.

Another atypical behavior was observed when we used males' mating attempts as a proxy of sexual attractiveness of virgin queens in *M. flavolineata* under laboratory conditions (Veiga et al., 2017). We observed two interesting behaviors: (i) *M. flavolineata* males permanently lose their genital capsules
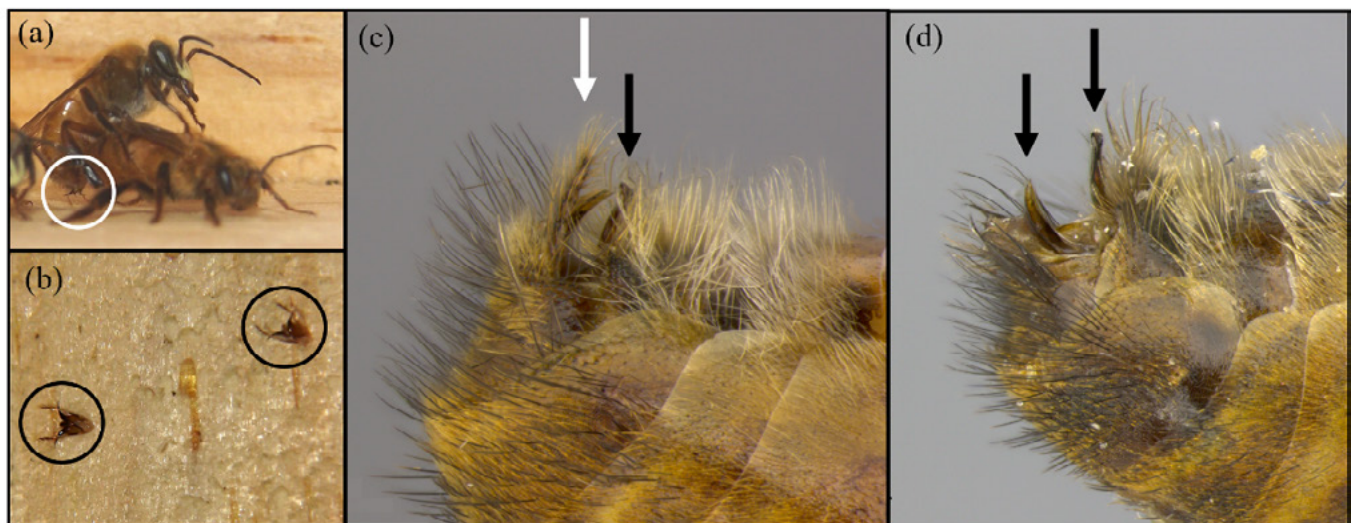

Fig 3. Atypical phenomena observed in *Melipona flavolineata* males: (a) loss of male genital capsule without a successful copulation, (b) male genital capsules lost after mating failures, (c) male with complete reproductive apparatus, including the genital capsule (white arrow) and genital sterna (black arrows), and (d) a penisless male found at congregation sites, presenting only genital sterna (black arrows) and with absent genital capsule.

when they copulate, also when they fail to do so (here failing in copulation means that males which attempted to copulate everted their genital capsule outside female's body); and (ii) these penisless (sterile) males can stay alive, reengage in congregation sites and behave similarly to virgin males. When males failed in copulation, they simply everted their genital capsules, losing it in the air (Fig 3a-b). We repeatedly observed such behavior under laboratory conditions, where 13.23% of 650 (in the first experiment), and 7.83% of 600 (in the second experiment) males permanently lost their genital capsules without a successful copulation. Additionally, when we sampled reproductive aggregations, we found males lacking genital capsules (10% in the first congregation site, from a total of 50 sampled individuals; 5% in the second, from 60 samples, and 7.7% in the third, from 90 samples). Penisless males stay alive on average for a further 2.62 days (±1.41 s.d.) and can be easily identified by the absence of the genital capsule (Fig 3c-d).

The life history of males of stingless bees has just started to be told. The main message is that their life histories are much more diverse than previously thought, and many aspects are yet to be described and understood.

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Supplementary Material

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Authors' Contributions

JC Veiga, FAL Contrera conceived the sampling designs; JC Veiga, KL Leão, BWT Coelho collected and analyzed data; JC Veiga, FAL Contrera wrote initial draft of the manuscript and all authors contributed to subsequent revisions and gave final approval for publication.

References

Engels, W. & Imperatriz-Fonseca, V.L. (1990). Caste development, reproductive strategies, and control of fertility in honey bees and stingless bees. In W. Engels (Ed.), Social Insects (pp. 167-230). Berlin Heidelberg: Springer

Jaffé, R. & Moritz, R.F.A. (2010). Mating flights select for symmetry in honeybee drones (*Apis mellifera*). Naturwissenschaften, 97: 337-343. doi: 10.1007/s00114-009-0638-2

Kerr, W.E., Zucchi, R., Nakadaira, J.T. & Butolo, J.E. (1962). Reproduction in the social bees (Hymenoptera: Apidae). Journal of the New York Entomological Society, 70: 265-276.

Koffler, S., Meneses, H.M., Kleinert, A. de M.P. & Jaffé, R. (2016). Competitive males have higher quality sperm in a monogamous social bee. BMC Evolutionary Biology, 16: 195. doi: 10.1186/s12862-016-0765-2

Nogueira-Neto, P. (1954). Notas bionômicas sobre meliponíneos: III–Sobre a enxameagem. Arquivos do Museu Nacional, 42: 419-451.

Paxton, R.J. (2005). Male mating behaviour and mating systems of bees: an overview. Apidologie, 36: 145-156. doi: 10.1051/apido: 2005007

Pereboom, J.J.M. & Biesmeijer, J.C. (2003). Thermal constraints for stingless bee foragers: the importance of body size and coloration. Oecologia, 42-50. doi: 10.1007/s00442-003-1324-2

Santos, C.F., Menezes, C., Vollet-Neto, A. & Imperatriz-Fonseca, V.L. (2014). Congregation sites and sleeping roost of male stingless bees (Hymenoptera: Apidae: Meliponini). Sociobiology, 61: 115-118. doi: 10.13102/sociobiology.61i1.115-11

Schorkopf, D.L. (2016). Male meliponine bees (*Scaptotrigona* aff. *depilis*) produce alarm pheromones to which workers respond with fight and males with flight. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology, 202: 667-678. doi: 10.1007/s003 59-016-1109-9

Sommeijer, M.J. & de Bruijn, L.L.M. de (1995). Drone congregations apart from the nest in *Melipona favosa*. Insectes Sociaux, 42: 123-127. doi: 10.1007/BF01242448

Van Veen, J. W., Sommeijer, M. J. & Meeuwsen, F. (1997). Behaviour of drones in *Melipona* (Apidae, Meliponinae). Insectes Sociaux, 44: 435-447. doi: 10.1007/s000400050063

Veiga, J.C., Menezes, C. & Contrera, F.A.L. (2017). Insights into the role of age and social interactions on the sexual attractiveness of queens in an eusocial bee, *Melipona flavolineata* (Apidae, Meliponini). Naturwissenschaften, 104: 31. doi: 10.1007/s00114-017-1450-z