GUSTATORY CONDITIONING BIASES ODOR PREFERENCE IN *Tetragonisca fiebrigi* STINGLESS BEE: INSIGHTS FROM CONTROLLED LABORATORY Y-MAZE ASSAY

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1. INTRODUCTION

Bees are essential pollinators, playing fundamental roles in maintaining biodiversity and agricultural productivity (Klein *et al*., 2007; Potts *et al*., 2010). However, studies on the biology, behavior and ecotoxicology of bees have predominantly focused on *Apis mellifera*, given its global economic importance and relative ease of management (Human *et al*., 2013). This restricted approach limits the understanding of the ecology of native species, whose biology and behavior differ substantially from those of honey bees (Human *et al*., 2013).

Among the native social bees of Brazil, the stingless *Tetragonisca fiebrigi* (*Jataí-do-sul*) stands out, being widely distributed and of great agricultural importance as a pollinator of several economically important brazilian crops (Giannini *et a*l., 2015). In addition, *T. fiebrigi* represents a valuable scientific model due to its complex social behavior and cognitive capabilities that allow for studies of learning and memory (Mc Cabe; Farina, 2010). Recently, research has shown that *Apis mellifera* may have its floral choices biased by gustatory conditioning — that is, previous experiences with gustatory rewards can alter subsequent floral preferences (Farina *et al*., 2023). However, this specific type of conditioning had not yet been described for *Tetragonisca fiebrigi*, which represents an important gap in the understanding of the foraging behavior of this species.

This study is therefore the first to experimentally demonstrate an induced floral selection bias in *Tetragonisca fiebrigi* in a laboratory approach, using a Y-maze test. Although the capacity for associative learning has been demonstrated in stingless bees via the proboscis extension reflex (PER) (Mc Cabe *et al*., 2007), such studies did not address the choices between options, a critical aspect for assessing real floral preferences. To this end, we fed bees a syrup containing a specific scent (geranium), generating gustatory conditioning that mimics the learning that occurs during floral visits in natural environments. This conditioning was later successfully tested in a Y-maze preference test, where free bees chose between two scents, one conditioned (geranium) and one naive (rosemary).

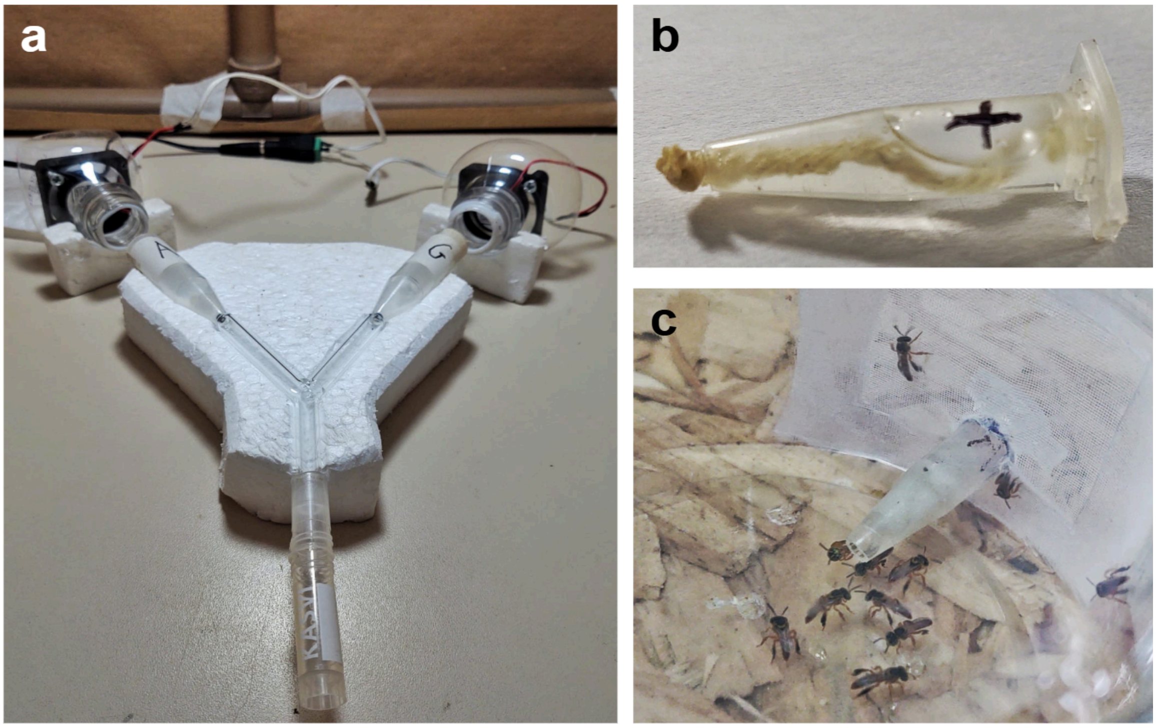
This finding is particularly relevant for its potential implications for agriculture and pesticide risk assessments. These studies with native bees face methodological difficulties, such as small size, high susceptibility to stress, and difficult laboratory management, that hinder the use of traditional protocols such as PER classical conditioning (Mc Cabe *et al*., 2007). The success of this Y-maze conditioning test thus offers a practical alternative to investigate learning and memory capacity in pesticides risk assessments for *Tetragonisca fiebrigi*. Furthermore, it allows for more detailed study of behavioral aspects such as discrimination between olfactory stimuli.

