

Time-place learning and the ecology of recruitment in a stingless bee, *Trigona amalthea* (Hymenoptera, Apidae)

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Abstract – The stingless bee, *Trigona amalthea* (Olivier), has the ability to associate time and place with feeding. Bees learned to visit a training site during a feeding interval in order to acquire a reward. A lengthy period of anticipatory visits to the training site preceded the feeding interval. When a reward was not presented, visitation was high preceding and during the feeding interval but was rare following the feeding interval, behavior indicative of time-place learning. Additionally, we demonstrate that *T. amalthea* scouts recruit other workers to nectar baits, however, there is no relationship between the flight time to the colony and either the number of visits prior to recruitment or the number of workers recruited. Due to territoriality in this species, foraging ranges are truncated so that the expected reductions of foraging effort at the margins of the foraging territory are not observed. In *T. amalthea* a combination of time-place learning and recruitment allow for rapid mobilization of workers when food is available.

stingless bee / Meliponinae / *Trigona* / foraging / learning / recruitment

1. INTRODUCTION

We present studies on time-place learning and on the recruitment system of a meliponine (stingless) bee, *Trigona amalthea* (Olivier). When combined, time-place learning and recruitment give a distinct advantage in competition with other

species that lack these abilities. To our knowledge, time-place learning has not been investigated in stingless bees. Additionally, while recruitment is well known in stingless bees (Roubik, 1989; Nieh, 1999) and has been demonstrated in *Trigona amalthea* (Kerr et al., 1963), most studies of stingless bee recruitment have focused on

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the mechanism of recruitment (Lindauer and Kerr, 1960), rather than on the ecological correlates of recruitment which we investigate.

Time-place learning, an association between time and place in foraging, can be an important component of efficient foraging. Animals that can learn when and where to expect resources out-compete animals that must rediscover a resource each time it is renewed. Time-place learning may be common in both eusocial and solitary insects, but has been studied only in the honey bee (*Apis mellifera* L.), in which it is well known (Aschoff, 1986; Frisch and Aschoff, 1987; Gould, 1987a; Gould, 1987b; Moore and Rankin, 1985), and in ectatommine ants (Beugnon et al., 1996; Harrison and Breed, 1987; Schatz et al., 1994; Schatz et al., 1999).

One of the principal benefits of sociality that eusocial insects have over their solitary counterparts is the sharing of information among foraging individuals. Information concerning the location of food resources can be conveyed by a scout directly leading a recruit to a food source, as in tandem running ants (Möglich et al., 1974) and some stingless bees (Lindauer and Kerr, 1960), by a scout laying an odor trail that is followed by recruits, exemplified by many termites (Pasteels and Bordereau, 1998) and many ants (Vander Meer and Alonso, 1998), or by complex coded signals, such as the dances of the honey bee (Von Frisch, 1967) and a stingless bee (Nieh, 1998; Nieh, 1999). Recruitment may be tuned to features usually considered in optimal foraging models, such as time or effort to reach a resource, and the nutritive value of the resource (Breed et al., 1996; Fewell et al., 1996). In addition, distance of the resource from the colony is sometimes considered to be a reflection of time or effort (Von Frisch, 1967).

Our first objective was to test the hypothesis that *T. amalthea* workers can use time-place learning to find renewed re-

sources. Our second objective was to determine if there is a relationship between travel time from a resource to a colony and the number of bees recruited or the number of scouting trips prior to recruitment. Answering these questions increases the depth of our understanding of stingless bee foraging strategies.

2. MATERIALS AND METHODS

We observed *Trigona amalthea* at the La Selva Biological Station, Sarapiquí Canton, Heredia Province, Costa Rica during the dry season, 1997. Bait stations, established approximately 1.5 meters above ground, consisted of a paper towel hung vertically from a string tied between two trees. In order to lure *T. amalthea*, we saturated the paper towel with a 40% honey solution, a concentration level that was attractive to bees in initial observations. Using these bait stations, we conducted time-place learning and recruitment experiments.

2.1. Time-place learning

We used two sets of four bait stations, each set within the foraging territory of a single *T. amalthea* colony. The distance between the two sets was more than 1 km. Honey solution served as bait at each station for a single thirty min period at the same time each day for three consecutive days. At the end of the baiting period, we replaced the wet paper towel with a dry towel that remained at the bait station until the following day when it was re-baited. The paper towel was not baited on the fourth day. In order to determine if the bees had learned the baiting time.

