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Associative learning in relation to foraging of Neotropical stingless bee *Trigona*

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ABSTRACT

Organisms, if capable, will invest in learning when it results in the improvement of overall fitness. Learning ability may allow organisms to outperform competitors or better survive in a variable environment. Order Hymenoptera exhibits a broad spectrum of learning abilities, including landmark navigation, time-place learning, and associative learning. These abilities are fairly well studied in North American genera such as *Apis*, but remain relatively unexplored in Neotropical species such as those in the genus *Trigona*, an essential stingless bee pollinator of rainforest ecosystems. In this study, I test whether *Trigona* bees in Monteverde, Costa Rica possess the capacity to associatively learn concerning foraging and extraneous markers. I conclude that like other Hymenoptera, the *Trigona* species studied have the capability to both associate an extraneous landmark with a reward and use this information to maximize foraging strategy. This association minimizes *Trigona*'s time and energy spent searching for a food source, thus improving colonial resource stores and supporting a larger colonial population.

RESUMEN

Los organismos, de ser capaces, van a invertir en el aprendizaje si resulta en una mejoría de su éxito reproductivo. La capacidad de aprendizaje puede permitir a los organismos a superar competidores o a sobrevivir en ambientes variables. El Orden Hymenoptera presenta un amplio espectro de habilidades de aprendizaje, incluyendo navegación por medio de puntos de referencia, aprendizaje tiempo-espacio, y aprendizaje por asociación. Estas habilidades son bastante estudiadas en géneros norteamericanos como *Apis*, pero permanecen relativamente inexploradas en especies neotropicales como aquellas del género *Trigona*, una abeja sin aguijón polinizadora de ecosistemas del bosque lluvioso. En este estudio, puse a prueba si las abejas *Trigona* tienen la capacidad de aprendizaje por asociación con lo que respecta a forrajeo y marcas externas en Monteverde, Costa Rica. Concluí que como otras Hymenopteras, la especie estudiada de *Trigona* tiene la capacidad de crear puntos de referencias externos o por asociación con una recompensa y usar esta información para maximizar su estrategia de forrajeo. Esta asociación minimiza el tiempo y energía invertido por *Trigona* en buscar fuentes alimenticias, por lo tanto mejorando las reservas de recursos de la colonia y apoyando una población mayor de la colonia.

INTRODUCTION

Learning is the adaptive modification of behavior based on experience (Alcock 2005). The process of learning is a costly investment (Kroodsma & Canady 1985) and therefore animals will not commit resources to it haphazardly. Only when an animal can account for environmental instability, providing a counter-balancing effect to the associated cost, will an organism make an investment in learning (Alcock 2005); the benefits must outweigh the cost. Benefits may include maximizing food quality and supply, communication, risk avoidance, and many others. These in turn can lead to an organism's ability to better adapt to a variable environment, thus outcompeting competitors and ensuring an ecological niche. Learning itself varies drastically in degrees of complexity and rewards among organisms.

Members within the Order Hymenoptera are able to learn along a broad spectrum of difficulty. Some Hymenoptera exhibit relatively simple learning abilities such as habituation, where the insects learn to ignore unimportant stimuli (Hölldobler & Wilson 1990), while others show much more complex abilities such as time-place learning, where they learn to associate a reward with a specific time and place (Breed 2002, Murphy & Breed 2008).

Trigona bees (Apidae: Meliponinae) display time-place learning abilities, a skill reserved for those foragers who specialize on food sources renewed each day on a fairly regular schedule, such as nectar supply in flowers (Murphy & Breed 2008). When regular nectar feeding stations were set up, it was found that *Trigona* bees anticipated feeding each day and appeared to previously resource-rich flowers when no incentive was being offered, showing they learned to associate a location with a reward (Murphy & Breed 2008).

Somewhat less complex than time-place learning is the ability of Hymenoptera to associate an external factor with a given specific result (Wilson 1971, Hölldobler & Wilson 1990). One of the most famous examples is when Pavlov (1927) trained his dog to associate a bell with food. Each time he would ring the bell he fed the dog, and eventually, the dog would salivate when it heard the bell, with or without a reward presented. Again, this learning method is on a spectrum of simple to complex. A simple behavior is the ability of individuals to learn the colony scent. Scent is learned almost immediately after metamorphosis from the pupa stage, as this is crucial to not only find and recognize fellow colony members, but also to recognize and defend against intruders (Hölldobler & Wilson 1990). On the complex end of the spectrum, Hymenoptera, along with other insects, may also learn to associate visual cues with an expected response (Weiss & Papaj 2003, Leadbeater & Chittka 2007). In nature this “landmark learning” often occurs when foraging (Hölldobler & Wilson 1990).

