

Hymenopteran Group Foraging and Information Transfer about Resources

Guest Editors: Felipe Andrés León Contrera, Margaret J. Couvillon,
and James Charles Nieh





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Psyche

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Editorial

Hymenopteran Group Foraging and Information Transfer about Resources

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1. Introduction

“Knowledge is of two kinds. We know a subject ourselves, or we know where we can find information on it [1].” In social insects, discovery of a resource is often coupled with communication of this discovery to nestmates in order to exploit fully the resource before competitors find it or it is naturally depleted. Although it may seem simple, this process of information transfer is influenced by several factors, both intrinsic to the colonies and individuals (e.g., satiation status, the perception of colony’s food storage) and external factors (e.g., climatological conditions, available sources at the moment of foraging), which also interact with each other. This special issue explores some aspects of the regulation of foraging, recruitment behavior, and information transfer in Hymenopteran species, and it is divided into three sections: (1) the regulation of foraging by intrinsic factors, (2) the regulation of foraging by external factors, and (3) the regulation of recruitment.

2. Nest-Based, Individual, and Group Foraging Regulation (M. J. Couvillon)

Foraging is costly. Searching for resources necessitates the consumption of energy and time, and leaving shelter increases predation exposure. Of course, solitary organisms have no option. However, for group-living animals with division of labor, an individual is confronted with a “decision” either to forage or to engage in another safer task. What factors impact

this decision? More specifically, how do colonies utilize these factors to regulate foraging, maximizing the gain while minimizing the costs? These next three papers examine the causes and cues in foraging regulation. The first two look at how different proximate cues such as intrinsic (physiology) and social status may impact an individual’s foraging decision. The third paper investigates the ultimate benefit of a specific type of foraging organization.

In honey bees, all workers eventually forage. However, do all workers forage alike? What physiological factors influence an individual’s foraging behavior? Higginson and co-authors report on data in which they experimentally clip forager wings to simulate naturally acquired wing wear. They demonstrate that wing damage has deleterious effects on survivorship and foraging behavior. Bees with light damage took shorter but more frequent trips; foragers with heavy damage took less frequent trips. In this way, foragers adjust behavior according to individual physiology.

The eusocial wasp, *Mischocyttarus cerberus styx*, forms dominance hierarchies, and an individual’s rank impacts her propensity to forage. However, what happens to foraging organization when ranking is disrupted? Filho and co-authors removed 2-3 individuals from nests and then monitored the number of foraging trips. They found that while the removal of lower-ranked females did not cause an effect, the removal of higher females caused a 66% decline in foraging trips. The authors hypothesize that the disruption of the dominance hierarchy by removing the higher ranking

individuals necessitated a reorganization that negatively impacted foraging effort.

Lastly, in “*Sequential load transport in grass-cutting ants (Atta vollenweideri): maximization of plant delivery rate or improved information transfer*,” J. Röschard and F. Roces ask why the grass-cutting ant *Atta vollenweideri* evolved sequential transport. They test the traditional explanation “economic-transport”, where forming transport chains increases individual efficiency, against an alternative explanation “information-transfer”, where transport chains, while decreasing individual efficiency, may benefit the colony by providing information for foragers. They show that while manipulating fragment size did not increase the probability of a transport chain, manipulating fragment quality did increase sequential transport, thus supporting the “information-transfer” hypothesis. In this way, colonies evolve exquisite—and different—mechanisms by which essential tasks are organized.

3. Recruitment Regulation (J. C. Nieh)

In this special issue, three papers examine two aspects of how social insect colonies use information transfer to regulate recruitment, a process by which the colony allocates foragers to food sources. Such information transfer can occur in multiple ways, including the use of odor trails or the famous honey bee waggle dance.

Odor trails are used in many ant species. In the paper “*Trail laying behaviour as a function of resource quality in the ant *Camponotus rufipes**,” P. Schilman shows that the probability of foragers depositing a recruitment odor trail varies with the quality (sucrose concentration) of the food source. A greater proportion of foragers deposited odor trails for higher as compared to lower concentration sucrose solution. This behavior could contribute to how a colony allocates labor among food providing different reward levels.

Such odor trails are used by other social insect species. An interesting question is whether a wasp that uses odor trails during nest swarming can also use olfactory information to guide nestmates. Taylor and his collaborators examined this possibility in the social wasp, *Polybia occidentalis*. This species lays odor trails to guide migrating swarms. In “*Recruitment in swarm-founding wasps: *Polybia occidentalis* does not actively scent-mark carbohydrate food sources*,” they show that foragers did not exhibit a preference, in a paired-feeder assay, for the feeder that multiple foragers previously visited while being trained. Thus, a species that can use odor trails to guide mass movements in one context (swarming) does not necessarily use them in a different context (foraging).

Finally, O. Duangphakdee and coauthors review our understanding of the role of celestial information in the waggle dances of different honey bee species. The honey bee waggle dance recruits nestmates to resources and provides orientation information that uses the sun’s position in the sky. Dancers transform the resource’s location relative to the sun’s azimuth into the angle of the waggle phase with respect to gravity (for dances on a vertical surface) or directly in the angle of the waggle phase on a horizontal surface. Accurately determining the sun’s azimuth (its direction projected onto a horizontal plane) can be difficult when the sun

is at its highest point in the sky. This is particularly true at locations near the equator and at times of the year when the sun is almost directly overhead at its zenith. In these situations, small errors in estimating the correct solar azimuth can generate large errors in the direction communicated in the waggle dance. Duangphakdee and his co-authors review this fascinating problem and discuss the ingenious solutions that different species of honey bees have evolved.

4. Role of Environment in Foraging Regulation (F. A. L. Contrera)

The foraging of social insect colonies is a complex behavior, regulated by several internal and external factors, such as climate conditions and the availability of resources. The contributions in this section deepen our understanding of the role of environment in foraging regulation in two species of social insects; *Melipona capixaba* (Hymenoptera: Apidae: Meliponini) and *Polybia paulista* (Hymenoptera: Vespidae: Epiponinae). Luz and collaborators show that in the stingless bee, *M. capixaba*, there is a preference for pollen sources from native species, but in disturbed regions, they shift to a high preference (~80%) for pollen from an introduced cultivar, *Eucalyptus*. The authors discuss the importance of the recovery of native flora for the nourishment of colonies, since introduced plant species may lose their economic attractiveness and be substituted for other cultivars that do not provide as much food for the colonies. In another study, N. C. de Souza Canevazzi and F. B. Noll examine the influence of weather on the foraging efforts of the wasp *P. paulista* throughout the year and also evaluate the importance of the resources that wasps forage for. They showed that temperature is the most relevant environmental factor influencing foraging behavior. In addition, nectar and water are the most relevant items collected, because they are involved in metabolism (water and nectar), thermoregulation (water) and nest construction (nectar).

Felipe Andrés León Contrera
Margaret J. Couvillon
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- [1] S. Johnson, quoted in Boswell’s Life of Johnson, (1709–1784), <http://www.quotationspage.com/quote/26259.html>.

Research Article

Experimental Wing Damage Affects Foraging Effort and Foraging Distance in Honeybees *Apis mellifera*

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Bees acquire wing damage as they age, and loss of wing area affects longevity and behaviour. This may influence colony performance via effects on worker behaviour. The effects of experimental wing damage were studied in worker honeybees in observation hives by recording survivorship, how often and for how long bees foraged, and by decoding waggle dances. Mortality rate increased with both age and wing damage. Damaged bees carried out shorter and/or less frequent foraging trips, foraged closer to the hive, and reported the profitability of flower patches to be lower than did controls. These results suggest that wing damage caused a reduction in foraging ability, and that damaged bees adjusted their foraging behaviour accordingly. Furthermore, the results suggest that wing damage affects the profitability of nectar sources. These results have implications for the colony dynamics and foraging efficiency in honeybees.

1. Introduction

The lifespan of worker honeybees (*Apis mellifera*) has been reported as ranging from two to four weeks in the summer [1, 2] while overwintering bees that rarely leave the hive can live for several months [2, 3]. The foraging period, defined as the length of time from the first foraging trip to death, is between seven days and two weeks [2, 4–7], is not affected by the in-hive length of life before the first foraging trip [4], and is negatively related to work rate [8], but see [9].

If mortality was always due to effects extrinsic to the bee (e.g., predation and weather), then the mortality risk per unit time over a bee's foraging career would not change. Indeed, bee foragers appear to experience a constant hazard rate, as measured by the constant probability of death per unit foraging time [6]. However, [6] and other studies (see [8]), used a simple analysis that may not be accurate because it does not allow for the reduced sample size at older ages

[10]. When the same data were analysed using a Weibull plot and a more appropriate calculation of the hazard rate that controlled for the skewed data distribution, an increasing probability of death as foragers aged was apparent [10]. Re-analysis using Weibull plots also showed that increasing mortality rates during the foraging period were also present in bumblebee studies that had previously claimed a constant mortality rate [8].

It has been suggested that the flight machinery of honeybees has a fixed lifespan [4]. In an experimental study [11], removing 10% of the wing area in bumblebees results in such bees having a reduced lifespan, but the cause of mortality in these cases was unknown. However, the rate at which the flight machinery reaches some finite limit may be increased by damage to the wings, since increasing damage increases the wingbeat frequency [12]. Increasing mortality risk does not necessarily imply that lifespan is physiologically limited by the flight mechanisms [4], as age and/or wing

damage may affect an insect's ability to avoid or escape from predators [11]. However, an increasing mortality rate in the absence of predators and inclement weather has been observed in bumblebees (*Bombus terrestris*, [11]), suggesting some intrinsic limit on lifespan.

If costs increase with age, we might expect bees to change their foraging strategy as they age. However, there appears to be no effect of the age of worker honey bees on foraging distance from the hive [1]. No studies have yet been carried out on the effect of age on energy use by flying insects but some have looked at the effects of wing damage. Wing damage is widespread across all insects [13] and is highly correlated with age [14–17]. In bumblebees, wing damage does not seem to affect energy use but does affect wingbeat frequency [12], because when a wing is smaller it needs to beat faster to generate more lift, but each beat requires less energy. Furthermore, wing damage has a negative effect on lifespan [11]. We have previously [18] proposed that the foraging lifespan is limited by the number of wingbeats the wing is capable of performing. If wing damage decreases lifespan by increasing the rate at which bees reach their wingbeat limit, then damaged bees may be expected to show shorter foraging periods, attempt to spend their wing beats at a slower rate and hence forage less frequently or closer to the hive, and may travel a shorter distance to forage in order to maximise the energy gain per wingbeat spent. These responses may explain such phenomena as the reduction in foraging efficiency observed after 10 days of foraging effort [19]. Also, since damaged individuals suffer increased costs associated with foraging, they may also perceive nectar sources as less profitable [20].

The current study investigated the effects of experimental wing damage on the foraging decisions of individual worker honeybees. To look for these effects, observation hives were established within which individually numbered workers could be manipulated in terms of wing damage and monitored for variation in foraging period duration and foraging behaviour. The results show that wing damage reduces survivorship, foraging trip duration and frequency, and the distance foragers travelled from the hive. Furthermore, the results suggest that wing damage might affect the perceived profitability of nectar sources, as reported by the waggle dance.

2. Methods

2.1. Experimental Manipulation. Data were collected in August and September of 2003 and 2004 in the Laboratory of Apiculture and Social Insects at the University of Sheffield, South Yorkshire, U.K. Each year, two colonies of 3000–5000 workers, plus queen and brood, were studied in observation hives. Honeybees were individually marked with coloured numbered disks (E. H. Thorne Ltd., U.K.) and had their wings clipped using entomological scissors to simulate wing damage (see [17]). Most bees were treated at eclosion by taking brood comb from the hives and incubating until bees emerged. Bees were introduced back to the hive via a box attached to the top of the hive separated by gauze. They were left in the box for several hours (to allow the bees to acquire

the hive scent) before the gauze was removed, enabling the newly emerged bees to enter the hive. Additional bees were marked by capturing foragers as they came out of the hive and chilling them in a domestic fridge for marking and clipping of wings. These bees were placed back outside the entrance to their own hive. In 2003, all the bees were marked when they eclosed, but in 2004, the second and third cohorts were marked when adult in order to compare the effects of the time of experimental damage.

There were four treatment groups, to which a total of 855 bees were assigned randomly. Each treatment involved a different degree of wing damage as follows: *Control*: undamaged control, bees were handled in the same way as other bees but their wings were not clipped; *Light*: light damage, 3–7% reduction in the area of each wing, and *Heavy*: heavy damage, 8–17% of each wing. We also attempted to damage bees asymmetrically but did not achieve a sufficiently small range of damage and had very low sample sizes throughout, resulting in unreliable results, and so these data are not presented. The amount of wing area removed from each individual was quantified using a gridded graticule by placing the wing clipping under a microscope. The wing areas removed were then converted to percentage wing damage. The area removed per wing per individual was relatively small within groups and differed among groups ($F_{3,219} = 30.183$, $P < .001$). The average percentage wing area removed per wing for the *Heavy* group was approximately twice that for the *Light* group. Three cohorts of 30 individuals per treatment group were treated and marked in each hive in each year.

2.2. Behavioural Observations. Between three and five times a week, marked bees were recorded by noting each bee seen during a 15 minute scan of the hive through the glass. This provided data on survivorship, including any bees that did not forage or were otherwise not observed at the hive entrance. The entrance to each observation hive was a tunnel through the outside wall of the building and a box in which there was a small camera (Micromark MM40010) that recorded the bees as they entered and exited (Figure 1). The cameras were linked to video recorders (Matsui TVR 162S) that recorded on VHS videotape from approximately 10.00–18.00 h each day of observation to yield the daily time each bee spent outside the hive and the number of times it left the hive.

Waggle dances were recorded opportunistically onto DAT tape using a digital video recorder (Sony DCR TRV 16E) by an observer watching the colonies from approximately 10.00–18.00 h each day of observation. The cameras were focused on the dancing area, the half of the lower comb near the hive entrance and positioned to maintain the dance floor in full plane view on the screen. The camera was held on a retort stand and clamp, so was steady but movable. Upon the observation of a waggle dance by a marked individual, the camera was moved if necessary to keep the dance in the centre of the screen. The recording continued until the focal individual stopped dancing or moved off the dance floor. Vertical lines 5 cm apart were drawn on the outside

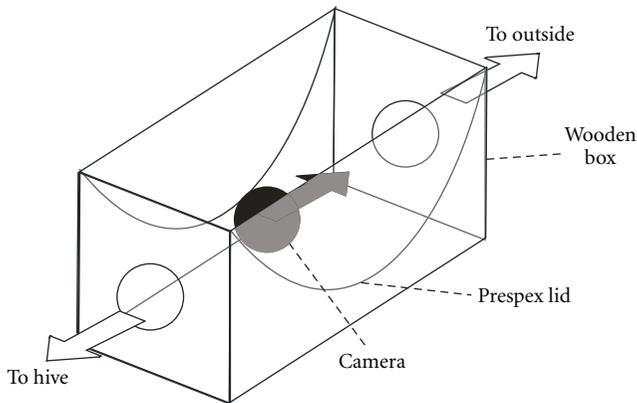


FIGURE 1: The camera box that bees must pass through to leave the hive. Bees are forced in the lower half of the box, under the curved perspex lid. This design forces all bees entering and exiting the hive to show their backs (and hence any numbered disc) to the camera positioned on top of the perspex lid.

of the observation hive in thin permanent marker to enable calibration of the angle of the dance to vertical.

All recordings were converted to Quicktime (Apple Computer, Inc., Cupertino, CA, USA) format, and the dances were cropped from the film. The dances were analysed using the Videopoint (Lenox Softworks, Inc., Lenox, MA, USA) path recording software. The program combines a spreadsheet and video. The user clicks on any part of the image to record the relevant pixels in the spreadsheet, yielding coordinates and frame number. Thus, for instance, clicking on the head of the bee at the start and the end of the waggle run of the dance would provide the duration and angle of the run. Additionally, clicks before each dance on the top and the bottom of a drawn line on the outside of the hive recorded the vertical, to control for imperfect orientation of the camera. The spreadsheet was entered into Microsoft Excel where the data were compressed, deleting all the frames where no click occurred. From the four recorded points (start of vertical line, end of vertical line, start of waggle run, end of waggle run), it was possible to calculate the angle of the waggle run on the honeycomb and its duration. Multiple waggle runs of the dance by each bee were analysed.