The total number of bees observed either in direct contact with the paper towel or flying within 0.5 meters of the towel was recorded every 3 min for at least 90 min. On day one observations began at the

beginning of the baiting period; control observations prior to bait placement indicated that no bees were flying in the area where the bait was to be placed. On days 2 and 3 observations began 0.5 h before the bait was presented so that the arrival of bees prior to bait presentation could be recorded. On day four this early observation period was extended to 1.0 h prior to the scheduled bait time. Also on day four, observations were terminated 0.5 h after the bait time because no bees remained in the area. For statistical purposes, we divided the total observation time into 15 min intervals and recorded the maximum number of bees present during the interval. This resulted in the 90 min observation period being divided into six 15 min observation periods.

2.2. Recruitment

We observed recruitment at 21 bait stations representing 3–4 *T. amalthea* colonies. After saturation of the paper towel with honey solution we observed the station area. When a *T. amalthea* worker landed directly on the bait we noted the time of arrival and marked this scout with a dot of paint (Testor's enamel) on the thorax, abdomen, wing or leg for future identification. Each potential scout that landed on the paper was marked until a recruitment event

was observed. Recruitment was defined as the arrival of a previously marked bee at the baiting station accompanied by one or more unmarked bees.

The following variables were recorded: the arrival times of potential scouts, the number of potential scouts that visited the station before recruitment occurred, the time away from the bait station (indicating approximate distance to colony), the mean number of visits by one scout and the number of recruits brought with each individual scout visit. Observations continued until either an hour had passed without any signs of recruitment or the bees were so numerous that efficient counts could no longer be taken.

2.3. Data analysis

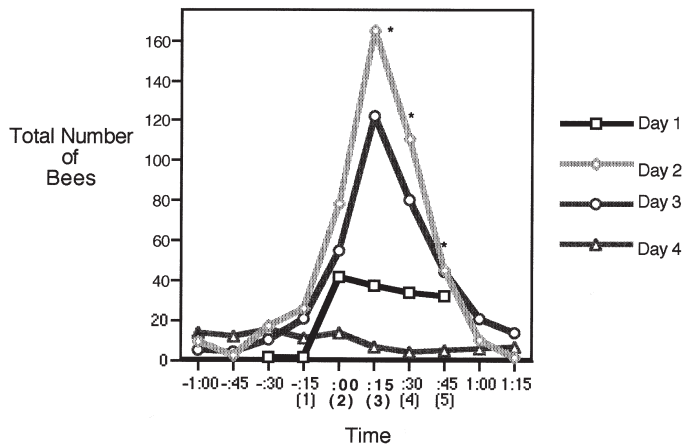
The data were analyzed using StatView (Version 4.1, 1992-4 Abacus Concepts, Inc.).

3. RESULTS

3.1. Time-place learning

The total number of bees in the vicinity of the bait station increased during the baiting period (Fig. 1). In the third, fourth and

Figure 1. Total number of *T. amalthea* at each time period on days 1 through 4 in the time-place learning experiment. The maximum number of bees observed during each 15 min intervals is indicated. We baited the stations with a 40% honey solution during intervals 2 and 3. Asterisks (*) indicate significant differences in number of *T. amalthea*.



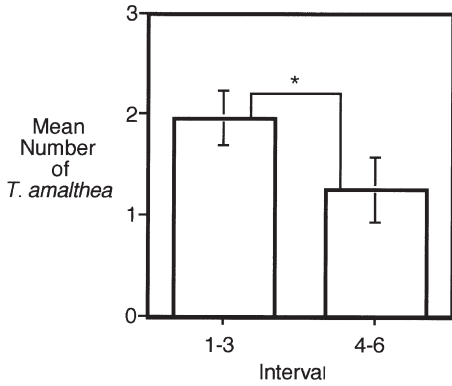


Figure 2. Comparison of the mean number of *T. amalthea* in the vicinity of the bait stations on day 4 before and during the feeding interval to the intervals following the feeding interval. Bars indicate the standard errors of the means. There is a significant ($P < 0.05$) difference between interval 1–3 and intervals 4–6, indicated by the * in the figure.

fifth time intervals there were significant differences in the total number of bees in the bait station vicinity between days 1, 2, 3 and 4 ($F = 8.51$, $P = 0.0004$; $F = 3.8$, $P = 0.024$; $F = 4.3$, $P = 0.016$). This indicates place learning but does not demonstrate time-place learning.