Optimal foraging theory dictates that animals have evolved to select for the highest reward possible with the least cost associated – gain the most calories, but spend the least amount of time and energy (Alcock 2005). This allows the animal to most efficiently dedicate resources toward biomass production rather than a waste product of metabolism. Therefore, if an animal can distinguish a pattern that maximizes foraging efficiency, they will commit to the cost of learning this pattern. It has been shown that insects can associate cues, such as flower color or landmarks, with higher caloric rewards (Leadbeater & Chittka 2007). Ants of the genus *Cataglyphis* (Formicidae) are able to use geographical landmarks to navigate to and from food sites (Hölldobler & Wilson 1990). Honeybees (Apidae: *Apis mellifera*) demonstrate the ability to associate landmarks with caloric rewards if trained (Cheng *et al.* 1987). Not only do the bees recognize patterns in the landmarks, but also relative sizes of the landmarks. They most often used the landmarks that were closest, then the largest, to the resource. The honeybees would then use these landmarks to calculate their flight vectors back to their colonies.

Like honeybees, *Trigona* bees are eusocial insects. They exhibit age-based polyethism: younger bees are assigned jobs within the nest, while older bees carry out the more dangerous foraging duties (Robinson 1992). As these older bees’ only duty within the colony is to collect food, they make their efforts as efficient and rewarding as possible. If they are capable, it would be a worthy learning investment to minimize search efforts by associating a visual cue with caloric rewards. I expect that *Trigona* bees do possess this ability, much like the honeybees and *Cataglyphis* ants (Cheng *et al.* 1987, Hölldobler & Wilson 1990). Scientists have directly observed the ability of *Trigona* to associate parameters such as flower color and odor with a reward (Pessotti & Lé’ Sénéchal 1981), but within the genus, species have variable learning and

communication capacities (Biesmeijer & Slaa 2004) and it is not known whether they have the ability to learn more complex associations.

In this study I investigate whether *Trigona* bees can associate landmarks with a food source, and then use these landmarks to determine the location of a reward in the absence of the ability to follow a set vector. I expected that after training the bees to associate a given landmark with a sugar incentive, they would follow the landmarks even in the absence of a reward as Murphy and Breed (2008) found in their time-place learning experiment. I predicted that *Trigona* bees do indeed have the ability to associate an extraneous landmark with a reward, as demonstrated by their Formicidae and Apidae relatives (Cheng *et al.* 1987, Hölldobler & Wilson 1990).

METHODS

Study Sites

I worked at three sites in pre-montane wet forest (Holdridge 1967), each with an individual *Trigona* colony, from April 19 to May 6, 2011. Colonies were found in the roots and trunks of mature trees. Two nests were located on the Campbell property and one at the Monteverde Biological Station. On the Campbell property, one nest was located in a regenerating bullpen and the other in the back of the pasture very near the forest edge, while the nest at the station was located in the garden. Both nests on the farm had large open areas to arrange flower patches, whereas at the station, flower patches were set up on a walkway due to limited area.

Preparation and Observation

Using 60 *Impatiens walleriana* potted plants of varying colors, I organized two plots (30 plants in each) approximately 5 m away from the nest and 5 m away from each other (Fig. 1). Though these 60 plants consisted of five different colors, I made the color composition of each patch essentially the same. I chose *I. walleriana* because, according to Murphy & Breed (2008), they are effective attractors for *Trigona* bees, and were also relatively inexpensive and easy to maintain.

In one patch, I added 20 microliters of a 50 percent honey-water solution to each flower, as performed by Murphy & Breed (2008), with another 5 percent per volume of vanilla added. While the honey acted as a reward, the vanilla was an attractant, as suggested by Mui (2005). I used the vanilla with the purpose of attaining a bigger sample size. Around each of these plants, I placed makeshift yellow landmarks (30 cm tall) (Fig. 1). I made these landmarks by taping together three painted barbecue skewers, selecting the color yellow because it is attractive to *Trigona* (Mui 2005). To the second patch of flowers, I added 20 microliters of water, again adding 5 percent vanilla per volume to the water. No landmarks were placed in this patch (Fig. 1).