The waggle dance comprises a rough figure of eight pattern on the comb, with the direction of movement of the dancer tending to alternate [21]. Three main kinds of information are passed on to the waggle dance follower [20, 22]. The duration of the waggle run represents the distance between the hive and the food source, with one second corresponding to 500–1400 m, with significant variation between subspecies and landscapes [2, 22, 23]. The angle of the waggle dance relative to the vertical represents the angle of the flight path relative to the solar azimuth. The duration and rate of performing the waggle run, and, therefore, the time between each waggle run, is related to the profitability of the food source in that the shorter the time between waggle runs the more profitable the nectar source [20]. The mean waggle run duration and direction were calculated for each waggle dance. The angle of the dance on the comb

was translated by means of a program (Sun 97 provided by W. F. Towne) that calculated the position of the solar azimuth at the time of each dance. The frequency of waggle-run production was estimated as the mean length of time between waggle runs.

Data were analysed by ANOVA, with normalising transformations as necessary, to check for differences in survivorship, foraging effort, foraging distance and reported quality between the treatment groups. Where normalization was not possible, equivalent nonparametric tests were used. Hazard rate and Weibull analysis (to assess mortality over time) followed the procedure detailed in [10]. Hutchinson [10] suggested that survivorship should be plotted using the Weibull distribution to see if it differed from an exponential distribution. If mortality risk is constant, the number of deaths will be exponential, which yields a gradient of unity when the Weibull distribution is plotted. Analysis was carried out in SPSS (SPSS Inc., Chicago, IL, USA) and R (Free Software Foundation, Boston, MA, USA). Unless otherwise stated there were no differences between colonies so data were pooled for most analyses.

3. Results

3.1. Survivorship and Hazard Rate. The first and last occasions on which individuals with known date of eclosion were noted in the hive and at the entrance provided an estimate of lifespan. We found that *Control* bees lived for around 20.5 days on average, whereas experimentally damaged bees lived for around 18 days, and there was no difference between the two levels of wing area removal (Figure 2(a)). The difference between treatment groups in lifespan was statistically significant (Kruskal-Wallis test: $H_4 = 14.415$, $P = .006$).

The probability of mortality per day (hazard rate) was initially low at around 0.02 but showed an accelerating increase as bees age such that for the oldest foragers it was greater than 0.25 (Figure 2(b)). The hazard rate actually fits an exponential distribution ($F_{1,26} = 45.65$, $P < .001$), showing a sharp increase in mortality at around 28 days of age. Furthermore, damaged bees experienced a 50% higher hazard rate when young, but around a 25% higher hazard rate when older. These observations are confirmed by fitting hazard rate models. The data provided a better fit to the Weibull distribution than to an exponential distribution ($F = 402.6$, $df = 1$, $P < .001$), implying that the hazard rate increased over time. Furthermore, a model fitting slopes to the three treatment groups provided a better fit than a model that assumed a single slope for all groups ($F = 8.99$, $df = 1$, $P = .011$), but was not a better fit than a model that fitted a single slope to both the *Light* and *Heavy* groups together ($F = 1.515$, $df = 1$, $P = .218$), implying that their hazard rates did not differ. That is, both groups of artificially damaged bees experienced a consistently higher mortality rate than control bees, but all individuals were subject to an increasing risk of mortality as they aged.

3.2. Length of Foraging Trip. The 288 hours of video data of bees entering and exiting the hive were subsampled using the random number generator in Microsoft Visual Basic

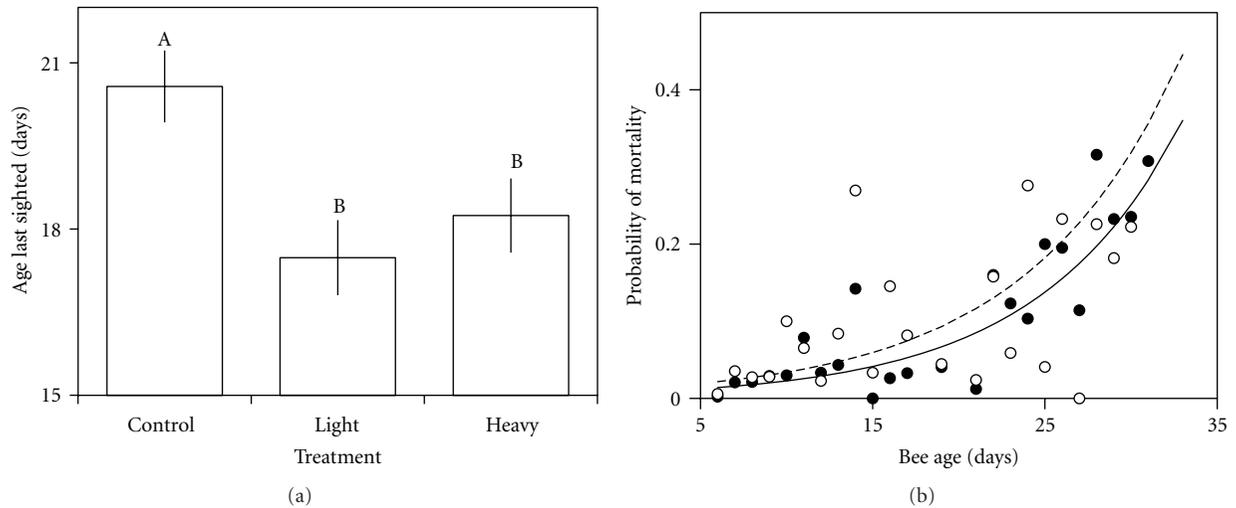


FIGURE 2: (a) Mean (± 1 SE) lifespan of workers in the treatment groups. Bars that share the same letter did not significantly differ. (b) Hazard rate (probability of death per day) against bee age for Control (closed circles, solid line) and Light treatments (open circles, dashed line). The lines represent exponential fits of the data ($0.0068 e^{0.1t}$, $0.0148 e^{0.0861t}$, respectively). Only two groups are shown for the sake of clarity and the curve for the *Heavy* group did not differ significantly from that for the *Light* group.

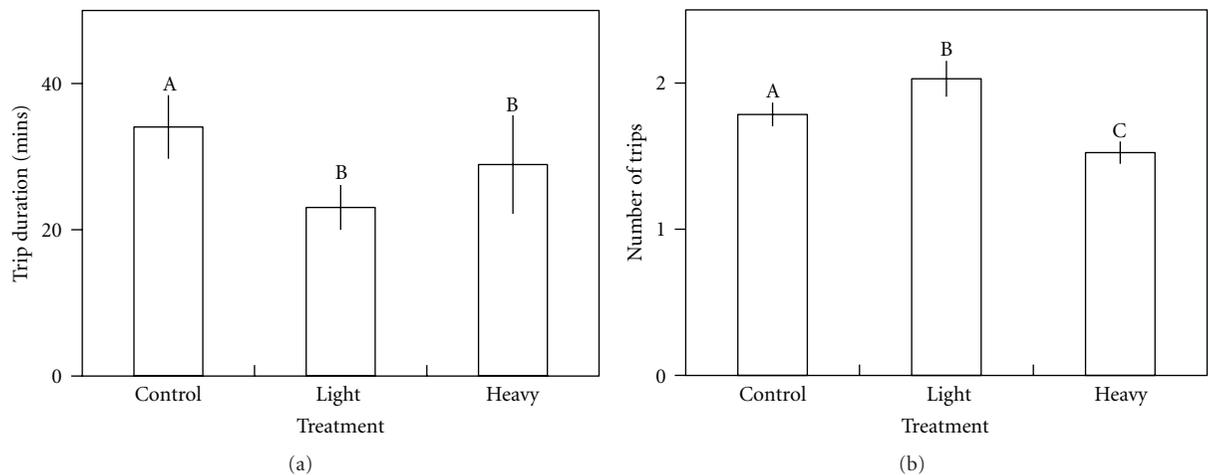


FIGURE 3: Mean (± 1 SE) (a) duration in minutes and (b) number of foraging trips per bee for each treatment group. Bars that share the same letter did not significantly differ using *post hoc* tests.

(Microsoft Inc, Redmond, WA, USA). The data on marked bees entering and exiting the hive was used to calculate the duration of 172 foraging trips (up to five for any given bee). Trips were omitted from analysis (a) if they were shorter than two minutes (31 trips), since it was likely that the bee had not left the outside tunnel, and (b) if they were longer than 3 hours, since the bee was likely to have been missed when reentering the hive (8 trips).

ANOVA of trip duration (natural log transformed to normalise) with colony and treatment group as factors and date and time of day as covariates showed there was a difference between the two colonies in the duration of foraging trips ($F_{1,163} = 3.98$, $P = .048$). There was a positive effect of date ($F_{1,163} = 25.92$, $P < .001$), and a negative effect of time of day ($F_{1,163} = 5.903$, $P = .016$) on trip

duration; trips became longer over the observation period, and shorter trips were carried out in the afternoon. Trips carried out before noon were more than twice as long, on average, as those carried out after 2 pm. Crucially, there was a difference between the treatment groups in trip duration ($F_{3,163} = 3.518$, $P = .016$). Trips carried out by *Control* bees were almost 50% longer, on average, than those carried out by *Light* bees and 17% longer than those carried out by *Heavy* bees (Figure 3(a)). To look for potential effects of bee age, the same analysis was carried out on the bees whose date of eclosion was known, but there was no effect of age on trip duration ($F_{1,123} = 0.406$, $P = .525$). The number of foraging trips recorded for each bee also differed between treatments (Kruskal-Wallis test: 18.2, $N = 287$, $P < .001$): *Heavy* bees performed around 17% fewer trips than *Control* bees and

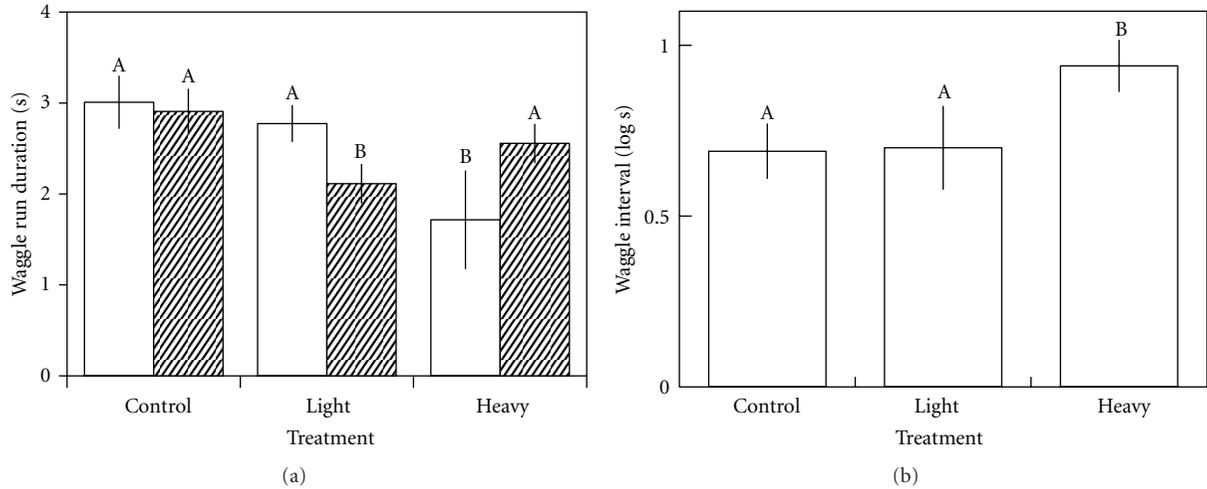


FIGURE 4: (a) Mean (+ 1 SE) duration of waggle run per dance for each treatment group and whether they were damaged after they started foraging (open bars) or at eclosion (hatched bars). (b) Mean (\pm 1 SE) interval between waggle runs per dance (*return run*) for the three treatment groups. Bars that share the same letter did not significantly differ. *Heavy* bees indicated lower quality nectar sources than *Control* and *Light* bees.

33% fewer trips than *Light* bees, and *Control* bees performed 13% fewer trips than *Light* bees (Figure 3(b)).

3.3. Choice and Perceived Quality of Patches. The observations provided 828 waggle runs from 164 dances by 132 individuals (up to three dances per bee). ANOVA showed that there was a difference in waggle run duration between colonies ($F_{3,152} = 3.11$, $P = .047$) and a difference between treatment groups ($F_{3,152} = 7.463$, $P < .001$, Figure 4(a)): whilst waggle run duration was approximately equal for the two groups of damaged bees, it was 20% shorter, on average, than for *Control* bees. The same analysis was carried out on the bees whose dates of eclosion were known, with age as a covariate, but there was no significant effect of bee age on run duration ($F_{1,75} = 2.427$, $P = .123$). There was also no difference in waggle run duration between bees that had been treated at eclosion and those that were treated after they started foraging ($F_{1,128} = 0.061$, $P = .806$), but there was a significant interaction between this factor (time of manipulation) and treatment group ($F_{3,128} = 3.598$, $P = .015$), *Heavy* individuals that were wing clipped after they started foraging and *Light* individuals clipped at eclosion travelled shorter distances than other groups, whereas there was no difference in distance between the two times of manipulation for the *Control* bees (Figure 4(a)). Wing clipping at eclosion rather than after foraging started was associated with a 23% reduction in waggle-run duration in *Light* bees, but a 48% increase in *Heavy* bees.

ANOVA (using dances where at least three waggle runs were recorded) was performed on the mean length of time between runs (normalised by natural logarithms), with treatment group, colony and time of manipulation (eclosion or adulthood) as factors, and mean waggle-run duration as a covariate, the latter to assess whether reported profitability and distance were related. There were no differences between

colonies ($F_{3,120} = 0.0082$, $P = .93$) nor an effect of the time of manipulation ($F_{1,120} = 1.34$, $P = .24$). There was, however, a positive relationship between the mean length of time between waggle runs and mean waggle run duration ($F_{1,120} = 10.58$, $P = .0014$); nectar sources further from the hive were reported to be less profitable. There was also a significant difference between treatment groups ($F_{3,120} = 4.26$, $P = .041$); *Heavy* damaged bees reported lower profitability of nectar sources (Figure 4(b)). Interestingly, there was an interaction between treatment and waggle run duration ($F_{3,120} = 3.93$; $P = .05$); the slope relating waggle run duration and time between waggle runs became less positive as the amount of added wing damage increased across groups. Hence, while control bees perceived more distant sources as less profitable, both groups of damaged bees perceived all sources as less profitable than controls, and even when nectar sources were at the same distances.

Fifty-three bees were observed both dancing and carrying out foraging trips, but there was no relationship between trip duration and waggle-run duration ($r_{53} = -0.051$, $P = .719$) or return run duration ($r_{53} = -0.007$, $P = .958$); trips to sites further away did not take longer. This might be unsurprising since bees will spend much more time foraging within a flower patch than flying to and from the patch.

4. Discussion

The results suggest that experimentally induced wing damage had deleterious effects on the survivorship and foraging behaviour of honeybees, and since wing damage is acquired naturally [13, 17], this has implications on foraging ecology. Since many other insects acquire wing damage, these effects may have analogues in an ecologically and taxonomically broad range of species.

The effects of experimental wing damage on lifespan expand on the results of other studies. Hutchinson (2000)

plotted the hazard rate against foraging life duration for the data of [6] and showed that there was a sharp increase in the final (80 hour) age group of their bees. This could be because many bees do not die as a result of predation but do reach a physiological limit, and this was typically after 80 hours foraging times in those data [6]. The data in the present study were based on total lifetime so they include one or two weeks before the foraging period starts. Therefore, they are not directly comparable with the foraging period duration data of [6], but they do show a similar pattern, namely a sharp increase in hazard rate late in life, after 25 days in our study. Caution must be exercised in interpretation because the end of the curve is based on little data, as few bees are still alive, and so is imprecise [10], but the data suggest there is a finite limit on foraging performance or lifespan. As experimentally damaged bees apparently died sooner than undamaged bees, the results suggest they may have reached this limit sooner. Since damaging wings is likely to have required an increase in wingbeat frequency [12] throughout the bees' lifetime, the flight mechanisms may have worn out sooner. If damage simply reduced the ability of bees to avoid predators, the probability of predation would not change as the bees aged, so there would have been no increase in hazard risk over time. We also found that the hazard rate for experimentally damaged bees increased more rapidly than that for undamaged individuals, as previously observed by [11], (reanalysed by [8]), suggesting that damaged bees reach such a physiological limit sooner.

Similar survivorship curves have been obtained for several bumblebee (*Bombus* spp.) species. Estimates of mean lifespan vary between 13 and 41 days [9, 24, 25], but all show increasing mortality rate with age. As [25] state "mortality rate was nearly constant for the first 14 days of adult life, after which it increased sharply." This suggests that there is a physiological limit on longevity in social insects, and this is true in laboratory conditions in the absence of weather or predation [8]; longevity has also been shown to be inversely related to physical activity in other insects, such as the house fly *Musca domestica* [26] and *Drosophila* [27]. A limit on flight performance would also explain the inverse relationship between work rate, or amount of flying, and longevity of bees [4, 24, 28].