From an agricultural perspective, this result also paves the way for well-founded speculations about the use of *jataí-do-sul* bees in directed pollination protocols, a practice consolidated only for honey bees (*Apis mellifera*). This possibility could benefit some crops such as strawberries, which significantly benefit in productivity and quality with small bees' pollination (Giannini *et al.*, 2020). Thus, this study contributes to the advancement of basic knowledge of stingless bees' cognition, development of risk assessments alternatives, and effective agricultural management strategies.

2. METHODOLOGY

Thirty stingless bees (*Tetragonisca fiebrigi*) were collected from the entrances of hives located in Capão do Leão, Rio Grande do Sul, during the fall of 2025. The bees were placed in a ventilated plastic container with a screened side opening (**Figure 1c**). The container held a centrifuge tube (1 mL) open at the end, allowing the bees access to a solution of sucrose (1.75 M) mixed with geranium oil (0.1% v/v). The end of the tube was closed with cotton string, preventing the solution from leaking, but allowing free access for feeding (**Figure 1b**). The container was kept in a controlled environment (25°C, 60% RH) for a period of 24 h for taste conditioning.

Figure 1 — (**a**) Y-tube showing the neutral zone (bottom) and the choice zones (right or left) of aromas driven by 5W fans (**b**) Feeding tube containing sucrose (1.75M) and geranium oil, allowing bees access and taste conditioning. (**c**) Plastic vial keeping bees for 24h.

