Salt foraging of stingless bees at La Selva Biological Station, Costa Rica

Nicholas N. Dorian[1] and Rachael E. Bonoan[2*]

[1] Department of Biology, Tufts University, 163 Packard Ave, Medford, MA, Current Address: Harry H. Laidlaw Jr. Honey Bee Facility, 1 Bee Biology Road, Davis, CA, 95616
ndorian1@yahoo.com

[2] Department of Biology, Tufts University, 163 Packard Ave, Medford, MA, (617) 627-4849,
rachael.bonoan@tufts.edu
*Corresponding author

Introduction

Nutrients are essential to the growth and reproduction of all organisms. As such, the distribution, health, and behavior of organisms are often dictated by the availability of nutrients. In the tropics, nutrients are heterogeneously distributed in both space and time (Silva, Souza, & Abreu, 2015). Across the tropical landscape, dissolved ions from bedrock, or animal feces and urine, are rarely found in soils due to high rates of weathering and leaching (Oesker, Homeier, Dalitz, & Bruijnzeel, 2011; Yavitt et al., 2009). Temporally, some tropical regions experience distinct seasons of rainfall throughout the year, whereas others are more aseasonal with respect to precipitation (Oesker et al., 2011). Therefore, nutrient availability can vary greatly throughout a year.

When dietary nutrients are scarce, herbivores often seek alternative nutrient sources to supplement their plant-based diet (Denton, 1982). This phenomenon is found across a wide diversity of taxa: mountain goats in British Columbia seek out natural salt licks (D. M. Herbert, 1971); African elephants make their own salt licks by digging in the soil during the dry season (Weir, 1969); and Amazonian frugivorous bats supplement their diets with mineral-enriched water (Ghanem, Ruppert, Kunz, & Voigt, 2013). This nutrient-specific foraging is particularly prevalent in social insects that simultaneously sustain several different life stages in the colony (Lihoreau et al., 2015). For example, leaf-cutting *Atta* ants will choose to forage on sodium treated baits (Kaspari, Yanoviak, & Dudley, 2008; Pizarro, McCreery, Lawson, Winston, & O'Donnell, 2012), *Reticulitermes* termites are attracted to potassium-rich nest sites (Botch & Judd, 2011; Judd & Fasnacht, 2007), and halictid bees (also known as "sweat bees") prefer a sodium solution over pure water (Barrows, 1974; Roubik, 1996).

Neotropical stingless bees likely exhibit a similar behavior. Their primarily floral diet contains trace amounts of essential minerals such as sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) (Cohen, 2004) however, floral quantities are likely not enough to sustain multi-generational, perennial colonies (Lihoreau et al., 2015). These nutrients are essential for physiological processes such as neurotransmission, immunity, and muscle movement (Cohen, 2004). To cope, colonies are thought to supplement their floral diet with non-floral resources such as resin, muddy water, ash, sweat, and even carrion (Lorenzon & Matrangolo, 2005; Wille, 1983). Indeed, Roubik (1996) found that *Trigona* and *Apis spp.* in Brunei preferentially sought sodium, potassium, and magnesium salt solutions at artificial feeders. Little is known, however, regarding whether stingless bees in the *neo*tropics exhibit this behavior.

In this study, we investigate the salt foraging behavior of stingless bees (*Trigona silvestriana)* at La Selva Biological Station (hereafter, "La Selva") in Puerto Viejo de Sarapiquí, Costa Rica (Figure 1). Specifically, we asked: do neotropical stingless bees prefer certain minerals when foraging for non-floral resources (i.e. water)? We predicted that stingless bees would show strong preference for sodium (compared to deionized water); the aboveground parts of land plants rarely contain much of this important micronutrient (Cohen, 2004; Oates, 1978) and herbivores are consistently limited by sodium (Denton, 1982). We expected stingless bees to show weaker preferences for potassium, calcium, and magnesium. Although they are equally physiologically important as sodium, they are more prevalent in floral resources (namely pollen)

(E. W. J. Herbert & Miller-Ihli, 1987) and therefore unlikely to be sought when foraging for non-floral resources.

Materials and Methods

Study Site

La Selva receives an annual average of 3,993 mm of rain and experiences weak seasonality (Wet = May-December, 395.6 mm/month; Dry = January-April, 197.5 mm/month) (McDade, Bawa, Hespenheide, & Hartshorn, 1994). La Selva's soil fertility is variable and salt-laden winds off the Caribbean Sea (50 km away) are important for maintaining soil ion abundance (McDade et al., 1994). Rainfall is highly dilute in the wet season, however, and ion concentrations can be inversely related to the amount of precipitation during the preceding period. Thus, at the start of the dry season in early January, rainfall at La Selva should still be fairly dilute with nutrients.

Study Species

Trigona silvestriana is an abundant stingless bee species in Costa Rica (Breed et al. 1999) (Figure 2). It builds perennial eusocial colonies around a queen and typically nests in cavities or at the base of mature trees (Hubbell & Johnson, 1977). Like all social insects, *T. silvestriana* raise dependent larvae that need to be fed both macro (protein and carbohydrate) and micronutrients (vitamins and minerals) for proper development.