The hypothesis that there is a limit on flight performance was also supported by the other findings of the present study. There was no effect of age on the length of foraging trips or the distance travelled from the hive, in line with [1]. However, there were clear effects of experimental wing damage on trip duration and distance travelled in that trips and distances were longest for undamaged bees. The finding that damaged bees travelled shorter distances suggests that flight is more costly and, therefore, foraging trips are less profitable at greater distances. It is possible that flight speed was affected by damage, or that damaged bees took longer on foraging trips because of a reduction in foraging ability. Earlier work by us [17] has shown that damaged bees were less choosy, which may have resulted in poorer gain rates, and so damaged bees to return to the hive quicker to find alternative nectar sources. Since there is an effect of the weight being carried on wingbeat frequency and energy

expenditure [29, 30], damaged bees may be more limited in how much they can carry.

However, since energy requirement does not increase with reduced wing area [12], these behavioural responses cannot be related to energetic efficiency or net rate. Wingbeat frequency has been shown to increase with reduction in wing area [12], so in both deciding on how far to travel, and when to return to the hive, bees may use a currency of energetic gain to per wingbeat [18]. This is in line with previous findings of times of returning to the hive that assumed a currency of energetic efficiency (net energy gain per unit energy spent), (e.g., [31]).

These findings also tie in well with our results showing that undamaged bees reported patches further from the hive as less worthwhile, which may be because patches further from the hive happened to be poorer, or because more costs are incurred in travelling to and from the patch. The interaction between treatment and distance on profitability is in line with this. Control bees report more distant patches as less profitable, but damaged bees report all patches as less profitable. If it is the case that damaged bees reported profitability from foraging trips differently from undamaged bees, it will mean that individual wing damage is likely to have colony-wide effects on foraging.

The differences between the damage treatment groups were interesting in that heavily damaged individuals carried out foraging trips of lengths intermediate between the controls and lightly damaged but carried out fewer trips. As they seemed to carry out fewer trips than lightly damaged individuals, it appeared that light damage caused shorter but more frequent trips, but heavy damage causes less frequent trips. These findings highlight the need for further experimental study in to the effects of wing damage on flight costs and load carriage. It was surprising that there were no differences between lightly damaged and heavily damaged individuals in survivorship and foraging distance. One might expect that doubling the amount of wing damage would at least increase the effects of damage. There may be a general effect of damage to the bee not due to the amount of wing removed, although our control should have controlled for handling effects. Alternatively, the effect of damage may be non linear, and the damage added here was sufficient to reach the saturation point of the behavioural response. Further work with finer differences between treatments are needed to test this possibility. Heavily damaged bees may have performed fewer foraging trips than lightly damaged bees, therefore, slowing the rate of exhaustion of the mechanisms of flight and, hence, surviving for the same length of time. A difference in work effort may explain the difference in the change in the hazard rate between the control and damaged groups, if undamaged bees flew further or carried out longer trips, and so the behaviour of damaged bees gradually over time reduced the apparent effect of damage on the hazard rate shown in Figure 2(b).

Overall, the results suggest there is strong selective pressure for honeybees to avoid accumulating wing damage, since it has marked apparent effects on the foraging efficiency of colonies, from flower choice and patch choice, to foraging time and even lifespan. As honeybees may avoid foraging

in vegetation-dense areas to reduce their risk of sustaining wing damage [32], these effects are likely to have profound implications for the impact of the spatial distribution, density and plant architecture of food sources exploited by bees.

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Research Article

The Effects of the Social Hierarchy Destabilization on the Foraging Activity of Eusocial Wasp *Mischocyttarus cerberus styx* Richards, 1940 (Hymenoptera: Vespidae: Polistinae)

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The genus *Mischocyttarus* comprises 245 species of neotropical basal eusocial wasps. They form small colonies (rarely more than few tens of individuals); castes are morphologically undifferentiated and determined behaviorally by agonistic interactions. The aim of this study was to verify the effects of the experimental disruption of social hierarchy on foraging activity of *Mischocyttarus cerberus styx*. We observed six colonies in postemergence phase and recorded data on the foraging activity under two experimental conditions: (1) removal of lower-ranked females and (2) removal of higher ranked females, except the queen. Our results showed that the removal of higher-ranked females had higher effect on the number of foraging trips of *M. cerberus styx* than the removal of lower-ranked females (the number of foraging trips/hour decreased by 66.4% and 32.7%, resp.). Such results are likely due to the social organization of this species and the presence of a distinct class of females, which in this study were regarded as intermediates. Our data also showed that, irrespective of the hierarchical status of the females, the removal of two or three individuals affected significantly the number of foraging trips in this species.

1. Introduction

The tribe Mischocytarini is arranged in a single genus (i.e., *Mischocyttarus*) with 245 species [1, 2]. This group of basal, eusocial wasps is widely distributed in South America, and only two species occur in southern and western of the United States [3, 4].

The colonies of *Mischocyttarus* are founded independently or by an association of few females, and castes are morphologically undifferentiated. The social organization of this group is determined by agonistic interaction which leads to the establishment of a dominance hierarchy [3, 5]. Queens occupy the highest rank in the social hierarchy. They are responsible for egg-laying, whereas workers perform other tasks such as brood care, nest defense, and foraging [5–8]. Since caste determination is mainly behavioral, the

social roles of females of *Mischocyttarus* are flexible. Trade-offs and superseding of queens are common features in the social hierarchy [3, 9]. Bruyndonckx et al. [10] carried out experimental manipulations on *Ropalidia marginata* and suggested that dominance and subordination interactions, beyond social control, also act as a signal for workers to collect more food. Physiological conditions of females, such as the amount of fat body and ovarian development, are positively related to the dominance rank in *Mischocyttarus cassununga* [11, 12]. Litte [13] verified that when the queens of *Mischocyttarus mexicanus* were removed the non-egg-laying foundresses were capable of developing their ovaries and started to lay eggs one week after the queen's removal. Similar results were found by Field and Foster [14] in *Liotenogaster flavolineata*, in which helper females began to lay eggs when queens were experimentally removed.

TABLE 1: Characteristics of the studied colonies in the beginning/end of the observations.

Colony	Hours of observations	Number of cells	Eggs	Larvae	Pupae	Females ¹	Males ²
M14	30	30/31	8/9	10/13	6/5	5/2	1/0
M15	30	52/53	7/7	11/12	11/12	6/2	0/0
M16	30	59/61	20/13	22/24	7/7	6/2	0/0
M17	30	70/71	19/11	21/24	5/7	5/2	3/0
M18	30	22/22	1/3	13/11	5/4	5/2	1/0
M19	30	46/46	11/12	13/16	4/5	5/2	1/0

¹Two females (one of colony M15 and another of colony M16) disappeared during premanipulation observations and were not considered in the data sets, thus in the end of the manipulations the same number of individuals were left in each colony.

²Males which emerged or were seen during the observations were immediately removed from the nests.

O'Donnell [15] argued that workers of *Polistes* differed in their behavior according to a set of physical and social stimuli, intrinsic (social and developmental) or extrinsic (environmental). These behavioral switches are necessary to maintain reasonable levels of food and pulp collection according to the needs of the colony.

The wasp *Mischocyttarus cerberus styx* builds stelocytarus, gymnodomous nests, and the colonies are populated by a few individuals [16]. Interestingly, Silva [17] firstly described a distinct class of females which were classified neither as foragers nor as dominant females. According to the author these females were characterized by resting the most part of their time in the back side of the comb and occasionally collecting and distributing the food; however, no further information on the social role of these females was provided in this study.

The aim of this study was to verify the effects of the experimental removal of females in different hierarchical positions upon the foraging activity of *Mischocyttarus cerberus styx* and to investigate the critical number of females necessary to maintain reasonable levels of foraging activity and prevent the colony from declining.

2. Material and Methods

The study was carried out from March to May 2010 in 6 postemergence colonies of *Mischocyttarus cerberus styx* (Table 1). These colonies were observed at the campus of São Paulo State University (UNESP), Rio Claro, São Paulo, Brazil (22°24'26''S; 47°33'36''W). All adults were marked with nontoxic acrylic paint to identify their position in the social hierarchy before the start of the observations.

The hierarchical position was determined by the dominance-subordination interactions of each female in the colonies. Dominance behaviors were defined as a female investing physically against another by attacking, biting, chasing, pecking, or holding wings or legs using the mandibles [3, 10]. This method of hierarchy establishment was widely used by several authors [5, 6, 8, 9, 11, 12, 18, 19]. In order to provide a better female categorization a set of other behaviors were recorded and analyzed: (1) permanence in the nest (in minutes), (2) foraging activity, (3) cell inspection, and (4) rubbing the gaster against the nest wall: the female typically rubs the ventral part of its gaster along the stem and upper part of the comb, this behavior is

associated with the defense against ants as tested by Jeanne [6]. The base of the terminal portion of the gaster bears a tuft of hairs that carries a glandular secretion which avoids ants to reach the wasp's brood [20].

To carry out this study six colonies were observed by 6 hours a day for 5 consecutive days, totaling 180 hours of observation. Observations were taken daily from 9:00 to 12:00 and from 14:00 to 17:00. The foraging activity was recorded when foragers landed in the nest carrying liquids or prey items. Then, foraging activity is herein defined as the act of bringing prey and liquids to the nest. As stated by Hunt [21] liquids can be described as nectar, nectar-like fluids, and body fluids of prey. In this study we also included water, once as it is extremely important to the maintenance of physiological activities of the individuals, thermoregulation, and building of the nest.

Initially the manipulations were taken by removing 50 and 75% of individuals of each colony. Such procedure was carried out in four colonies (M10, M11, M12, and M13). However, in these four colonies even the removal of 50% of individuals caused the abandon of individuals from the nest. Based on this previous test we concluded that the abrupt removal of females of *Mischocyttarus cerberus styx* led to the colony decline and abandon of females. Thus, a new approach based on gradual removal of the individuals in different hierarchical positions was conducted as follows.

- (1) During 12 hours (day 1 and day 2) the dominance hierarchy was determined and we also collected data on the foraging trips. This period represented the control observations (no manipulations).
- (2) At the end of the second day of observation, the first female was removed, and the data on the foraging trips was collected during day 3. At the end of day 3 the second female was removed, and data collection took place during day 4. Finally, at the end of day 4 the last female was removed from the nest, and the data collection occurred during day 5.
- (3) In the first three colonies (M15, M16, and M17) only the lowest-ranked individuals were removed, whereas in the other colonies (M14, M18, and M19) only the highest-ranked individuals were removed except the queen. On the last day of observation, only two individuals were left in each colony. The colonies

TABLE 2: Dominance and subordination behaviors (% absolute number) of each individual by colony during the determination of dominance hierarchy.

Colony	Rank				
	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a
M14					
Dominance	94.6 (35)	5.4 (2)	0.0 (0)	0.0 (0)	0.0 (0)
Subordination	0.0 (0)	48.7 (18)	43.2 (16)	5.4 (2)	2.7 (1)
M15	1 ^a /Q	2 ^a /I	3 ^a /W	4 ^a /W	5 ^a /W
Dominance	93.9 (31)	6.1 (2)	0.0 (0)	0.0 (0)	0.0 (0)
Subordination	0.00 (0)	66.7 (22)	6.1 (2)	6.1 (2)	21.2 (7)
M16	1 ^a /Q	2 ^a /I	3 ^a /I	4 ^a /W	5 ^a /W
Dominance	76.1 (35)	10.9 (5)	10.9 (5)	2.1 (1)	0.0 (0)
Subordination	0.0 (0)	39.1 (18)	41.3 (19)	10.9 (5)	8.7 (4)
M17	1 ^a /Q	2 ^a /I	3 ^a /W	4 ^a /W	5 ^a /W
Dominance	90.0 (36)	10.0 (4)	0.0 (0)	0.0 (0)	0.0 (0)
Subordination	0.0 (0)	40.0 (16)	27.5 (11)	10.0 (4)	22.5 (9)
M18	1 ^a /Q	2 ^a /I	3 ^a /W	4 ^a /W	5 ^a /W
Dominance	80.0 (24)	20.0 (6)	0.0 (0)	0.0 (0)	0.0 (0)
Subordination	0.0 (0)	70.0 (21)	16.7 (5)	0.00 (0)	13.3 (4)
M19	1 ^a /Q	2 ^a /I	3 ^a /I	4 ^a /W	5 ^a /W
Dominance	100.0 (2)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Subordination	0.0 (0)	50.0 (1)	50.0 (1)	0.0 (0)	0.0 (0)
Total	1 ^a /Q	2 ^a /I	3 ^a /I	4 ^a /W	5 ^a /W
Dominance	86.7 (163)	10.1 (19)	2.7 (5)	0.5 (1)	0.0 (0)
Subordination	0.0 (0)	51.1 (96)	28.7 (54)	6.9 (13)	13.3 (5)

M15 and M16 had originally 6 individuals each; however, during the control observations, one individual of each colony disappeared by unknown causes and were not considered in the analyzes. Therefore, at the end of the manipulations in these two colonies, like in the other ones, only two individuals remained in the nest. It is also important to mention that the two different manipulations were never carried out in the same colony. We studied six colonies, and, in three of them, we followed only with the removal of higher-ranked females and in the other three we only removed the lower-ranked females.

The statistics were conducted using STATISTICA 8.0 and SAS 9.2 statistical software packages. Once we spent 12 hours collecting data during the control and 6 hours for each treatment the absolute numbers of foraging trips were converted into frequency per hour. In order to achieve normality the data was transformed using Box-Cox Transformation. We used K-S Lilliefors and Levene's statistics to test normality and variance homoscedasticity, respectively. Since the tests revealed that the data were normally distributed ($d = 0.16$, $P = 0.15$) and the variances were homogeneous ($F = 1.44$, $P = 0.26$), a one-way ANOVA was chosen to test the effects of the experimental removal of different-ranked females against control observations. We used Dunnett's t -test to perform post hoc means comparisons to a control treatment (no experimental manipulation). The same procedures were used to test the effect of the number of removals on the foraging activity of *Mischocyttarus cerberus styx*.

3. Results and Discussion

3.1. Determination of Dominance Hierarchy. The percentages of dominance and subordination behaviors of each female by each colony are shown in Table 2. Among all the females of each colony the queens performed the higher percentage (86.7%) of dominance interactions and were not subordinated by any other female of the colony. The intermediate-ranked females (2nd and 3rd individuals of the hierarchy rank) may also perform dominance interactions; however, in a much lower percentages than the queens (10.1 and 2.7%, resp.). These individuals, however, are constantly attacked by the queens (they received 51.1 and 28.7% of all subordination acts). On the other hand, the lower-ranked females (the 4th and 5th individuals in the hierarchy rank, resp.) performed no aggressive acts towards any individual of the colony and also received few dominance interactions from higher-ranked individuals (6.9 and 13.3%, resp.).

The percentages/absolute number of a set of important behaviors related to the determination of the dominance hierarchy of *Mischocyttarus cerberus styx* are shown in Table 3. According to these results the time spent in the colony by each individual is proportional to its position in the social hierarchy: queens remained the most part of the time in the nest (95% of total time observation), followed by the intermediate-ranked females (2nd and 3rd females, 78 and 70%, resp.) and the lower-ranked individuals (4th and 5th individuals, 32 and 21%, resp.).

The higher percentages of agonistic interaction received by the intermediate-ranked females (Table 2) may be

TABLE 3: Time in the nest (T) of foraging activities (F), cell inspection (C), and gaster rubbing (R) of all individuals by colony during the determination of dominance hierarchy.