To determine if *T. amalthea* are capable of time-place learning, we compared the mean number of bees on day 4 in the vicinity of the bait station prior to and during feeding (first, second, and third intervals) to the mean number of bees in the bait station vicinity following the feeding interval

(fourth, fifth and sixth intervals) (Fig. 2). If a time-place association formed, we expected more *T. amalthea* at the bait station during intervals 1 through 3, regardless of the absence of food. There were significantly more bees in the bait station vicinity during intervals 1–3 than intervals 4–6 (Mann-Whitney U, $P < 0.05$).

Additionally, anticipation of the feeding interval is indicative of time-place learning behavior. In order to determine if anticipation occurred on day 4, the test day, we compared the mean number of *T. amalthea* present at the bait station during interval 1 to a hypothesized mean of 0, the number of bees that would randomly be found at any given location and time. We found significantly more bees than 0 present in the bait station vicinity during interval 1 (one sample t-test, $t = 4.13$, 8 d.f.; $P = 0.003$).

3.2. Recruitment

We determined the time the first scout took to discover a feeding station, the number of scouts discovering the station, flight time between the station and the nest, number of visits per scout, and the number of recruits (Tab. 1). If *T. amalthea* forages based on standard cost-benefit strategies, we would expect to find a significant negative correlation between the time potential scouts spend away from the bait station (indicative of the distance to the colony) and the number of bees that are recruited. However, there was no significant correlation

Table 1. Recruitment characteristics of *T. amalthea* ($n = 23$ bait stations, sample sizes for individual variables given in table), means \pm SE.

Time (minutes) to discovery of station, first scout	5.5 ± 1.17	$n = 23$
Number of scouts discovering station	3.1 ± 0.29	$n = 23$
Flight time (minutes) from departure of scout to return	5.3 ± 0.55	$n = 21$
Number of visits per scout	3.3 ± 0.28	$n = 22$
Number of recruits	9.1 ± 2.29	$n = 22$

between flight time and number of recruits ($r = 0.40$, $P = 0.09$) or between any of the other pairs of variables.

The number of scouts was recorded in 23 cases. In five of these cases no recruitment occurred, but there was no significant difference when the cases are divided into those with and without recruitment for flight time ($F = 1.07$, $P = 0.32$) or discovery time ($F = 0.27$, $P = 0.61$). Of the six baits at which discovery was delayed for more than five min recruitment took place at four (66.7%), which was only slightly less often than the 81.3% of cases with shorter flight times that had recruitment (χ^2 , $P = 0.87$).

4. DISCUSSION

We show that *T. amalthea* workers associate the presentation of food at a location with the time of presentation. To date such an ability has only been demonstrated in a small number of insect species, all of which are eusocial Hymenoptera. Like most nesting Hymenoptera, *T. amalthea* workers have a well developed ability to learn food locations and to return to those locations repeatedly; the unique aspect of our findings is the ability of these bees to associate place with time. In addition, we found that the number of foragers responding to a feeding station did not vary with distance from the colony. This lack of response to increased time and energy invested in visiting the stations that distant from the nest is consistent with the model of foraging territories for this species proposed by Breed et al. (1999).

The total number of bees present on day 4 in the time-place learning experiment is substantially lower than the other days, due to the lack of recruitment (Fig. 1) that day. Thus our comparisons focus on the behavior a small number of scout bees, which we hypothesize have learned the time and place that food has appeared on previous

days. The most convincing argument that time-place learning is exhibited by this species is the simple fact that bees appear at all at the test location when no bait is present.

The ability to learn time in association with a feeding location may be widespread in the nesting Hymenoptera. Future studies that emphasize cross-species comparisons will resolve this point. The discovery of time-place learning in both ants and bees suggests that, at least in the Hymenoptera, it may be relatively easy for insects to associate spatial learning with either an internal or external clock. Tests for time-place learning in a broader range of insects will reveal whether such learning is deeply rooted in the insect phylogeny, or if this ability has evolved independently in a number of taxa.