I then observed the site two hours per day counting how many bees were drinking nectar or hovering over a patch, for four days, except for the first site at which I observed only for three days. I alternated observing each patch (landmarks and no landmarks) every minute. I began taking observations when *Trigona* colonies became active in the mornings; this varied among colonies due to weather and site variation. Each evening after bees returned to their nests or each morning before they became active for the day, I refilled the flowers following the same procedure as discussed above. I also switched landmark and no landmark flower patch locations each day to prevent the bees from learning a set vector for reward location. This period served to train the bees to associate the extraneous landmarks with a reward.

After this observation period, I had one experimental day. I refilled the flowers following the same procedure except I transferred the landmarks to the water patch. I then observed the bees for only that next morning following the same procedure as discussed above: observe for two hours alternating between patches each minute.

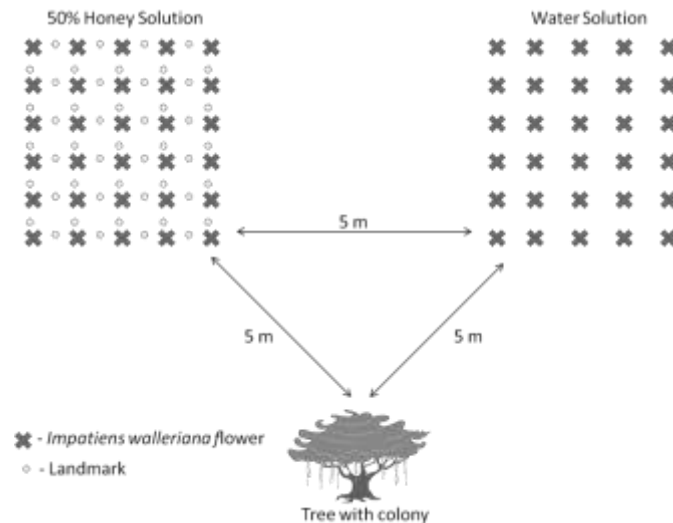


Figure 1. *Trigona* associative learning test-site set-up. All three sites had essentially the same set-up with 5 m gaps and 60 flowers; however, the Biological Station site had only 2 m gaps and 30 plants. Vanilla was added to both solutions as an attractant agent for the bees. Solution was added daily, and at this time, flower patch locations were also switched. On experimental days, the landmarks were transferred to the water solution patch.

After this entire process was completed at the bullpen site, I moved all of my materials and conducted the experiment again in the pasture using the same methods, and then again at the Biological Station. However, by the time I conducted my experiment at the station, half of the potted *I. walleriana* no longer had flowers due to temporal and physical stresses as a result of flower maturation and moving plants between sites. Therefore, only thirty plants were used at this third site, fifteen in each patch. Due to limited space in the garden, patches and the colony were also only separated by 2 m.

RESULTS

All together, I made 1680 observations: I collected 1320 data points during the observational period and 660 on the experimental days, ultimately equating 15 days spent observing the bee nests.