	Category/ranking	T (min)	% of behaviors by colony (absolute number)		
			F	C	R
Colony M14	1 ^a	720	0.0 (0)	42.3 (11)	64.9 (24)
	2 ^a	317	35.3 (6)	19.2 (5)	27.0 (10)
	3 ^a	643	17.7 (3)	26.9 (7)	2.7 (1)
	4 ^a	137	29.4 (5)	11.5 (3)	5.4 (2)
	5 ^a	31	17.7 (3)	0.0 (0)	0.0 (0)
Colony M15	1 ^a	719	0.0 (0)	32.6 (14)	25.8 (16)
	2 ^a	619	8.3 (1)	34.9 (15)	33.9 (21)
	3 ^a	415	25.0 (3)	7.0 (3)	14.5 (9)
	4 ^a	350	33.3 (4)	11.6 (5)	17.7 (11)
	5 ^a	260	33.3 (4)	14.0 (6)	8.1 (5)
Colony M16	1 ^a	720	0.0 (0)	35.9 (14)	45.7 (16)
	2 ^a	720	0.0 (0)	10.3 (4)	25.7 (9)
	3 ^a	690	0.0 (0)	28.2 (11)	14.3 (5)
	4 ^a	298	33.3 (5)	15.4 (6)	2.9 (1)
	5 ^a	123	66.7 (10)	10.3 (4)	11.4 (4)
Colony M17	1 ^a	523	0.0 (0)	44.0 (11)	35.7 (10)
	2 ^a	423	18.2 (2)	20.0 (5)	10.7 (3)
	3 ^a	342	9.1 (1)	28.0 (7)	17.9 (5)
	4 ^a	148	45.5 (5)	4.0 (1)	7.1 (2)
	5 ^a	122	27.3 (3)	4.0 (1)	28.6 (8)
Colony M18	1 ^a	712	0,0 (0)	33,3 (0)	33,3 (4)
	2 ^a	720	0,0 (0)	25,0 (3)	25,0 (3)
	3 ^a	358	16,7 (2)	0,0 (0)	8,3 (1)
	4 ^a	216	41,7 (5)	16,7 (2)	16,7 (2)
	5 ^a	186	41,7 (5)	33,3 (4)	58,3 (7)
Colony M19	1 ^a	720	0.0 (0)	43.5 (20)	31.5 (17)
	2 ^a	586	10.0 (1)	17.4 (8)	20.4 (11)
	3 ^a	569	20.0 (2)	15.2 (7)	14.8 (8)
	4 ^a	245	20.0 (2)	6.5 (3)	3.7 (2)
	5 ^a	392	50.0 (5)	17.4 (8)	29.6 (16)
Total (%)	1 ^a	95	0,0 (0)	40,0 (74)	37,3 (87)
	2 ^a	78	13,0 (10)	21,6 (40)	24,5 (57)
	3 ^a	70	16,9 (13)	16,8 (31)	12,4 (29)
	4 ^a	32	31,2 (24)	9,2 (17)	8,6 (20)
	5 ^a	21	39,0 (30)	12,4 (23)	17,2 (40)

TABLE 4: One-Way ANOVA results for the removal of females according to the social rank (control: no experimental manipulation, manipulation 1: removal of lower-ranked females, and manipulation 2: removal of higher-ranked females).

Effects	Df	Anova SS	One-Way ANOVA		
			Mean square	F value	P
Manipulation	2	2.44	1.22	10.14	<0.001
Error	21	2.51	0.12		

Post hoc comparisons (Dunnett's t -test)

Control versus manipulation 1

Control versus manipulation 2***

***Significance at the 0.05 level.

TABLE 5: Female category, ranking position, absolute frequency of egg-laying, and oophagy behaviors observed for each colony during the observation.

	Category/ranking	Egg-laying	Oophagy
Colony M14	Queen/1 ^a	5	1
	Intermediate/2 ^a	1	2
	Intermediate/3 ^a	0	1
	Worker/4 ^a	0	0
	Worker/5 ^a	0	0
Colony M15	Queen/1 ^a	2	1
	Intermediate/2 ^a	1	1
	Worker/3 ^a	0	0
	Worker/4 ^a	0	0
	Worker/5 ^a	0	0
Colony M16	Queen/1 ^a	2	0
	Intermediate/2 ^a	0	0
	Intermediate/3 ^a	2	2
	Worker/4 ^a	0	1
	Worker/5 ^a	0	0
Colony M17	Queen/1 ^a	1	1
	Intermediate/2 ^a	0	1
	Worker/3 ^a	0	0
	Worker/4 ^a	0	0
	Worker/5 ^a	0	0
Colony M18	Queen/1 ^a	0	0
	Intermediate/2 ^a	0	0
	Worker/3 ^a	0	0
	Worker/4 ^a	0	0
	Worker/5 ^a	0	0
Colony M19	Queen/1 ^a	3	1
	Intermediate/2 ^a	0	0
	Intermediate/3 ^a	1	1
	Worker/4 ^a	0	0
	Worker/5 ^a	0	0

explained by the behavioral role displayed by these females in the social scenario. Intermediate-ranked females exhibit typical dominance behaviors spending a large amount of the time in the nest performing relatively high percentages of cell inspection (21.6 and 16.8%, resp.) and gaster rubbing (24.3 and 12.4%, resp.) (Table 3). In colony M16 the queen and the 2nd ranked female remained in the nest during all the observation period, and in colony M18 the 2nd ranked female spent even more time in the nest than the queen (Table 3). Although these females remain a large amount of the time in the nest, as the queens, they typically occupy different positions in the comb. While the queens occupy mostly the front face of the comb right above the pupae, the intermediate-ranked females typically rest at the back face of the comb; such differences in the nest position of the different ranked females were also observed in *Polistes canadensis* [8]. The intermediate-ranked females

also contribute to the foraging activity (13 and 16.9%, resp.) of the colony, and only in colonies M16 and M18 there are no records for food collection by these females. On the other hand, the lower-ranked individuals spent more time in the field collecting prey and liquids and performed a higher number of foraging trips/hour (31.2 and 39%, resp.).

According to the behavioral repertoire showed by the females which occupied the 2nd and 3rd positions in the social rank they cannot be exactly classified as nonworkers of *Polistes fuscatus* [8] since they also contribute to the food intake of the colony and perform behaviors highly associated with dominance (e.g., cell inspection and gaster rubbing) (Table 3). Gadagkar and Joshi [18] found three different categories of females based on behavioral repertoire: sitters, fighters and foragers. Sitters were represented by the queens and other non-egg-laying females which did little or no foraging and rarely exhibited defense behavior. The author stated that these non-egg-laying females could represent replacement queens or naive workers. Table 3 showed that the intermediate-ranked females (2nd and 3rd positions) were responsible for a considerable portion (29.9%) of the total foraging trips recorded in this study, different than what was found for the nonworkers of *Polistes fuscatus* and for the sitters of *Ropalidia marginata* [18, 22]. In this context, the “sitters” are more likely to be compared to the dominant females of *M. cerberus styx* and the intermediates to the “fighters” of *Ropalidia marginata*. However, we observed that the intermediate females attacked each other much less frequently than was found among the “fighter” of *Ropalidia marginata*, and the dominant females were considerably more aggressive towards the intermediates than were the “sitters” against the “fighters.” As stated by Gadagkar and Joshi [18] the “fighters” could also reach the status of an egg-layer if the colonies of *Ropalidia marginata* become large and polygynous or if the queen disappears. In fact, Table 5 showed that the intermediate-ranked females and the queen could be competing for reproduction through differential oophagy. In this context, in the absence of queens (due to natural causes, such as predation or senescence and death) the intermediate females of *M. cerberus styx* would have more chances to assume the post of principal egg-layer than typical foragers, as occurring in *Polistes fuscatus* and *Polistes canadensis* [8]. Such comparisons indicate that, despite some similarities in the social organization of *Polistes*, *Ropalidia*, and *Mischocyttarus*, the different ranked females behaves differently according to the taxa and generalizations on the social roles of each group of females are difficult, especially if these groups of wasps are commonly compared in different regions and climates and are commonly under different environmental constrains. Similar results to these found in the present study were also found by Murakami [19], Murakami and Shima [9, 11], and Murakami et al. [12] in *Mischocyttarus cassununga*.

Based on the results showed above the females of *M. cerberus styx* were classified as follows.

- (1) Queen: it spends most of her time in the comb and occupies the front face of the comb and usually rests right above the pupae; it is the first female to request

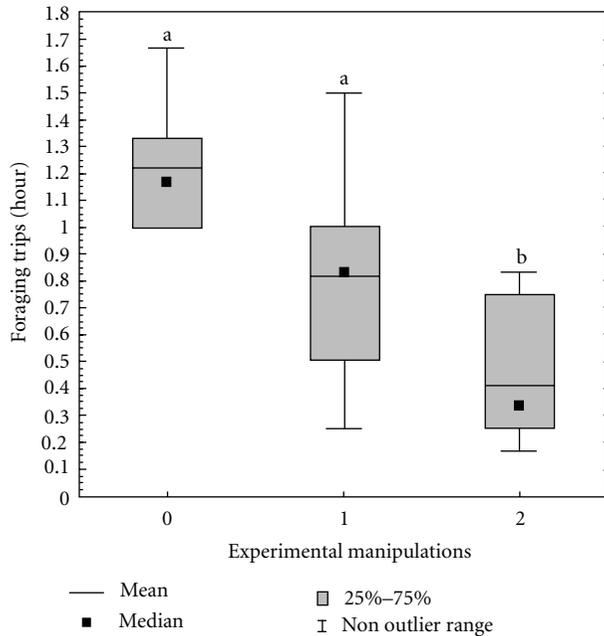


FIGURE 1: Box-plots indicating the means, median, 25th and 75th percentiles and non-outlier range of the data set used to compare the effect of the removals of females according to the hierarchical position. When compared with the control (0), only the removal of higher-ranked females decreased significantly the foraging activity of *Mischocyttarus cerberus styx* (0: no manipulation; 1: removal of lower-ranked females and 2: removal of higher-ranked females). Statistical significance at the level of 0.05.

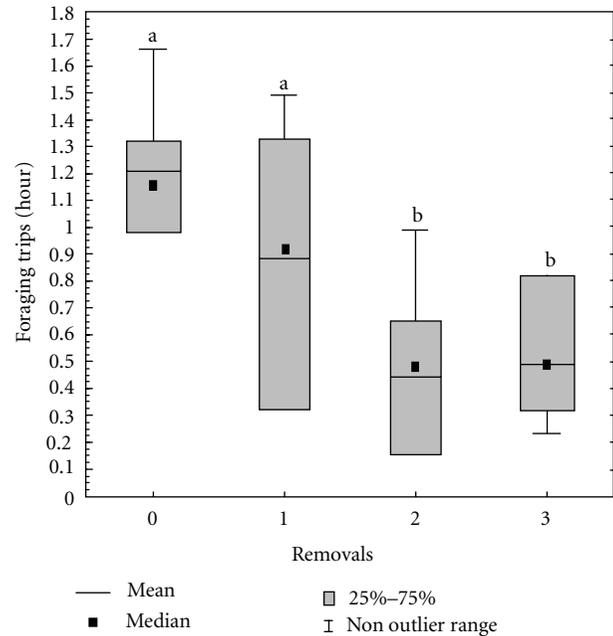


FIGURE 2: Box-plots indicating the means, median, 25th and 75th percentiles and non-outlier range of the data set used to compare the effect of the number of removals regardless the female hierarchical position. When compared with the control (0), the removal of 2 or 3 females, regardless of their social position, decreased significantly the mean number foraging trips/hour of *Mischocyttarus cerberus styx* (0: no manipulation; 1: removal of 1 female; 2: removal of 2 females; 3: removal of 3 females). Statistical significance at the level of 0.05.

food from foragers; it is usually the most aggressive individual of the colony.

- (2) Intermediate females: they spend most of their time resting in the back side of the comb and usually occupy the 2nd or 3rd positions of the social rank. They present intermediary behavior between queens and foragers: they perform high frequencies of cell inspection and gaster rubbing, as the queens [16]; however, they also forage. These female may also lay eggs, even in the presence of the queen (Table 5).
- (3) Workers or foragers: they spend most of their time in the field collecting food and liquids.

3.2. Experimental Manipulations. The results showed that both experimental removals of individuals decreased the foraging activity of *Mischocyttarus cerberus styx* (Table 4; Figure 1). However, the effects proved to be statistically significant only when higher-ranked females were removed. No statistical difference was found when lower-ranked females were taken away (Table 4; Figure 1). Although the gradual removals of the females could create a dilutive effect during the manipulations, the entire experiment was conducted in the very same conditions (the two different treatments were never carried out in the same colony, the same number of removals were performed in each colony, and the colonies were analyzed in the same phase of development and with

the same number of individuals), ensuring the consistence of the collected data. Furthermore, as cited in Section 2 the abrupt removal of females led to the abandon of the colony in 100% of attempts, colonies M10, M11, M12, and M13. Most importantly is that the social hierarchy system showed by this species may explain these results.

According to Silva [17], females that occupy the second and third positions in the hierarchical ranking usually exhibit the intermediate behavior. As previously described, these females remain most of their time resting in the back side of the comb performing dominance behaviors and also contributing to foraging activity (Table 3). We found that these females competed with the queens for the reproductive control of the colony, either by oophagy or by laying eggs in empty honeycomb cells (Table 5). Unlike the queen's substitutes described by Litte [13] the intermediate females of *M. cerberus styx* may lay eggs even in the presence of the queen.

This intermediate behavior may provide to these females the possibility of saving energy, which would be primarily spent on foraging trips and other tasks. This individual would have a much higher chance of assuming the position of an egg layer than a regular forager. Actually, in 5 out of the 6 colonies observed (M14, M15, M16, M17, and M19) we recorded oophagy by individuals that were not the queen, and in 4 of them (M14, M15, M6, and M19)

TABLE 6: One-Way ANOVA results for the removal of females regardless of their social rank (control: no manipulation; treatment 1: removal of 1 female, treatment 2: removal of 2 females, and, treatment 3: removal of 3 females).

Effects	Df	Anova SS	One-Way ANOVA		
			Mean square	F value	P
Removal	3	1.61	0.54	5.68	0.0056
Error	20	1.89	0.10		
Post hoc comparisons (Dunnett's <i>t</i> -test)					
Control versus 1 ^r removal					
Control versus 2 ^r removal***					
Control versus 3 ^r removal***					

***Significance at the 0.05 level.

we observed more than one individual laying eggs (Table 5). These behaviors were considered as an indicative of intense competition among females because, in most cases, before the oviposition, the intermediate female fed on the egg previously laid in the same cell, even with several empty cells available in the nest.

In the situation that intermediate females (typically the 2nd and 3rd females of the social rank) were removed experimentally, a typical forager assumed the post of intermediate immediately after the removal. Thus, besides the removal of an intermediate female, which also contributes to food collection (Table 3), there was also the loss of a typical forager which began to remain more time in the nest and to forage less. This dramatically reduced the daily number of food trips in the colonies observed (Figure 2). These results demonstrate the importance of the maintenance of social hierarchy in colonies of *Mischocyttarus cerberus styx*. According to Strassmann and Meyer [22], the maintenance of dominance hierarchy reduces considerably the conflicts for queen replacement when she disappears.

The removal of lower-ranked females did not affect the amount of foraging trips statistically (Table 4, Figure 1). As the dominance hierarchy was not disrupted, no rearrangement in the dominance rank was necessary and the remaining foragers continued food collection normally. Only when most of foragers were removed the intermediate females started to behave as typical foragers, spending more time collecting, and this may explain the reason that the removal of foragers did not affect significantly the foraging activity of *M. cerberus styx*.

O'Donnell [15] showed that the removal of foragers of *Polistes instabilis* decreased the rate of foraging activities, but it also resulted in the recruitment of new individuals to carry out the tasks affected. Robinson [23] argued that colonies of social insects respond to intrinsic and environmental changes by adjusting the ratio of working force allocated in the different tasks. Although *Mischocyttarus cerberus styx* have shown some flexibility when all foragers were removed, it is very unlikely that females recruitment occurs in this species, since it has a small population and may not have enough individuals to be reallocated for different tasks. Thus, the loss of few individuals in this species may prevent colonies to continue developing. In fact, our data showed that the removal of 2 or 3 individuals, regardless of their social position, decreased significantly the number of foraging

trips/hour (Table 6; Figure 2). To perform such analysis we isolated the effect of the removals based on the hierarchical position, since the prime objective here was to investigate the critical number of removals which would affect significantly the number of foraging trips/hour in *M. cerberus styx*. The results obtained were not a surprise for this species since it has a few individuals per colony and a single female in a colony of 5 nest mates representing 20% of the total population and 2 or 3 individuals representing respectively, 40 and 60% of the entire colonial population.

Finally, we concluded that (1) the foraging activity of the colonies of *Mischocyttarus cerberus styx* is more sensitive to the removals of higher ranked females than foragers since such treatment caused the disruption of the social hierarchy and forced a rearrangement in the social roles of females decreasing significantly the number of foraging trips/hour; (2) in this species, no evident recruitment of new individuals to perform foraging trips was observed after the experimental manipulations, possibly because of the small colonial population in this species; (3) the removal of 2 or 3 individuals, regardless the social rank, could bring serious implications to the food intake of the colony as it affected significantly the number of foraging trips/hour. In fact, our preliminary tests showed that the abrupt removal of at least 50% of the colony individuals led to the abandon of females preventing the colony from continuing developing.

This study represents the first step towards a better understanding of how the eusocial basal *Mischocyttarus* deals with internal conflicts and how these colonies adapt themselves to new social scenarios. Moreover, we approached for the first time the existence and probable role of intermediate females in neotropical basal eusocial wasps.

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Research Article

Sequential Load Transport in Grass-Cutting Ants (*Atta vollenweideri*): Maximization of Plant Delivery Rate or Improved Information Transfer?