Schatz et al. (1994, 1999) and Fourcassie et al. (1999) have argued that an animal's ability to return on a regular schedule to a single location is not adequate to test the hypothesis of time-place learning. Most of the hypotheses concerning the timekeeping mechanisms used by animals in time-place learning assume that a series of times and locations can be learned (Wilkie and Wilson, 1995). However, social insect workers may specialize on single resource types or single resource patches; they are able to serve appropriate foraging functions by learning only one time and location. Thus, more elaborate learning schedules can be biologically irrelevant. The more stringent criteria suggested by Schatz et al. (1999) and Fourcassie et al. (1999) are interesting in establishing correlates with solitary foragers, such as pigeons (Wilkie and Wilson, 1995), but learning a single time and location clearly meets a reasonable standard for time-place learning.

Our recruitment results suggest that *T. amalthea* do not conform to optimal foraging strategies, which predict a diminishment of foraging effort as the costs of foraging increase (Breed et al., 1996; Charnov, 1976). Breed et al. (1999) propose that *T. amalthea* workers forage in

territories that are limited in size by interactions with conspecifics from adjacent territories. We interpret our results as indicating that foraging home ranges are truncated by territorial boundaries to the extent that the expected reduction in foraging effort at the extreme limits of the home ranges are not observed.

The number of bees at the baits diminished between the third and fourth intervals, even though food was present until the end of the third interval. This was probably due to the fact that an initial group of recruits, present in the second time interval, had returned to their nest and had not yet appeared at the bait on a second foraging trip. Lacking the positive feedback of continued food, the number of foragers continued to decline through the later time periods.

These results raise several interesting questions for future work. Comparative studies of other Hymenoptera will resolve the issue of the phylogeny of time-place learning and the underlying mechanisms (for example, the use of an internal or external clock) which are used to accomplish it. Territoriality in stingless bees needs further exploration, as such constraints on foraging and colony distribution raise interesting issues in the conservation of these bees and the plants that they pollinate.

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Résumé – Apprentissage de l'association temps-lieu et écologie du recrutement chez une abeille sans aiguillon, *Trigona amalthea* (Hymenoptera, Apidae). L'apprentissage de l'association temps-lieu est un mécanisme complexe important pour les animaux qui exploitent des ressources que

l'on peut temporairement prédire, telles que le nectar des fleurs. Les butineuses qui apprennent quand et où trouver des ressources peuvent répartir efficacement leur temps et leur effort de butinage. Alors que ce phénomène est bien connu chez les abeilles domestiques (*Apis mellifera* L.) et chez certaines fourmis, on ne sait pas grand chose des capacités d'apprentissage temps-lieu chez les autres insectes sociaux.

Nous avons étudié cette question chez l'abeille sans aiguillon, *Trigona amalthea* (Olivier). Les ouvrières ont appris à visiter un site de nourrissage durant 30 minutes à la même heure chaque jour pendant 3 jours pour obtenir une récompense. Des visites sur le site précédaient la période de nourrissage. En l'absence de récompense, les visites étaient élevées avant et pendant la période habituelle de nourrissage, mais rares après. Le comportement d'anticipation est une preuve forte de l'apprentissage du temps-lieu puisqu'il n'y a pas de signal indiquant que de la nourriture va apparaître pour attirer les butineuses. Apparemment seules les éclaireuses se lancent dans l'apprentissage du temps-lieu, puisque le nombre d'abeilles présentes sur les stations était relativement faible jusqu'à ce que de la nourriture soit réellement offerte. Une fois que la nourriture est là, les éclaireuses recrutent des butineuses en grand nombre.

Nous avons étendu notre recherche au comportement de recrutement de *T. amalthea*. Les éclaireuses ont trouvé les stations de nourrissage rapidement, cinq minutes environ après leur installation. Le nombre moyen d'éclaireuses trouvant les stations a été légèrement au-dessus de trois et chaque éclaireuse a visité la station de nourrissage plusieurs fois au cours d'une heure d'observation. Les éclaireuses ont recruté en moyenne neuf autres ouvrières si bien que de nombreuses ouvrières ont pu prendre part à la récolte de nourriture. Chez certaines espèces d'insectes sociaux qui recrutent des ouvrières pour la nourriture, le nombre de recrues dépend de

la distance entre la source de nourriture et le nid. Mais chez *T. amalthea* nous n'avons pas trouvé de relation entre la durée de vol jusqu'à la colonie et le nombre de visites faites par les éclaireuses à la station avant le recrutement. Nous n'avons pas trouvé non plus de relation entre le nombre d'ouvrières finalement recrutées.