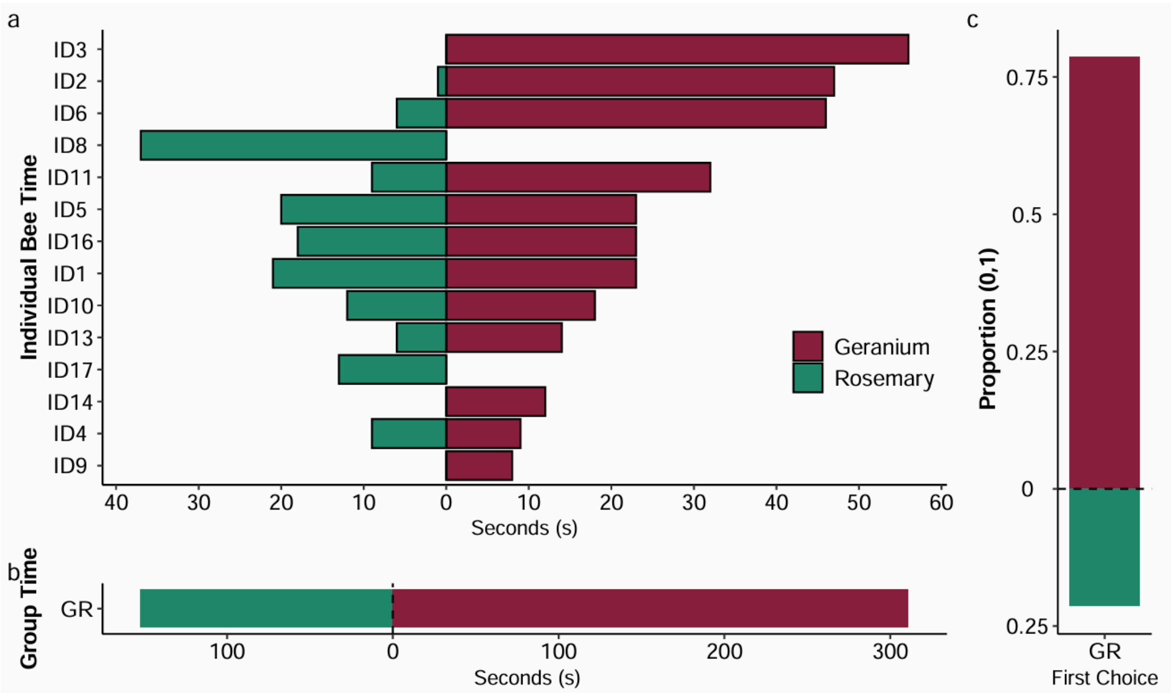


After the 24-hour period, 17 bees were randomly selected and subjected to a Y-maze olfactory choice test (**Figure 1a**). In this test, each bee (individualized 2 hours prior tests) was placed at the neutral end and allowed to freely explore the two ends containing different scents driven by fans: (1) Geranium (Previously conditioned odor); (2) Rosemary (Naive odor). Bees were recalled for a period of 60 seconds, recording: (1) The first side to be explored; (2) Time spent at each end. After each trial, the Y-tubes were washed and the position of the scents was randomized. The data obtained were fitted in generalized linear mixed models in the R environment. We discarded aroma preferences in previous pilots tests.

3. RESULTS AND DISCUSSION

The conditioning protocol proved efficient for the gustatory conditioning of *jataí-do-sul* bees. This is strongly highlighted by the average time spent by each bee (**Figure 2a**) exploring the conditioned geranium aroma: 22.2 ± 17.5 seconds, being 2.05 times higher than the time spent at the neutral rosemary scent: 10.9 ± 10.5 seconds ($p < 0.001$). The total time can be observed in **Figure 2b** for the two groups, where only two bees do not prefer to explore the conditioned scent. Considering the first chosen aroma for each bee (**Figure 1c**), the geranium side was chosen 3,67 times more than the rosemary side ($p = 0.046$), accounting 78,57% of the choices.

Figure 2 — (**a**) Time spent exploring each odor (Geranium; Rosemary) by each individual bee (ID) (n = 14). (**b**) Total time spent on each odor. (**c**) Proportion of odor chosen in the first exploratory choice.



Our results indicate that the conditioned memory lasts for at least two hours for *T.fiebrigi*, although the work of Mc Cabe and Farina (2009) with other stingless bee indicate that the memory can last longer than 24h periods. Further long-term experiments are required to clarify this issue. Also, It should be emphasized that laboratory data often don’t represent the real-world conditions. From that, it is necessary to design field experiments to investigate the real outcomes of gustatory conditioning in terms of flower-visiting biases.

Despite that, gustatory conditioning followed by Y-maze tests prove to be an adequate tool for assessing the cognition of *T.fiebrigi*, revealing their capacity for olfactory orientation. Bees took an average of 5,07 ± 1,69 seconds to discrimination between two complex odors introduced in the neutral zone, with a 78,57% accuracy rate. Altogether, these findings position this method as a promising tool for assessing cognition in stingless bees, as classical conditioning approaches typically achieve a maximum success rate of 20% for this genus (Mc Cabe; Farina, 2010).

4. CONCLUSIONS

From our results, we concluded that the presentation of a scented solution positively conditions *Tetragonisca fiebrigi* bees, and can be reliably tested with high precision using a Y-maze assay. Based on these findings, this approach is recommended for contaminant risk assessments and investigation of cognition abilities in stingless bees.

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