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Sequential transport of plant fragments was studied in the grass-cutting ant *Atta vollenweideri*. Two competing hypotheses concerning its occurrence were tested. Based on the “economic-transport hypothesis,” sequential transport occurs because of a mismatch between load size and ant body size, and it is therefore considered a way to improve size-matching and so the plant delivery rate. Alternatively, the “information-transfer hypothesis” states that sequential transport improves the information flow during foraging. By transferring its load, a worker may return earlier to the foraging site so as to intensify chemical recruitment. To distinguish between these two competing hypotheses, standardized paper fragments that differed either in size or in quality were presented to workers of a field colony, and sequential transport was quantified. Neither an increase in fragment mass nor in fragment length influenced the occurrence of transport chains. Sequential transport took longer than transport by a single carrier. However, the occurrence of sequential transport increased with increasing fragment quality. High-quality fragments were transferred more frequently and after shorter distances than less-attractive fragments. Taken together, these results strongly support the hypothesis that sequential load transport has been favoured during evolution because of an improvement in the information flow during foraging.

1. Introduction

Ant colonies are highly organized societies without central control, yet with mechanisms that permit workers to respond to colony needs, and consequently engage in different task required for colony function such as foraging, nest construction, and brood tending. In addition to simultaneously coordinated activities such as cooperative food transport and resource defense [1–3], ants also show serially organized work, in which a given task is partitioned into two or more sequential stages, for instance, when a food item or building material is passed consecutively from one worker to the next. Several investigations have highlighted details on sequential processing of material not only in ants [4–8], but also in wasps [9], bees [10, 11], and humans [12]. For social insects, in general, sequential transport was described mostly in the

context of foraging, but also during nest building [13] and waste transport [14].

Compared to a nonsequential mode, sequential material processing has several advantages, for instance, a decrease in both the time and energy required to perform the activity [12, 15, 16], which may result from a more efficient individual performance, or an improved co-ordination between individuals [9]. A sequential transport of collected material implies that the task is partitioned among different workers, and linked by material transfer. Task partitioning is defined as a process in which one task is split up between different worker groups, in contrast to division of labor in which different tasks are performed by different worker groups [9, 13, 16, 17].

Leaf-cutting ants of the tribe Attini show both division of labor [18, 19] and task partitioning to an extraordinary

extent [20], including different contexts such as foraging [4, 21], trail construction [22], or waste disposal [23]. Sequential load transport in leaf-cutting ants, involving both direct and indirect (via dropping) load transfers, has repeatedly been reported in the literature [4, 5, 21, 24–28], although the possible adaptive value of such transportation schemes remains unclear [29].

The question arises why loaded leaf-cutting ants decide to transfer their fragments on their way to the nest, and what are the advantages of a sequential load transport. Fragments might be dropped or transferred if a minimum transport speed is not met by the carrier [28]. Although it seems conceivable that loads carried particularly slowly may eventually be abandoned by the carrier, a low travel speed does not necessarily indicate that a worker is not capable of carrying the load, or of walking faster [30, 31]. Travel speed may be reduced because of trail-marking activity by the carriers. Or workers may slow down because they try to pass the carried fragment to an unladen nestmate, and not because of the burden, thus being able to return to the source after unloading [5].

It is conceivable that sequential load transport in leaf-cutting ants may have been favoured because of a faster load delivery rate. These arguments are the core of the so-called “economic-transport hypothesis” [25]. It should be noted that “economic” in this context refers to the maximization of the transportation speed of a leaf fragment [32, 33], which at the colony level may result in an increased overall rate of resource delivery. In fact, maximization of leaf transportation has been proposed as the adaptive value of sequential transport in three leaf-cutting ant species that transfer loads or cache fragments on the ground [4, 5, 28]. In *Atta colombica*, direct transfer of leaf fragments between workers indeed resulted in a higher transportation speed after transfer, but it occurred in only 9% of the transported fragments. However, transferred fragments did not travel faster than those not transferred [28]. In another study on the same species, fragments recovered from a cache were also transported back to the nest more slowly than singly-foraged leaf fragments, leading the authors to argue that “leaf dropping, and, therefore, the switch to task partitioning is not in itself adaptive” [26]. In the grass-cutting ant *Atta vollenweideri*, transport time of single fragments carried sequentially was 25% longer, in average 8 min longer, than that of fragments carried to the nest by single workers along a 28 m-trail [25]. Thus, in terms of foraging time and material transport rates, sequential load transport in *Atta vollenweideri* was less efficient than transport by single carriers.

Based on these results, an alternative hypothesis was advanced to account for the occurrence of sequential transport in grass-cutting ants [25]. It was argued that sequential transport was favoured during evolution as a way to enhance the information flow among foragers, thus leading to a quicker buildup of workers at particular harvesting places, and to an increased overall rate of resource transportation. These arguments are embodied into the “information-transfer hypothesis” [34–36], which states that at newly discovered food sources, foraging ants compromise their

individual transport rates of material in order to return early to the colony for information transfer. Foragers’ performance as food carriers is, therefore, reduced, but the colony as a whole increases its harvesting rate due to the workers that gained information and participate in the collective gathering activity [29, 31]. Based on the information-transfer hypothesis, the behavioural response of dropping or passing fragments in the grass-cutting ant, *Atta vollenweideri*, may have been selected for because of its positive effect on the information flow, rather than because of an improvement in the economics of load-carriage [25]. The importance of information transfer seems apparent when the colony-wide foraging patterns of leaf-cutting ants are considered. Foraging trails can exceed 100 m in length [18, 37] and are characterised by strong branching into several side trails. Thus, outgoing workers have to choose between several bifurcations leading to different food patches. Sequential load transport could enhance information transfer in several aspects. By dropping a load at a trail bifurcation, successful workers may be able to return to the foraging site earlier following a freshly deposited pheromone trail and to chemically reinforce that trail sector much stronger than if they walk all the way to the nest, thus enhancing recruitment. Furthermore, fragments dropped on the trail, or being carried along it, may themselves act as information cues about what plant is currently harvested [38–40].

Up to now, both the information-transfer and the economic-transport hypotheses remain at the descriptive level, as no predictions of one of them have been experimentally addressed. For instance, if sequential load transport speeds up leaf transport, it should be expected to occur when the transporting ants move too slowly because of their burden, for example, when ants carry relatively large fragments. Based on the information-transfer hypothesis, sequential load transport is expected to occur more frequently under conditions in which information is worth transferring, for instance, upon the discovery of high-quality resources or when the colony is starved [31, 36, 41]. Grass-cutting ants provide a particularly well-suited system for studies of sequential transport during foraging because ants harvest monocotyledonous plants near the ground [42], so that the whole process of cutting at the source until reaching the nest can be observed and experimentally manipulated.

The aim of the present study was to investigate the variables that trigger sequential load transport in the grass-cutting ant *Atta vollenweideri*, and to test predictions of the two competing hypotheses presented above. Based on the arguments of the economic-transport hypothesis, fragment dropping (or transfer) by foragers occurs because of a mismatch between load size and body size at the individual level. Chains are, therefore, considered as a way to maximize the delivery rate of the collected loads. The probability of occurrence of sequential transport would, therefore, be expected to strongly depend on fragment size, and not necessarily on fragment quality, and should be higher for larger fragments that are difficult to carry. Alternatively, the information-transfer hypothesis states that the behavioral response of transferring fragments has been selected for because of its positive effect on the information flow

during a foraging process. This hypothesis predicts that the formation of a transport chains should strongly depend on fragment quality rather than on fragment size. To distinguish between the different predictions, workers from an *Atta vollenweideri* field colony were presented with standardised paper fragments that differed either in size or in quality. The occurrence of transport chains was quantified by following marked grass fragments all their way to the nest and by recording when, where, and how fragments were transferred between carriers.

2. Methods

Field experiments were conducted at the biological field station of the “Reserva Ecológica El Bagual” in Formosa province, Chaco region of north Argentina, on a large mature colony of *Atta vollenweideri*. A single large colony was used because field colonies in the area varied greatly in size and worker-size distribution, making a standardization of size matching between load and worker sizes difficult to achieve. Ant foraging activity was strictly nocturnal, so that headlamps covered with a red filter were used for observations. Foragers showed no signs of disturbance because of the light.

The effects of load size on the occurrence of sequential transport were investigated on a natural trail of approximately 50 m length, at the end of which ants harvested a variety of grass species. Foraging ants were offered paper fragments of three different sizes, which were placed in the middle of the trail, at 26 and 33 m from the nest, over eight consecutive nights. Fragment sizes were chosen so as to separate the effects of load length and load mass on transport speed. Fragments differed either in length or in mass, but not in width, which was held constant at 3 mm, as follows. “Short” fragments were 15 mm long and weighed in average 4.25 mg. “Long” fragments were 30 mm long with an average mass of 8.5 mg. Finally, “double” fragments were made by sticking two wet fragments together, forming a short double fragment of 15 mm in length, and an average mass of 8.5 mg. It is known that for fragments of similar mass, fragment length had a marked negative effect on manoeuvrability during transport and, as a consequence, on material transport rate [43, 44]. Fragments were cut out of standard paper (80 g/m²), soaked with orange juice for at least one hour, and then dried. In order to increase the acceptance of the paper fragments presented on the trail, we additionally placed an artificial “paper plant” 20 cm beside the main trail. It was created by soaking paper stripes of 15 cm length in orange juice, and by putting the stripes into a small plastic vial that was “planted” on the ground, as previously described [25]. Ants readily cut fragments from the paper plant and dropped them on the trail as observed for natural grass plants. During the measurements, however, only the offered, previously cut fragments were monitored. Such fragments were identified with pencilled marks and placed on the trail. After retrieval by workers, fragments were followed all their way to the nest. The occurrence of sequential transport, the transport time, the transport distance by each involved worker, as well as the “waiting

times” of fragments, that is, the time a fragment was left on the trail before being retrieved by another worker, were recorded for each individual fragment. For those fragments transported sequentially, the total transport time included travel time, handling time by the foragers, and waiting times of the fragment. The workers involved in the sequential transport were collected after having passed their loads, and weighed (wet mass) at the nearest 0.1 mg.

The effect of food quality on the occurrence of sequential transport was investigated by presenting ants on a foraging trail, as described above, with paper fragments of constant size but different quality, as follows. Paper fragments were previously added either with pure orange juice (henceforth “orange fragments”), with a solution of 15% tannin in orange juice (henceforth “tannin-orange fragments”), or with a solution of 10% tannin in water (henceforth “tannin-water fragments”). Tannin is a natural plant secondary compound that has been shown to negatively influence leaf-cutting ant foraging and to inhibit the ant symbiotic fungus [45–47]. Fragments impregnated with these solutions are, therefore, expected to differ in quality and vary in their acceptance by the ants. Differences in acceptance were measured prior to the experiments by presenting simultaneously one fragment of each quality on the trail at 20 m from the nest, and by recording which one was taken first (see results). A trail that bifurcated into two branches at 31 m from the nest was used for the quality experiments. On the two arms, at 33 m from the nest, ants were presented with fragments of a given quality, that is, two different fragment qualities could be tested each experimental night. Initially, orange and tannin-orange fragments were offered over four consecutive nights, with the side of presentation alternating each night to control for potential side effects. In the following two nights, ants were presented in the same manner with orange fragments and tannin-water fragments, but due to methodological difficulties, only tannin-water fragments could be followed. All fragments were 20 mm long, 3 mm wide, and averaged 9 mg in mass. Again, “paper plants” of the respective quality were presented 20 cm beside the trail, yet only the experimental, previously cut fragments were followed. A total of 41 orange, 42 tannin-orange, and 35 tannin-water fragments were followed all the way to the nest.

3. Results

3.1. Behaviour of the First Carriers. The occurrence of sequential transport strictly depends on the behaviour of the first worker that collected a fragment. Sequential transport occurred when this “first carrier” transferred its fragment directly to an outgoing nestmate that then turned back and run loaded to the nest, or when the first carrier dropped the fragment on the trail, and it was collected and further carried by another worker. Single first carriers were gently marked with a small dot of Edding 780 paint marker while collecting one orange paper fragment close to the “paper plant”, on a side trail 1 m after a trail bifurcation, and its behaviour monitored ($n = 16$ carriers were observed). Observations lasted at least 45 to 60 min, or until the carrier

entered the nest. All first carriers were observed to continue foraging at the location where they had initially collected their first paper fragment, to deposit the fragment after a given distance, and to retrieve at least one further fragment. Most of them collected one or two additional fragments prior to entering the nest and were possibly at the end of their daily foraging period. Seven workers collected more than five further fragments and started to walk back once more to the patch during the observation time. Thus they were still within their foraging period when they were caught (Figure 1). Seven carriers returned to the nest during the observation time, and only one worker switched to another foraging site and continued foraging but collected small natural sticks.

Most first carriers did not cut any fragments, with the exception of two workers that cut one fragment each out of the paper plant after having collected several paper fragments. Regarding the load transfers, 36% of them occurred when the carrier reached the main trail. For those that continued to walk along the main trail, there was no preferred location for passing or dropping the fragments, that is, workers did not walk a fixed distance before fragment transfer. Even the distances covered by individuals that retrieved several fragments varied considerably. There was neither a relationship between ant body size and number of fragments retrieved (Spearman Rank Correlation Test: $y = 2.9 + 0.05x$, $r^2 = 0.02$, $R = 0.2$, $n = 16$, $P = .6$, NS) nor with the walking distance before fragment transfer ($y = 2.4 - 0.008x$, $r^2 = 0.002$, $R = 0.06$, $n = 68$, $P = .7$, NS).

3.2. General Description of Sequential Transport. Most transport chains consisted of two or three carriers, but occasionally up to five foragers were involved. When not directly transferred, fragments were dropped in the middle of the trail. Ants neither preferred certain places on the trail for dropping fragments, nor did they build up piles or caches at a given location. Dropped fragments attracted unladen foragers and were readily collected. Waiting time of fragments dropped by first carriers were significantly shorter than that of fragments deposited by us with forceps, suggesting that previously transported fragments had some chemical marks as a consequence of their handling by workers (waiting times of fragments deposited by first carriers, all fragment sizes pooled, mean \pm SD: 44 ± 88 sec, $n = 114$; untouched fragments: 139 ± 166 sec, $n = 141$; U -test: $U = 2412$, $Z = 7.2$, $P < .001$).

With regard to the mode of fragment transfer, 29% (range 14–54%) of all fragments were transferred directly between the first and second carrier, that is, most of the transfers were indirect, via dropping. The ratio of direct transfers to the total number of transfers was independent of both fragment size and food quality (Chi-Square-Test: $P > .05$ for all pairs).

Based on direct observations, it was difficult to reveal what variables triggered a direct transfer between workers. In some cases, the first carrier was observed to reduce its walking speed and to move very slowly until a nestmate approached and took the fragment. In other cases, the carrier kept approaching unladen nestmates coming from

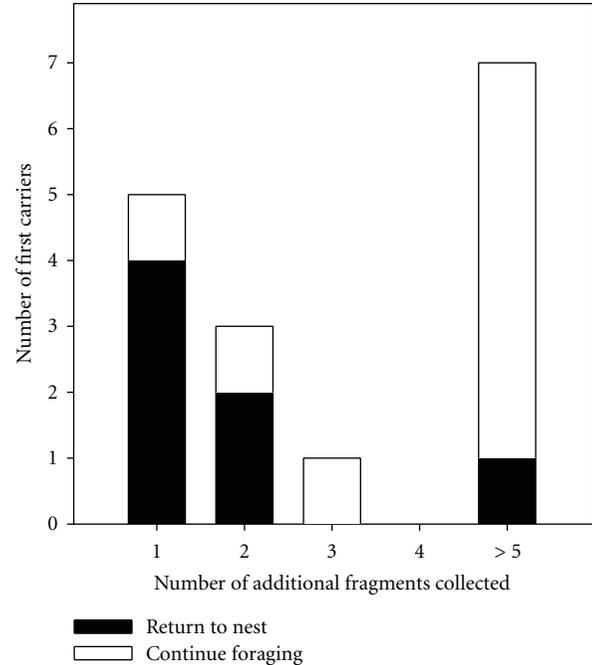


FIGURE 1: Number of additional fragments collected by the first carriers in a transport chain after the first fragment was dropped or directly transferred. Black bars indicate foragers that returned to the nest with a fragment during the observation time. White bars indicate foragers that continued to forage and returned to the patch at the end of the observation time.

the nest, whereby it rather meandered along the trail instead of walking straight ahead towards the nest. Additionally, unladen nestmates were observed to approach the first carrier, to antennate the fragment and then to take it. Several times, the two ants were observed to struggle for one or two minutes until one of them took the fragment.