Chez *T. amalthea* une combinaison de l'apprentissage du temps-lieu et du recrutement permet de mobiliser rapidement des ouvrières lorsque une source de nourriture devient disponible. La compétition alimentaire entre les colonies peut être intense et les colonies qui trouvent et exploitent rapidement les ressources ont un avantage dans la compétition. La preuve de l'apprentissage du temps-lieu chez *T. amalthea* soulève la question de savoir si ce mécanisme est largement répandu parmi les espèces d'abeilles.

Meliponinae / abeille sans aiguillon / *Trigona* / butinage / apprentissage / recrutement

Zusammenfassung – Zeit-Orts-Lernen und die Ökologie des Rekrutierens bei der Stachellosen Biene *Trigona amalthea*. Zeit-Orts-Lernen ist für Tiere, die an zeitlich vorhersagbaren Quellen nach Futter, wie z.B. Blütennektar, suchen, ein wichtiger Verhaltensmechanismus. Futtersuchende Tiere, die lernen, wo und wann Nahrungsquellen zu finden sind, können die Zeit und die Arbeit, die sie für die Futtersuche aufwenden, effizienter einteilen. Während dieses Phänomen bei Honigbienen und einigen Ameisen gut bekannt ist, weiß man über die Fähigkeit des Zeit-Orts-Lernens bei anderen sozialen Insekten wenig.

Wir gingen dieser Frage bei der Stachellosen Biene *Trigona amalthea* (Olivier) nach. Arbeiterinnen lernten, während einer bestimmten Zeit einen Trainingsplatz aufzusuchen, um eine Belohnung zu finden. Vorhergehende Besuche an dem Trainingsplatz gingen dem Fütterungs-Intervall vor-

aus. Wurde keine Belohnung angeboten, so waren die Besuche vor und während des Fütterungs-Intervalls hoch, aber im Anschluss daran gering. Vorausschauendes Verhalten ist offensichtlich wichtig für das Zeit-Orts-Lernen, da es keine Zeichen für das baldige Auftauchen einer Nahrungsquelle gibt, die futtersuchende Tiere anlocken könnten. Anscheinend sind nur die Scout-Bienen an Zeit-Orts-Lernen beteiligt, da die Anzahl der Bienen, die an den Futterstationen auftauchte, bevor tatsächlich Futter dort angeboten wurde, relativ gering war. Diese Scout-Bienen rekrutierten viele Sammelbienen sobald Futter angeboten wurde.

Wir dehnten unsere Untersuchungen auf das Rekrutierungsverhalten von *T. amalthea* aus. Scout-Bienen fanden die Futterstationen schnell, innerhalb von etwa 5 Minuten nach dem Ausbringen. Die durchschnittliche Anzahl Scout-Bienen, die die Stationen fanden, war etwas mehr als 3, und jede Scout-Biene besuchte die Futterstation mehrmals innerhalb des einstündigen Beobachtungszeitraumes. Die Scout-Bienen rekrutierten im Mittel 9 andere Arbeiterinnen zu den Nektar-Ködem, so dass viele Bienen am Futterholen beteiligt werden konnten.

Bei einigen sozialen Insekten, bei denen Rekrutierung zu Futterplätzen vorkommt, hängt die Anzahl der Rekrutierungen von der Entfernung des Futters zum Nest ab. Bei *T. amalthea* jedoch fanden wir keine Beziehung zwischen der Flugzeit zum Volk und der Anzahl an Futter-Besuchen durch Scouts vor der Rekrutierung. Es gab auch keine Beziehung zur Anzahl an letztlich rekrutierten Arbeiterinnen. Vorausgegangene Studien legen nahe, dass *T. amalthea* territorial ist. Territorialität könnte den Aktionsradius für die Futtersuche beschränken, so dass wir die erwartete Verringerung der Sammelbemühungen in größerem Abstand vom Nest nicht beobachten konnten.

Bei *T. amalthea* ermöglicht eine Kombination von Zeit-Orts-Lernen und Rekrutierung eine schnelle Mobilisierung der

Arbeiterinnen, sobald Futter zur Verfügung steht. Die Konkurrenz um Futter könnte zwischen den Völkern erheblich sein, und Völker, die Futterquellen schnell finden und ausbeuten können, haben einen Wettbewerbsvorteil. Der Nachweis von Zeit-Orts-Lernen bei *T. amalthea* wirft die Frage auf, ob dieser Mechanismus unter Bienenarten weit verbreitet ist.

Stachellose Bienen / Meliponinae / *Trigona* / Futtersuche / Lernen / Rekrutierung

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