Preference Assays

Preference assays were conducted from January 1-4, 2016 at La Selva. Stingless bee foragers were attracted to an elevated feeding station (a stool) using a 30 % sucrose solution and a fresh banana peel (Butler, 1940; Roubik, 1996). The feeding station was placed at the center of a gazebo (Figure 1a) which overlooked La Selva's arboretum (Figure 1b). Bees arrived within 36 hours. The feeding station offered six different mineral solutions in inverted petri dishes (diameter = 50 mm) (Figure 3a): 1 M sucrose (positive control) (Roubik, 1996), four chloride (Cl) solutions including 0.5 M NaCl, 0.5 M KCl, 0.5 M $CaCl_2$, 0.5 M $MgCl_2$, and deionized water (negative control) (Pizarro et al., 2012). These concentrations were used to attract congeneric bees in Brunei (Roubik, 1996), therefore we assume them to be biologically relevant for stingless bees. Solutions were randomly allocated to one of six equal areas on the circular feeding station at the start of each day (Figure 3b). Trials started around 0800 and ended around 1600, when bee activity and recruitment began to decline. Actively feeding bees were counted every 15 minutes for one minute. If competitors (i.e. wasps, ants) were present, we removed them from the feeder. To discourage presence of competitors in the first place, we placed the feeder base in moats of water.

Results

Stingless bees visited salt solutions (54 combined visits) almost as often as they visited the sucrose solution (59 visits) (Figure 4). Throughout the course of the preference assays, deionized water was visited the least (9 visits) (Figure 4).

In comparison to deionized water, stingless bees tended to prefer $CaCl_2$ the most (+10 visits), and $MgCl_2$ the least (-2 visits) (Figure 5). Stingless bees did not show a strong preference for NaCl (+6 visits) or KCl (+4 visits) (Figure 5).

Discussion

In general, stingless bees preferred mineralized water over deionized water (Figure 3). These results corroborate findings in halictid bees (Barrows, 1974; Roubik, 1996). Also, preference strength tended to differ between salt solutions (Figure 5).

Stingless bees were attracted to $CaCl_2$ and KCl solutions, which could highlight a scarcity of these essential nutrients in the environment (Figure 5). The strong preference for $CaCl_2$ was interesting since in excess, calcium can cause paralysis in bees (Somerville, 2005). In invertebrates, potassium is a component in the structure of lipids and some proteins (Cohen, 2004). Unexpectedly, stingless bees had no interest in sodium (Figure 5). In insects, when coupled with potassium, sodium aids in the regulation of pH in cells and body fluid (Cohen, 2004). Although herbivores tend to be limited by sodium (Denton, 1982; Oates, 1978), sea-spray from the coast (only 50km away) may have a larger effect on available nutrients than we had anticipated.

$MgCl_2$ was avoided by bees at La Selva, however, it was favored by stingless bees in old world tropics (Roubik, 1996) (Figure 5). Although the physiological requirement of magnesium is unknown in stingless bees, the discrepancy in results highlights a likely geographic variation in nutrient preferences. Another explanation for this finding is that stingless bees already get enough of these micronutrients from both floral and non-floral resources in the environment. We may have provided solutions that were too dilute to be worth foraging effort, and it is possible that bees in the dry season are not as nutrient-limited as bees in the wet season when nectar and water sources are more dilute (*Monteverde : Ecology and Conservation of a Tropical Cloud Forest*, 2000). Indeed, apart from our feeders, the only non-floral resource we observed stingless bees visiting was prepared fruit (i.e., watermelon) near the field station kitchen. It is likely bees were seeking out sucrose, as there remained unvisited sources of salt ions nearby (i.e., muddy water; pers. obs). In addition, samples of rainwater, fruit, or nectar could be analyzed for specific ions to understand whether our solution concentrations were truly ecologically relevant.

This exploratory study has implications in both basic and applied science. While there is much known regarding insect nutrition and foraging in general, insect micronutrient requirements remain poorly understood (Cohen, 2004). Understanding micronutrient requirements in social insects is particularly interesting as social insects are not only foraging for themselves but for the colony as a whole (Lihoreau et al., 2015). Future manipulative studies can be used to understand the fitness consequences of nutrient-limitation at the level of the individual, the level of the colony, or both (Lihoreau et al., 2015).

On the applied side, understanding the nutritional requirements of stingless bees in the tropics is of particular economic value to meliponiculturists, people who raise stingless bee colonies and harvest honey. Meliponiculture holds tremendous historical and cultural value to indigenous groups in Costa Rica. With the loss of many stingless bee populations due to urbanization and deforestation, meliponiculturists in certain sites may have to adopt practices such as diet supplementation with mineral solutions to keep their colonies healthy and productive (*Pot-honey: A Legacy of Stingless Bees*, 2013).

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Fig. 1 (a) Distribution of *Trigona silvestriana* centered on Costa Rica, map created at discoverlife.org (b) Our study system, *T. silvestriana*. Photo: Rachael E. Bonoan

Fig. 2 (a) *Trigona silvestriana* foraging at an inverted petri dish. (b) Preference assay set-up with *T. silvestriana* foraging at artificial feeders. Photos: Rachael E. Bonoan

Figure 3. Total number visits *Trigona silvestriana* made to deionized water, all the salt solutions combined, and the sucrose solution.

Figure 4. Total number visits *Trigona silvestriana* made to each experimental mineral solution in comparison to deionized water. Bars above 0 indicate solutions that were visited more often than deionized water, bars below 0 indicate solutions that were visited less often than water.