3.3. Fragment Size and Sequential Transport. The probability of occurrence of sequential load carriage was independent of the size of the transported fragments (Figure 2). Fifty-five percent of the short fragments ($n = 47$), 69% of the double ($n = 52$), and 57% of the long fragments ($n = 46$) were carried sequentially, these figures being not statistically different (Chi-Square-Test: $P > .2$).

As *Atta* workers are highly polymorph, the body size distribution of the different task groups (single, first, and last carrier within a transport chain) was compared, in order to elucidate whether the position along a transport chain was influenced by body size (Table 1). For all fragment types presented, the first carriers in a transport chain were smaller than the last carriers. First carriers were also smaller than single carriers, that is, those that transported the fragments all the way to the nest, for long and short fragments, but not for double ones. Body size of single and last carriers did not differ statistically. Furthermore, first carriers of long fragments were larger than first carriers of short fragments ($H_{1,52} = 10.9$, $P < .005$), and the same was true for last ($H_{1,50} = 9.7$, $P < .005$) and single carriers

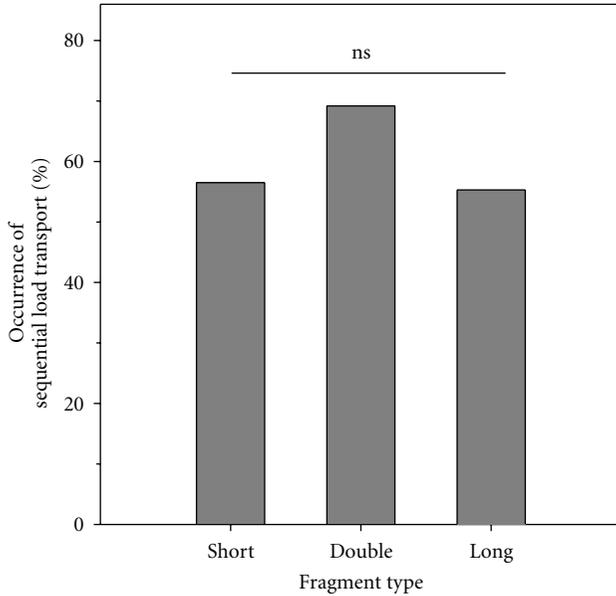


FIGURE 2: Occurrence of sequential load transport when ants were offered paper fragments of different size but similar quality (see text for Chi Square statistics).

($H_{1,39} = 6.4, P < .05$). Thus, the size of the carriers in a transport chain depended on both their position within it and the size of the carried load.

The distance each fragment was carried by the first carrier until transfer did not depend on fragment size (Figure 3; mean \pm SD: short: 8.2 ± 7.2 m; double: 7.0 ± 6.0 m; long: 11.9 ± 9.9 m; Kruskal-Wallis-Test: $H_{2,88} = 2.9, P = .2, NS$). Transfer distance was also independent of ant body size: for short and long fragments, a relationship, though not significant, between body mass of first carriers and walked distance was found, but the sign of the correlation differed between the two groups. No correlation was found for the double fragments (Spearman Rank Correlation Test: long: $y = 1.0x - 0.6, R = 0.39, t = 2.09, n = 26, P = .05, NS$; short: $y = -0.3x + 10.5, R = -0.41, t = -2.02, n = 24, P = .06, NS$; double: $y = -0.2x + 9.9, R = -0.24, t = -1.41, n = 34, P = .2, NS$).

The transport time of fragments carried sequentially along 33 m was significantly longer than that of fragments carried by a single carrier all the way through, with differences ranging from 9 to 18 min (Figure 4, mean \pm SD; short fragments: 30 ± 18 min (single carrier), 44 ± 23 min (transport chain), $U = 103, Z = 3.5, P < .001$; double fragments: 32 ± 9 min (single carrier), 41 ± 14 min (transport chain), $U = 174.5, Z = 2.3, P < .05$; long fragments: 31 ± 11 min (single carrier), 49 ± 27 min (transport chain), U -test: $U = 86.5, Z = 4.0, P < .0001$).

3.4. Fragment Quality and Sequential Transport. The fragments of three different qualities initially presented as a choice on the trail were indeed ranked by the ants. Workers took first the orange fragments in 56% of the cases, the tannin-orange fragments in 28%, and the tannin-water

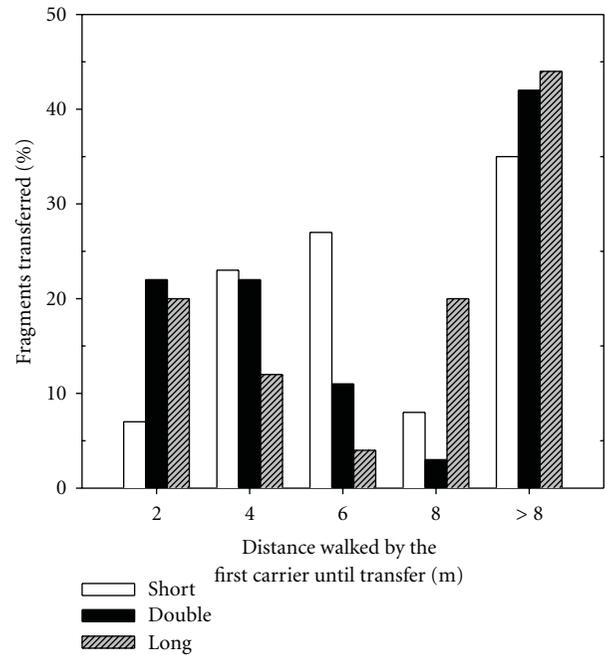


FIGURE 3: Distance at which first carriers in a transport chain transferred their fragments, for the three different fragment sizes assayed. Bars indicate how many loads were transferred within a distance category (bin width 2 m). Fragments were offered and collected at “0 meters,” and 100% refers to all transferred fragments of a given size. Trail length was 33 m. Transport distance for loads of different size did not differ significantly (statistics in text).

fragments in 16% of the cases (Chi-Square Test, $P < .05$ for all pairs, $n = 39$). Thus, orange fragments were clearly the most preferred ones.

Sequential transport occurred significantly more often for the most preferred fragments (orange) than for the two others (Figure 5), that is, 81% of the orange fragments ($n = 41$), 57% of the tannin-orange fragments ($n = 42$), and 40% of the tannin-water fragments ($n = 35$) were carried by transport chains (Chi-Square Test: orange versus tannin-orange: $P < .05$; orange versus tannin-water: $P < .0005$; tannin-orange versus tannin-water: $P = .2, NS$). These values correspond to all fragments presented over the entire experimental period.

Fragment quality also affected the distance the first carrier walked before transferring the carried fragment. The highly attractive orange fragments were transferred after a significantly shorter distance than the less attractive tannin-orange fragments (Figure 6; mean \pm SD; orange: 6.1 ± 7.2 m; tannin-orange: 12.6 ± 9.9 m; $H_{1,57} = 7.6, P < .01$). Thus, highly attractive fragments were transferred more often (Figure 5) and after shorter distances (Figure 6) than less attractive fragments. In addition, if only the direct transfers are considered, the place where they occurred significantly depended on the fragment quality. Orange fragments were directly transferred after distances much shorter than those at which tannin-orange fragments were directly transferred (mean \pm SD: orange: 6.9 ± 5.5 m, $n = 8$; tannin-orange: 21.2 ± 6.2 m, $n = 6$; $H_{1,14} = 7.4, P < .01$). As for the fragments

TABLE 1: Body mass (mg) of carriers in a transport chain and single carriers that transported fragments of different sizes: short, double or long fragments. Data are means \pm SD; N numbers in brackets. Comparisons after Kruskal-Wallis-ANOVA.

	Short fragments	Double fragments	Long fragments
First carrier	8.7 \pm 4.5 (26)	11.4 \pm 6.1 (35)	12.7 \pm 4.2 (25)
Last carrier	13.7 \pm 9.3 (25)	15.5 \pm 7.1 (36)	19.3 \pm 10.7 (26)
Single carrier	12.1 \pm 4.0 (18)	14.0 \pm 7.6 (16)	16.7 \pm 5.1 (21)
Kruskal-Wallis-ANOVA:	$H_{(2,69)} = 12.1$ $P < .005$	$H_{(2,87)} = 7.7$ $P < .05$	$H_{(2,72)} = 10.3$ $P < .01$
Paired comparisons:			
First vs. Single	$H_{(1,44)} = 8.4$ $P < .005$	$H_{(1,51)} = 1.4$ NS	$H_{(1,47)} = 5.8$ $P < .05$
First vs. Last	$H_{(1,51)} = 8.8$ $P < .005$	$H_{(1,71)} = 7.2$ $P < .01$	$H_{(1,51)} = 8.8$ $P < .005$
Single vs. Last	$H_{(1,43)} = 0.002$ NS	$H_{(1,52)} = 1.8$ NS	$H_{(1,46)} = 0.3$ NS

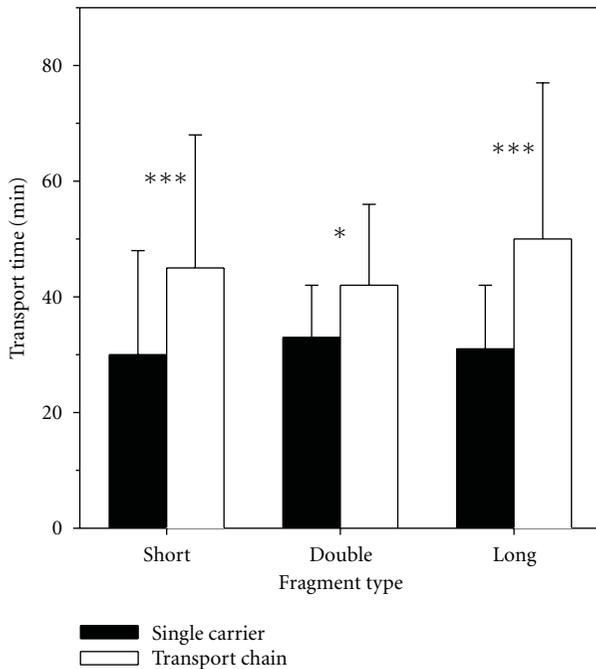


FIGURE 4: Transport time (mean \pm SD) of fragments transported by one carrier all the way to the nest (black bars) or by a transport chain (white bars). Transport time includes handling time of foragers and waiting times of a dropped fragment until transport was continued (statistics in text).

of different size, the distance walked by the first carriers until the transfer was independent of their body mass (Spearman Rank Correlation Test: orange: $R = -0.16$, $n = 32$, $P = .4$, NS; tannin-orange: $R = -0.38$, $n = 23$, $P = .07$, NS).

3.5. Load Transport in Other Contexts: Collection of Dry Plant Fragments and Building Materials. Under natural conditions, not only freshly cut grass fragments are transported back to the nest along foraging trails, but also fallen, dry plant

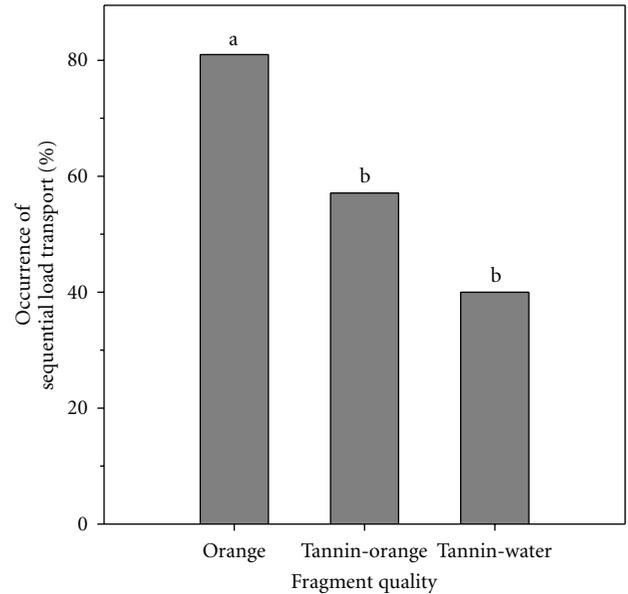


FIGURE 5: Occurrence of sequential load transport when ants were offered paper fragments of similar size but different quality. Bars sharing the same letter are not statistically different (see text for Chi-Square statistics).

fragments and twigs used as building materials to stabilize the nest structure. Such alternative scenarios allow the analysis of sequential transport in contexts other than the foraging one and may shed light on the benefits provided by a sequential load transport in more general terms. Detailed observations of fragment transport were performed at the study site both after a prolonged dry season, when colony foraging was reduced and workers mostly collected fallen plant fragments, and later after heavy rains, when workers moved along the trail and collected twigs, fallen fragments and even soil crumbs that were dropped around the nest entrances as building materials [48].

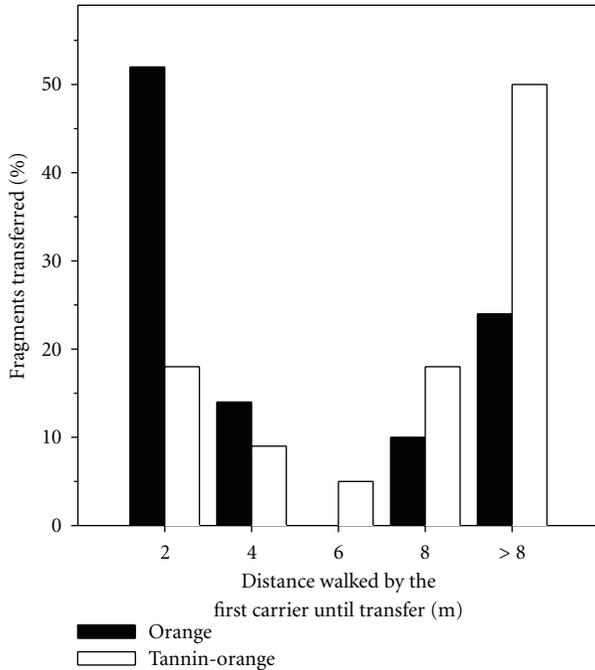


FIGURE 6: Distance at which first carriers in a transport chain transferred their fragments, for the different fragment qualities assayed. Bars indicate how many loads of a specific quality were transferred within a distance category (bin width 2 m). Fragments were offered and collected at “0 meters,” and 100% refers to all transferred fragments of a given quality. Trail length was 33 m. High-quality fragments were dropped at a significantly shorter distance than low-quality fragments (statistics in text).

When collecting fallen, dry plant fragments, workers moved in small numbers on the trails (in average 7 loaded ants per minute), and laden ants were observed to carry their loads all the way to the nest, along a 15 m trail. No sequential transport of loads occurred ($n = 45$ fragments were followed). Although no direct comparisons with highly active colonies could be made, a previous study reported that 36% of the fragments harvested from the sedge *Cyperus* (and 16% of the collected paper fragments) at 10 m from the nest were transported sequentially [25]. After heavy rains, workers collecting wet plant fragments and twigs as building materials, 11 m away from the nest, transported them all the way to the nest without transfers ($n = 50$ loads observed). Workers moved in large numbers on the trail, comparable to a foraging context (in average 21 loaded ants per minute). They did not incorporate the building materials into the nest but dropped all them at the nest entrance.

4. Discussion

The present study tested predictions of two competing hypotheses concerning the adaptive value of sequential load transport in grass-cutting ants, *Atta vollenweideri*. In general terms, sequential transport via so-called “bucket brigades”, in which all fragments and workers are always on the move, is expected to enhance the performance of individuals, so

that workers are more likely to become specialists and thus more efficient [12, 17]. True bucket brigades may in addition reduce queuing delays at both the source and destination, resulting in a higher group’s overall rate of resource transportation [8, 13]. In fact, an increased rate of load delivery has been proposed as the main benefit of sequential transport for several ant species [4–6, 28]. However, in the ants *Atta vollenweideri* and *Anoplolepis gracilipes*, sequential transport took longer than transport by a single carrier all the way through (the present study and [25, 49]). In *Atta*, this resulted from delays associated with the careful lying down of the carried fragment, the waiting time until retrieval by another worker, and the subsequent handling time. Sequential load transport was therefore time consuming and resulted in a lower material transport rate than the nonsequential mode.

In the framework of the economic-transport hypothesis, fragments should be transferred whenever the carrier experienced a reduced transport speed, either because the fragment is too large for the carrying ant or the ant too small for the carried load. The present results, however, do not support this prediction, as neither an increase in fragment mass nor in fragment length affected the probability of occurrence of transport chains, even though they are known to markedly affect transport rates [43, 44]. Because of stability constraints [44], larger loads should in addition be transferred after a shorter distance than lighter ones, yet this was not the case. Finally, if fragments should be transferred because of a mismatch between body and fragment size, fragments of a given size carried by small workers should be dropped at shorter distances than those carried by larger workers, yet there was no significant relationship between these variables. On the contrary, observations of the first carriers revealed that a high percentage of loads were transferred after the carrier reached the main trail, suggesting that the perception of a strong chemical trail, or the interactions with nestmates, triggered the load transfer [5, 50]. Taken together, the available data on the relationship between fragment size and occurrence of sequential transport do not provide support for the predictions of the economic-transport hypothesis.