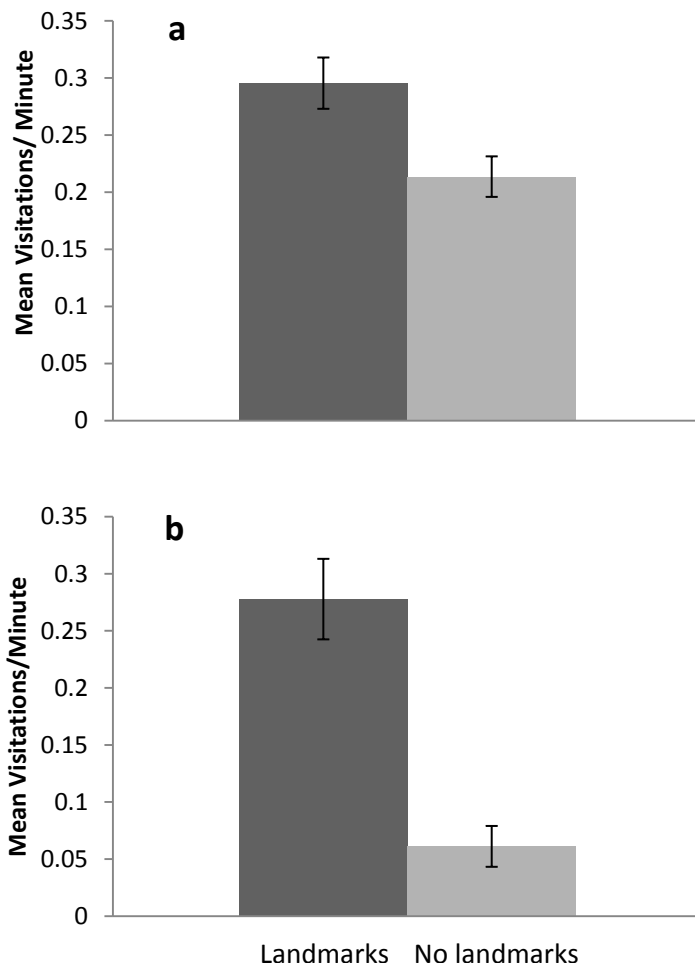


Figure 2. Mean (± 1 SE) visitations/minute of *Trigona* to flower patches a) with honey and landmarks, and with water and no landmarks during training periods (N = 660 for each patch) and b) with water and landmarks, and with honey and no landmarks for the experimental day (N = 180 for each patch) in Monteverde, Costa Rica. Differences are significant in both trials.

The *Trigona* bees significantly followed the landmarks not only when the landmarks were next to the honey (One-Way ANOVA, $F_{1, 1319} = 8.17$, $P = 0.004$; Fig. 1a) but also when they were moved to the water patch (One-Way ANOVA, $F_{1, 359} = 29.99$, $P < 0.001$; Fig. 1b). The observational period depicts the bees' significant tendency to visit the landmark patch with the honey nearly 50 percent more often than the patch with only water, a mean of 0.30 visitations/minute (SE = 0.022) at the landmark patch versus a 0.21 mean visitation rate (SE = 0.017) at the patch without landmarks (Fig. 1a). On the experimental day, the mean visitation rate was still higher at the landmark patch at 0.27 visitations/minute (SE = 0.035) than the 0.06 visitation rate (SE = 0.018) at the honey patch without landmarks – bees were over four times more likely to visit the landmarks patch even without a reward.

DISCUSSION

Trigona bees can and do learn to associate external factors with rewards. I established that *Trigona* prefer sugar-filled flowers with landmarks present over water-filled flowers without landmarks. Then, even though no reward was offered with the landmarks on the experimental day, the bees still followed the landmarks over visiting the flowers offering honey. These findings correlate with the conclusions of Cheng *et al.* (1987) for their honeybee experiment (*Apis mellifera*) and Hölldobler and Wilson's (1990) conclusions for *Cataglyphis* ants. *Trigona* bees seem to possess similar landmark learning abilities to other social Hymenoptera.

This fairly complex behavior could be partly explained by this order's generally social nature (Wilson 1971). If individuals are able to specialize, as they do in a caste system, they can commit more resources to excelling at a given task rather than partitioning resources to attempt all necessary tasks for survival. Within *Trigona* which exhibits temporal polyethism, learning plays a critical role; throughout a lifetime, individuals must learn to fill different roles and all associated tasks. As bees age and move into foraging roles within the colony, it is in their best interest to optimize foraging efficiency, making associative learning in relation to landmarks and food sources extremely beneficial. Relating these findings back to optimal foraging theory, by discerning an association for where the greatest rewards are found, these foragers decrease the amount of time and energy they invest, increasing their net energy gain. This in turn results in a larger resource base for the entire colony, allowing higher reproduction rates and a longer colony lifetime. A higher population would allow a colony to better outcompete or defend against other colonies, and make it more resilient to disturbances. Associative learning in foragers could increase the entire colony's fitness.

However, finding a bright yellow stick next to a reward does not often occur in nature, nor do 50 percent nectar rewards and giant patches of flowers directly in front of the nest. Future studies may consider gauging *Trigona* bees' associative responses in relation to more natural markers, rewards, and flower patch locations.