Interestingly, first carriers were smaller than last carriers in a transport chain, as also reported for other ant species [4–8, 49], and also smaller than single carriers that transported the fragment all the way to the nest. This appears *a priori* to support the arguments of the economic-transport hypothesis, that is, fragments might be initially dropped because the first carriers were too small for the task to be efficiently performed. However, ants may drop the fragment not because they are too small for the task, but because they restrict their work on a short trail segment. Observations on the behaviour of foragers collecting dropped fragments make very unlikely that most of them (up to 100% of the orange fragments in our experiments) “erroneously” collected fragments they were unable to carry because of their size, thus needing to drop them after a short distance. Foragers seem to be very selective when collecting dropped fragments; workers were observed first to try to lift the load, before they either abandon the fragment and search for another one or take it up for carriage. And more important, why should small

workers drop high-quality fragments more often than low-quality ones, if their decisions are based alone on fragment size and are the result of a mismatch between load and body sizes? The arguments of the economic-transport hypothesis fail to provide a compelling explanation.

Fragment quality was observed to markedly affect both the probability of occurrence of sequential transport and the distance walked by the first carrier before transfer. The first carriers transferred very attractive fragments more often, and after a shorter distance than less attractive ones. Intuitively, one would expect a worker carrying a high-quality fragment to be more motivated to carry it all the way back to the nest. So why should carriers transfer their fragments? Observations on the subsequent behaviour of the first carriers, as described in this study, may shed some light on this issue. All first carriers continued foraging, and all but one returned to the exact place where they collected the first fragment. Dropping or passing a load at the main trail after having carried it for a short distance has several consequences. First, fragment dropping after a given distance may allow workers to quickly go back to the harvested plant, making it easier for them to find the source again by following the freshly deposited pheromone trail. Moreover, an early return to the source may shorten the time needed to update information about the discovery, analogous to the reduction of dead time in a control system, thus allowing workers to switch to alternative resources whenever needed. Second, moving along a short-trail sector may enable workers to locally reinforce the pheromone marking better than after walking all the way to the nest, leading to a quicker establishment of a foraging column and a faster monopolisation of the discovered source, as demonstrated for foragers of the leaf-cutting ant, *Atta sexdens* [5]. It is important to note that field foraging trails can commonly exceed 50 m in length. In our experiments, ants usually needed 30 to 60 min to walk along the 33 m to reach the nest. This means that one single roundtrip would last one or two hours. In contrast, the observed first carriers managed up to 13 roundtrips per hour by foraging just on their short trail section.

Leaf- and grass-cutting ants forage along well-defined trunk trails leading to the harvested trees (in case of leaf cutters) or grass patches [4, 18, 37, 51]. Trunk trails split up into several side branches, and foraging patches are not necessarily located directly beside a trail nor are they defined spots. This means that between patch and main trail, a distance without or with a poorly defined trail has usually to be covered, thus making the finding of a newly discovered source difficult. *A. vollenweideri* ants, like other leaf-cutting ants [52, 53], usually did not deplete sources, but switched to other plants within a few days (personal observations). The reasons for this behaviour remain unclear. Rapid induction of secondary deterring components in the harvested grasses may be involved [54], but no studies have been carried out to investigate this phenomenon. Hence, the dynamic pattern of trail use and the strong branching of the existing trails may have favoured the evolution of a system that allows quick information transfer.

In the context of information transfer, fragments dropped on the trail may in addition act as “signposts.” It

has been shown that leaf-cutting ant foragers learn the odors of the harvested resources [39, 40], and that the foragers’ choices once at the patch depend on the material that is currently transported on the trail by their nestmates [38, 50]. The odor of the carried fragments, or of those found on the trail, might function as stimulus for olfactory learning. In fact, as soon as a worker started to lay down its load, outgoing nestmates coming from the nest were strongly attracted to it. Most foragers, even those that continued on their way to the cutting site without a load, were observed to antennate the dropped fragments they encountered on the trail. Thus, outgoing foragers may obtain information about the harvested resources both by contacting laden nestmates along the trail and by finding dropped fragments on it. This information may stimulate them to search for the experienced plant species, thus leading them from the trail to the newly discovered plant. Such an active search for information about the loads being transported was described for *Atta cephalotes* as early as 1929 by Lutz [32], who observed “frequently, a returning laden forager is stopped momentarily by an outgoing nestmate which is apparently interested in what is being carried.” The shorter waiting times of fragments that had been carried by workers, compared to those of “naive” fragments deposited by us on the trail, strongly suggests that dropped loads may have in addition been passively or actively marked by the ants.

We hitherto discussed scenarios that may have led to the evolution of sequential transport in ants, yet, at the proximate level, the question about what triggers load transfer remains open. Our results provide no indication that load size or ant body size influenced the workers’ decision to transfer their loads. The obtained correlations between body mass and covered distance were weak, sometimes of different sign, and showed in addition high variation. Moreover, individually marked ants dropped their loads in successive trips at very different distances. As mentioned above, a low transport speed because of a mismatch between load and body size was proposed to trigger fragment transfers in *Atta colombica* [28]. We did not record transport speed but measured the total time spent by each carrying ant, including handling times, interactions with nestmates, and so forth. Considering that the trail structure changes very much with distance, that is, trails are generally narrower, less cleared of vegetation, and with obstacles further away from the nest, average walking speed over the complete distance does not seem to represent a useful measure. More importantly, we often observed foragers walking very slowly or even stopping walking before dropping a load. These ants started at the source with a higher speed and then reduced their speed on the way, suggesting that they would have been able to continue walking at the same pace. The observed reduction in speed was often accompanied by a continuous approach to unladen nestmates, and a typical zig-zag walking pattern from one trail side to the other. Therefore, it could be argued that ants walked slowly because they were going to drop their loads, instead of that they dropped their loads because the burden forced them to walk at a slow pace. In addition, ants covering the section from the patch to the main trail were possibly involved in trail-marking, which

may also have slowed them down. Further conditions that were shown to cause fragment dropping in leaf-cutting ants under laboratory conditions, such as “bottle necks” during transport [20, 21], were absent in our field study. There were in addition no specific dropping places that could have triggered dropping via positive feedback or the presence of pheromone marking [21], since the short waiting times of dropped fragments prevented the formation of piles. One possible trigger could be the interaction with unladen, outgoing workers, but this aspect needs further investigation.

Summing up, the present study demonstrated that neither an increase in fragment mass nor in fragment length alter the probability of occurrence of sequential transport. In addition, sequential load transport took longer than transport by a single carrier. However, the frequency of occurrence of sequential load transport increased with increasing fragment quality, independently of fragment size. High-quality fragments were not only transferred more frequently but also after much shorter distances than less attractive ones, which suggests that sequential transport is driven by the need to transfer information about the discovery. The lack of occurrence of sequential load transport in two other contexts that are not necessarily associated with high information demands, that is, the more opportunistic collection of fallen dry plant fragments or building materials, provides indirect support for the hypothesis that a sequential load transport in grass-cutting ants has been favoured during evolution because of an improvement in the information flow during foraging.

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Research Article

Trail-Laying Behaviour as a Function of Resource Quality in the Ant *Camponotus rufipes*

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Chemical trails have been shown to act as an orientation cue in some ant species. Here, I report that the trail-laying behaviour in the nectar-feeding ant, *Camponotus rufipes*, varies with the concentration of the sucrose solutions collected. Single workers collected solutions of different sucrose concentrations (5%, 20%, and 40% in weight) during 4 consecutive visits to the resource, and their trail-marking behaviour was recorded on soot-coated slides during their first and last visits. Results suggest that these chemical trails provide both an orientation cue between the nest and the food source, as previously suggested for *Camponotus* ants, as well as information about food quality.

1. Introduction

The recruitment techniques employed by different ant species vary considerably and involve the use of mechanical and chemical signals either singly or in combination [1]. Mass-recruitment communication, in which information can be transferred from one group of workers to another, is organized almost exclusively by odour trails, as studied, for example, in the fire ants genus *Solenopsis* [2] and in *Atta* leaf-cutting ants [3]. For such species, the number of individuals leaving the nest is controlled by the all-or-none trail-laying response of the individual workers. Responses to the quality or quantity of the discovered food source are modulated by the thresholds of individual foragers, each of which decide whether or not to reinforce the existing trail depending on their success upon reaching the source. In a recent and complete set of studies [4–8], Detrain and coworkers demonstrated that for the ant, *Lasius niger*, the decision of a scout to return to the nest and lay a trail is governed by an internal response threshold that is based on the desired volume of solution ingested [4]. The desired volume is specific to each individual ant and is kept constant over successive visits to the food source [5]. Thus, the threshold is modulated by starvation; that is, more starved

foragers have lower trail-laying threshold [6]. Previous studies found that food distance also modulated the trail-laying behaviour [7] and that food type, that is, proteinaceous or sucrose droplets, changed the proportions of individuals that laid trails but not the individual trail-laying intensity [8]. However, these individual recruitment responses are plastic. Workers of *Solenopsis geminata* will increase the continuity of their pheromone trail with increasing colony starvation, increasing food quality, and decreasing distance to the resource [9]. Moreover, trail-laying workers of *Acanthomyops interjectus* [10] and *Monomorium pharaonis* [11] can modify the intensity of the trail with respect to food quality.

During food recruitment, workers of the ant *Camponotus socius* employ multimodal signals, involving a specific motor display, that is, waggle-movements, and chemical signals that emanate from the hindgut and the poison gland. The chemical signals are (1) a short-lived recruitment substance discharged from the poison gland, that is, formic acid, which elicits an unequivocal recruitment and trail following behaviour, and (2) long lasting trails laid with the contents of the hindgut, consisting of (2S,4R,5S)-2,4-dimethyl-5-hexanolide and possible 2,3-dihydro-3,5-dihydroxy-6-methylpyran-4-one, which apparently acts as a chemical orientation cue between the nest and the discovered food

source [12–14]. In addition, scout ants of *C. pennsylvanicus* also use alerting motor displays to recruit nestmates to new food sources; colony starvation will intensify these motor displays, evoking a strong recruitment response [15]. However, such modulation of individual trail-laying responses to food profitability has not been investigated for any *Camponotus* species. It is conceivable that workers may be able to respond to changes in food profitability by varying either their mechanical displays, the amount of trail pheromone laid, or both.

Contrary to predictions of optimal foraging theory, *Camponotus rufipes* (Figure 1) returns to the nest with partial crop loads of sugar solution even at a source with a constant crop loads or flow rate [16]. This early return has the clear disadvantage of resulting in less nectar being collected, but it has distinct advantages: reducing the time and energy spent by the forager at the source. Further studies by Schilman and Roces [17–19] showed that decreasing foraging time is more important than increasing individual energetic efficiency. This may reduce the risk of predation while foraging and any time saved could be used for increasing information transfer, for example, by depositing more trail pheromone. Any subsequent increase of recruitment would increase foraging efficiency of the whole colony at expenses of reduction in individual foraging efficiency. Behavioural studies have shown that *C. rufipes* workers lay trails with hindgut contents during recruitment of nestmates to food sources or to new nest sites (Hölldobler, personal communication), with 3,4-dihydro-8-hydroxy-3,7-dimethylisocoumarin being the most effective trail pheromone component [20].

In this study, I addressed the question of whether individual workers of *C. rufipes* collecting different concentrations of sucrose solution show graded trail-laying behaviour. In addition, the effect of a novel or a familiar food source on trail-laying response was evaluated by comparing the behaviour of individual workers during their first and fourth visits to the source.

2. Materials and Methods

2.1. Insects. A colony of *C. rufipes* comprised of one queen, approximately 500 workers, and brood was used for this study. The founding queen was collected in November 1994 in Misiones, Argentina. The colony was maintained at 25°C, 50% RH, and 12:12 LD regime (light from 7:30 to 19:30 local time); see [16–19]. In nature, colonies of *C. rufipes* build semispherical nests made of dry leaves attached together. Foragers are active throughout the day but show peak activity at the beginning of the night, both in the laboratory [21], and in the field [22]. In the Atlantic forest of southeastern Brazil, *C. rufipes* was found in almost 90% of the trunks of 8 species of *Magnoliophyta* analysed, likely attracted to the presence of extra floral nectaries [23]. It was also the most common ant species harvesting honeydew from aggregations of the treehopper *Guayaquila xiphias* on inflorescences [22].

Many social insects will collect and store food, when available, for times of resource dearth. Ant colonies of *C. rufipes* differ from other social insects such as honeybees, in that they store nectar internally in workers' crops. This



FIGURE 1: Worker of the nectar-feeding ant *Camponotus rufipes*, walking on a wooden stick to collect sugar solution from an artificial feeder. The ant was marked with yellow powder to allow for individual recognition (photograph by Helga Heilmann).

suggests that, for *C. rufipes*, individual foraging behaviour including trail-laying may be affected by their level of starvation or crop loading. To avoid changes in trail-laying responses, the physiological state of the colony was standardized following [16, 17]. Briefly, the colony was fed sugar solution *ad libitum* for 3 or 4 hours or after all workers exhibited fully expanded gasters after feeding. Ants were provided access to cockroaches and water *ad libitum*, but were deprived of sugar solution for 3–7 days prior to measurements. Under these conditions, *C. rufipes* colonies can survive at least 14 days of sugar deprivation (unpublished data), indicating that a period of 3–7 days does not constitute severe starvation because of the presence of crop reserves. In addition, I used a single colony to control for potential inter-colony differences in nutritional state, colony age, and/or size; these factors may affect the behavioural responses under investigation and are quite difficult to standardize for different colonies. Thus, the number of workers, rather than the number of colonies, was used as sample size for statistical analyses.

2.2. Experimental Device and Food Sources. The experimental apparatus consisted of a *C. rufipes* colony kept in a plaster nest inside an open-top Plexiglas container (37 × 57 cm and 27 cm high) with fluon-coated walls to prevent escape. A vertical wooden stick extended out of the container and could be connected to the food source via two mobile wooden bridges, one 50 cm and the other 10 cm long (Figure 2). A soot-coated slide (ca. 56 × 26 × 1 mm) was placed on the bridge (ca. 10 cm from the food source) while the worker was collecting the solution; so the marks left on the soot-coated glass allow direct observation of the trail-laying behaviour and pheromone deposition [9–11]. The entire experimental device was mounted on a vibration-buffered table (Figure 2).

The food source on the bridge consisted of a 1 mL droplet of sugar solution. Knowing that *C. rufipes* foragers of similar size have maximum crop loads of about 6–7 μL [16], a 1 mL droplet of sugar solution is an *ad libitum* source. In independent assays, solutions of three different concentrations

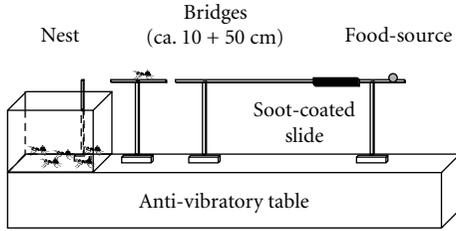


FIGURE 2: Schematic diagram of the experimental apparatus.

of sucrose (Sigma-Aldrich; Deisenhofen, Germany) were used. The concentrations were 5%, 20%, and 40% weight in weight (w/w). Solutions were prepared the morning of the experiment to prevent any change of concentration by evaporation, contamination, or fungal growth that would affect the food quality.

2.3. Experimental Procedure. Experiments were carried out in March 2000 at the Department of Zoology II of the Theodor Boveri Institute of the University of Wuerzburg, Germany.

Each assay began by connecting the laboratory nest to the food source (Figure 2). Ants in the nest spontaneously explored the whole nest including the vertical wooden stick located inside it. To allow only one ant at a time to cross onto the bridge and go to the food source, I placed the small bridge close to the vertical stick until one ant passed onto it. Then, I moved the small bridge carrying the focal ant into contact with the 50 cm long wooden bridge, thus, allowing the ant to reach the food source. To quantify trail-laying, a soot-coated slide was placed on the bridge while the worker was collecting the sucrose solution, so that chemical trails were laid on the slide upon the ant's return to the nest [9–11]. When the ant had passed the soot-coated slide on its way over the bridge, I gently marked it with coloured powder (yellow pigment from Lukas-Farbe, Wuerzburg, Germany; see Figure 1). Upon arrival at the nest, the marked worker was allowed to enter and to unload the collected fluid via trophallaxis with nestmates. Immediately after unloading, which took about 1 minute, the marked ant searched for the bridge to return to the food source and was therefore free to decide when to cross the bridge and to visit the food source again. Each individual ant was followed for four consecutive visits to the food source and the trail-laying behaviour recorded when coming back to nest during the first and fourth visits. At the beginning of the fifth visit, the unloaded worker was caged before feeding and weighed to the nearest 0.01 mg (Ohaus Model AS60; Karlsruhe, Germany) and discarded.