We must also consider the repercussions of these findings. If stingless bees are more sensitive to landmark placement than we originally thought, this makes them much more vulnerable to habitat disturbances such as deforestation or road and trail development: if they cannot find their previous landmarks, they may have difficulty finding the food. This would be devastating to both the environment and human society if *Trigona* bees were to start disappearing at the same rates as the honeybees in the U.S. dying of Colony Collapse Disorder (CCD). Bees provide a cosmopolitan ecosystem service; just within the United States, bee

pollination services are estimated at nearly \$10 billion per year (Nabhan & Buchmann 1997). As pollinators, *Trigona* bees are critical to the rainforest plants, and therefore to all the animals who eat those plants, and so on and so forth. The role of small-scale landmarks in the activity of these stingless bees must be understood in greater detail if we are to be able to guess how continuing deforestation and even climate change might affect these pollinators.

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LITERATURE CITED

- ALCOCK, J. 2005. *Animal Behavior*. Pp. 88, 89, 210. Sinauer Associates, Inc., Sunderland, Massachusetts.
- BIESMEIJER, J.C. AND E. J. SLAA. 2004. Information flow and organization of stingless bee foraging. *Apidologie*. 35: 143-157.
- BREED, M.D., E.M. STOCKER, L. K. BAUMGARTNER, AND S.A. VARGAS. 2002. Time-place learning and the ecology of recruitment in a stingless bee, *Trigona amalthea* (Hymenoptera, Apidae). *Apidologie*. 33: 251-258
- CANE, J. H. AND V. J. TEPEDINO. 2001. Causes and extent of declines among native North American invertebrate pollinators: Detection, evidence, and consequences. *Conservation Ecology*. 5:1.
- CHENG, K., T.S. COLLETT, A. PICKHARD, AND R. WEHNER. 1987. The use of visual landmarks by honeybees: Bees weight landmarks according to their distance. *Journal of Comparative Physiology A*. 161: 469-475.
- DYER, F. AND J. DICKINSON. 1996. Sun-Compass Learning in Insects: Representation in a Simple Mind. *Current Directions in Psychological Science*. 5: 67-72.
- HÖLLDOBLER AND WILSON. 1990. *The Ants*. Pp. 366, 368. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.
- HOLDRIDGE, L.R. 1967. Lifezone ecology. Tropical Science Center, San Jose, Costa Rica.
- KROODSMA, D. E., AND R. A. CANADY. 1985. Differences in repertoire size, singing behavior, and associated neuroanatomy among Marsh Wren populations have a genetic basis. *Auk* 102: 439-446.
- LEADBEATER, E. AND L. CHITTKA. 2007. Social learning in insects – From miniature brains to consensus building. *Current Biology*. 17: 703-713
- MUI, C. Y. What kind of flower attracts stingless bees? An experiment. 2005. International Field Biology Course, Center for Tropical Forest Science.
- MURPHY, C. M., AND M. D. BREED. 2008. Time-place learning in a neotropical stingless bee, *Trigona fulviventris* Guérin (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*. 81: 73-76.
- NABHAN, G.P. AND S.L. BUCHMANN. 2009. Services provided by pollinators. In: *Nature's services: societal dependence on natural ecosystems*, G. Daily, ed. pp. 37-46. Island Press, Washington, D.C.
- PAVLOV, I.P. 1927. *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. Oxford University Press, London, England.
- PESSOTTI I., AND A. M. LÉ'SÉNÉCHAL. 1981. Aprendizagem em abelhas. I- Discriminação simples em onze espécies, *Acta Amaz.* 11: 653-658. As cited in: BIESMEIJER, J.C. AND E. J. SLAA. 2004. Information flow and organization of stingless bee foraging. *Apidologie*. 35: 143-157.
- ROBINSON, G.E. 1992. Regulation of division of labor in insect societies. *Annual Review of Entomology*. 37: 637-665.
- WEHNER, R., B. MICHEL, AND P. ANTONSEN. 1996. Visual navigation in insects: Coupling of egocentric and geocentric information. *The Journal of Experimental Biology*. 199: 129-140.
- WEISS, M. R. AND D. R. PAPAJ. 2003. Colour learning in two behavioural contexts: how much can a butterfly keep in mind? *Animal Behaviour*. 65: 425-434.
- WILSON, E. O. 1971. *The Insect Societies*. Pp. 217. The Belknap Press of Harvard University Press, Cambridge, Massachusetts, and London, England.