2.4. Data Analyses and Statistics. At the end of the consecutive visits to the food source by one ant, the two soot-coated slides (from first and fourth visits) were directly observed and the marks left by the ant categorized as (1) only footprints, (2) hair marks, and (3) gaster-tip marks. The latter were always combined with hair marks and in all cases footprints were present. Afterwards, the whole slides as well

as close up of the marks were recorded with video camera (Panasonic F15) with a zoom lens 18-108/2.5 connected to a VCR (Panasonic AG 7355). Few examples were digitalized with Screen Machine II and Unimark software and graphic card (Fast Electronics GmbH, München, Germany).

A total of 37 different ants and 71 trails were analysed, 12 ants (23 trails) for 5%, 13 ants (25 trails) for 20%, and 12 ants (23 trails) for 40% sugar solution treatments. Three trails were missing from analysis: two fourth visits from 5 and 20% treatment were missing because the ants lost their mark before the fourth visit and were impossible to identify. The third missing trail was the first visit from an ant of 40% treatment where the soot of the slide was damaged prior to analysis of the marks. Each day, data from all treatments were taken, excluding the possibility of a daily variation of any other parameter different from the nectar concentration. For statistical analysis, the G-test of Independence was used to analyse the frequency of different marks left by ants collecting sucrose solution of different concentration, that is, 5, 20, and 40%. The G-test was performed in Microsoft Excel based on formulas from Box 17.8, page 738 from [24]. McNemar's Paired Test was used to compare differences between the marks left by the ants in the first versus fourth visits. Because the data of the ant mass met the requirements for parametric analyses, that is, normal distribution and homoscedasticity, ANOVA was used to compare ant mass across treatment [24].

3. Results and Discussion

Like most ant species, *C. rufipes* lays chemical trails for recruitment and orientation during foraging. This deposition is a conspicuous behaviour occurring when foragers run back to the nest bending their gaster downward and dragging the tip along the ground. Single workers left three different types of marks (Figure 3(a)), instead of four as reported for *Solenopsis geminata* (i.e., only footprints, hair, combined, and sting marks) [9]. Since *C. rufipes* does not sting, I found only footprints, hair combined with footprints, and gaster-tip marks combined with hair marks and footprints. Significant variation of the trail-laying according to food quality was observed (Figure 3(b), G-test of Independence, $G_{(4)} = 14.1836614$, $P = .0067$). The proportion of workers laying a trail increased with sucrose concentration. For the 40% treatment, all workers were observed to lay either hair or gaster-tip marks (Figure 3(b)). Such increased proportion of hair or gaster-tip marks with higher concentration of the solution indicates changing intensity in the pressure of the gaster tip against the substrate. These results show that workers of the ant *C. rufipes* modify their trail-laying behaviour according to the richness of the food source, suggesting a control of recruitment responses to food quality. Similarly, in Pharaoh's ant (*Monomorium pharaonis*), the frequency of individuals marking with high intensity is significantly greater with a high-quality food source [11]. For *C. rufipes*, the differences in trail-laying responses only depended on the richness of the food source because (1) data of all treatments were taken each day and at different times of day, thus, excluding the possibility of bias due to daily variation, and (2) the body mass of the foragers was

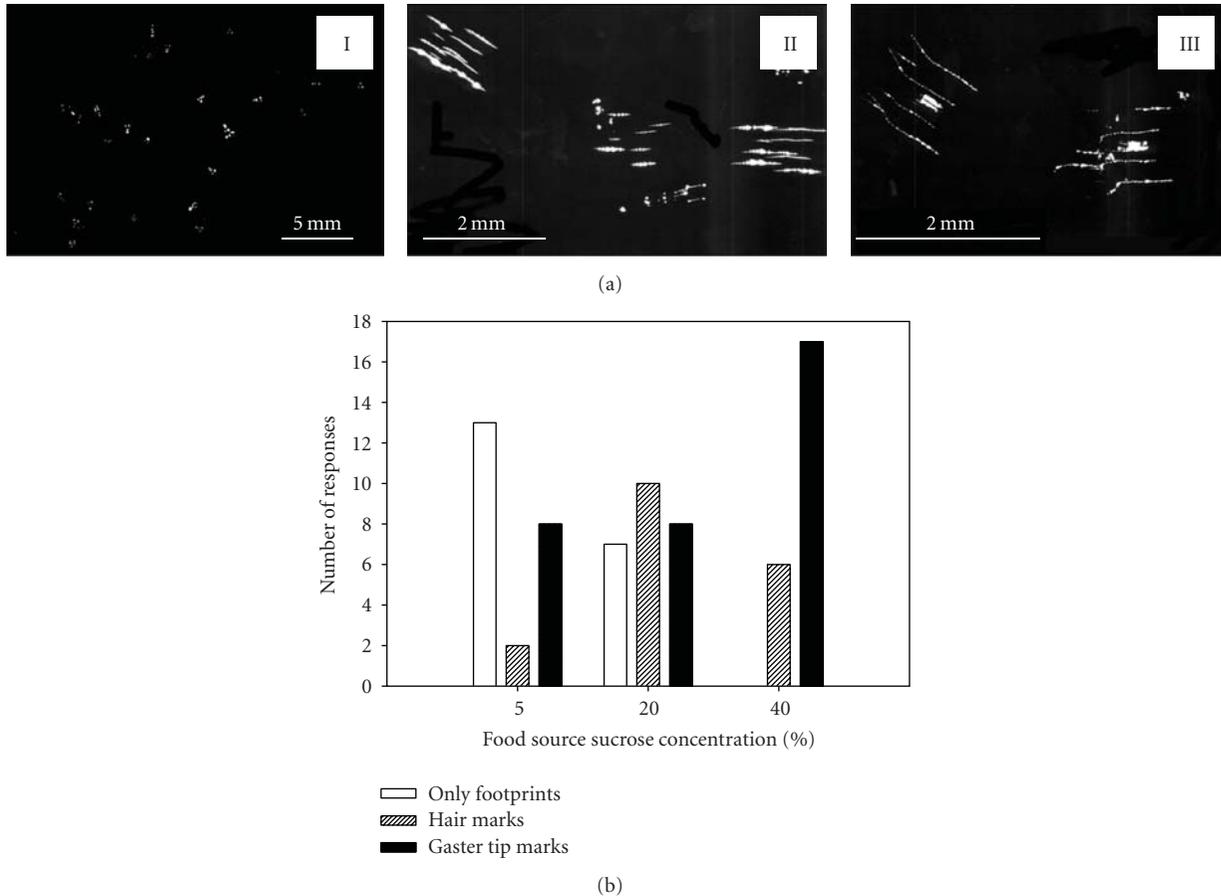


FIGURE 3: (a) Examples of the different marks left by *C. rufipes* ants: (I) only footprints, (II) hair marks, and (III) gaster-tip combined with hair marks; (b) number of marks left by the foragers after the first and fourth visits to the bait for 5%, 20%, and 40% sucrose concentrations.

similar across treatments (ANOVA test, $F_{(2,33)} = 0.2652$, NS, range between 8.046 and 13.992 mg). However, Jaffé and Sánchez [21] showed no change in recruitment rates and total number of recruited *C. rufipes* workers as a function of colony starvation and food quality, suggesting that no modulation of recruitment was occurring. The discrepancy between the findings of this and a previous study [21] could be explained by the fact that I did not measure recruitment responses in nestmates, or by the great variability reported by Jaffé and Sánchez in their study.

About how trail-laying ants respond to a novel versus a familiar food source, I analysed the dynamics of individual trail-laying behaviour by comparing the first and fourth visits to the food source. In another Formicine ant, *Lasius niger*, it is known that the number of marks laid per passage per forager decreases with time [25]. However this decline seems unlikely to occur in Pharaoh's ant, whose trails are essential for their orientation [11]. For *C. rufipes*, I did not detect significant differences in the trail-laying behaviour between the first and the fourth visits to the source for any sugar concentrations (McNemar's Paired Test, NS). This suggests that the marking frequency did not change as foraging bouts progress. Thus, the two visits (1st and 4th) were pooled in Figure 3(b); for statistical analysis, the mean of the two visits

was used to avoid pseudoreplication [26]. However, these results cannot completely exclude the possibility that this variation could occur under other conditions. First, a new soot slice without any trail mark was used for each new visit; so the ant did not find any scent odour on that part of the bridge. Second, while the trail-laying behaviour should stimulate trail following in nestmates, the experimental design used (open loop) did not allow the forager to recruit nestmates to the food source, regardless of the behavioural trail-laying response of the worker. Therefore, the worker did not experience any feedback from nestmates on the bridge or at the resource. Third, if there is any decay in the trail-laying response with successive visits to the food source, it may be possible that more than four visits are required to observe it.

I can conclude that, at least for *C. rufipes*, the trail-laying behaviour is not an all-or-none response but a graded response to the richness of the food source. Similar changes in individual marking were found in *S. germinata* [9] and *M. pharaonis* [11]; this suggests that the individual response to food is key to modulating trail strength in these three species, which contrasts with the all or none response of *L. niger*. Assuming that *C. rufipes* uses the trail pheromone as a communication channel and not solely as an orientation cue, these results indicate that trail pheromone would provide

information about the profitability of the food source to recruited workers.

Finally, it is noteworthy that *Camponotus* ants, which use a waggle display as a graded signal to enhance trail following [13], may also use the chemical trail as a graded signal, showing that recruitment control in ant communication is more complex than it was thought.

Acknowledgments

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Research Article

Recruitment in Swarm-Founding Wasps: *Polybia occidentalis* Does not Actively Scent-Mark Carbohydrate Food Sources

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Scent marking food resources is expected to enhance foraging efficiency reducing search time. Many social bees exhibit this behavior, but scent-marking is absent in social wasps, except for *Vespa mandarinia*. We tested for scent marking in the swarm-founding wasp, *Polybia occidentalis*. This wasp has moderately large colonies and utilizes resources that are concentrated in time and space, making scent marking profitable. Also, this wasp uses chemical markings to lead nestmates to a new nest site during swarm emigration, making it possible that it could use the same behavior to recruit nestmates to a food source. Foragers from 11 colonies were given a choice between a previously visited feeder and an unvisited one, both containing a rich, unscented sucrose solution. There was no difference in the number of visits to the two treatments. However, some individuals chose the feeder on one side more often. We conclude that foragers of this species of wasp do not use odor marks left behind by nestmates to find food, but they do exhibit the tendency, when returning to a food source that has not been depleted, to choose a resource based on its relative position, presumably by using visual cues.

1. Introduction

Recruitment is communication that brings nestmates to an area where work is required, and in social insects it enhances the efficiency of colony resource acquisition by increasing the number of foragers exploiting a food source [1]. A number of recruitment mechanisms have evolved, including the well-known waggle dance of honey bees [2] and the trail pheromones of ants [3] and stingless bees [4, 5]. Both the occurrence and sophistication of recruitment mechanisms tend to correlate positively with colony size [6].

In contrast to the bees and ants, no recruitment signals that encode distance or direction have been found in the social wasps [7, 8], but foragers in some species utilize social cues. Olfactory cues from the food brought back by successful foragers can stimulate foraging and bias a colony's collection efforts to resources with the same scent in the field. This behavior has been demonstrated in yellowjackets [9–12]

and the swarm-founding wasp *Polybia occidentalis* [13, 14]. In addition to this nest-based information, wasps also utilize social, field-based information; many species are attracted to food sources where others are feeding (local enhancement) [8, 13, 15, 16].

The majority of social wasp colonies have populations in the 10^2 – 10^3 range, but some species reach the 10^4 – 10^5 range [17], and individuals number as many as 7 million in *Agelaia vicina* colonies [18], far beyond the colony sizes of honey bees, stingless bees, and many ant species. It is puzzling that wasps have not evolved food recruitment behavior despite achieving colony sizes that exceed those of other recruiting insects. The reason for the unequal distribution of recruitment mechanisms across the social Hymenoptera has not been explained. However, the range of recruitment mechanisms also has not been fully explored, especially for the social wasps. Thus, there remains the possibility that food recruitment exists in species that have

not been thoroughly studied, or for which the full gamut of recruitment mechanisms has not been examined.

Chemical markings (either repellent or attractive) left at food sources by previous visitors can also play a role in the rejection or acceptance of a resource. These marks are beneficial because attractive marks can increase the rate of exploitation of a resource, while repellent marks can prevent useless probing of depleted sources. These markings can be categorized as either signals, which are actively left at food sources to convey information, or cues, which provide information but are left passively as a byproduct of sender behavior. Evidence for both attractive and repellent marks has been adduced for some bumble bees [19, 20] and stingless bees [21, 22]. Recent evidence suggests that these marks are cues, footprints of cuticular hydrocarbons left behind passively by foragers walking on food sources [5, 23–26]. The rejection or acceptance of a resource is a context-dependent, learned association between the marking and the presence or absence of a reward. Thus, when a bee encounters a scent mark on a resource that is paired with a reward, she positively associates the scent mark with the presence of a reward and visits scent-marked flowers more frequently. The opposite occurs when a flower has previously been visited and depleted.

Repellent and attractive marks are also left on food by foraging honey bees [27–29]. Studies comparable to those performed on bumble bees and stingless bees have not been carried out to determine whether these marks are cues or signals. However, foraging honey bees actively release Nasonov gland pheromone at some resources, which is attractive to other foraging bees [29, 30]. This pheromone is most often released at water sources, unscented artificial feeders, and highly profitable resources [30, 31]. Taken together, the foraging contexts under which Nasonov pheromone is released suggest that its function is to pinpoint the location of profitable, unscented resources to potential recruits.

Previous work on *Vespula germanica* wasps suggested that feeders are attractive to foragers after being visited heavily over a period of days or walked over extensively [32, 33]. However, a better-controlled study on this species found that feeders visited 50 or 100 times are no more attractive than unvisited ones [34]. The only wasp that has been found to actively apply scent marks to food sources is the hornet *Vespa mandarinia*, which applies secretions from the sixth sternal (van der Vecht's) gland that are attractive to nestmates [35]. This behavior occurs during autumn raids on honey bee and wasp colonies, when these hornets switch to group hunting and attack these colonies en masse [35, 36].

In contrast to the vespine wasps, food site marking has not been studied in the swarm-founding Polistinae. Swarm-founding wasps may be more likely to exhibit scent-marking behavior than independent-founding species. Many species of the swarm-founding Polistinae, including *Polybia occidentalis*, deposit attractive scent marks that guide nestmates to a new nest site during colony emigration [37, 38]. During emigration, scout wasps drag their gasters on leaves to deposit an attractive pheromone from a gland at the base of the fifth gastral sternite [37, 38]. This pheromone could easily be co-opted for use in a foraging context.

The benefits of food-site marking are expected to be greater for species with large colony size. Larger colonies have more foragers and therefore are more likely to find scent-marked resources quickly. *Polybia occidentalis*, like many wasps, exploits a variety of carbohydrate resources that are often concentrated in time and space, including fruit, human refuse, honeydew, and extrafloral nectaries ([39], B. Taylor pers. obs.). *Polybia occidentalis* also gathers nectar from flowers of at least 15 families of plants, and the families of plants preferred by wasps tend to be those that produce large numbers of flowers [40]. Furthermore, *P. occidentalis* forms moderately large colonies, numbering up to several thousand individuals [38]. Thus, in addition to the presence of scent-marking glands used during swarm emigration, the large colony sizes and the types of resources exploited by *P. occidentalis* suggest this species may benefit from food-site marking.

Here, we test the hypothesis that the swarm-founding wasp *Polybia occidentalis* marks carbohydrate food sites using a chemical attractant signal. We also test whether foragers use a visual cue—relative position on a feeder stand—to relocate food upon return.

2. Methods

The study was performed at Centro de Rescate Las Pumas, approximately 5 km west of Cañas, Guanacaste, Costa Rica (10°25'N, 85°7'W). Experiments were conducted between 23 June and 9 July 2008. This is the wet season in this area of Costa Rica, and colonies were in a stage of active growth.

To facilitate training and following of foragers, nests were moved into a field with scattered trees, where they were attached to branches at eye level using either nylon cable ties or wire. Tanglefoot (The Tanglefoot Co., Grand Rapids, MI, USA) was applied to the branches to prevent predation by ants. All nests were moved at night to minimize the number of workers lost. Experimental trials were conducted between the hours of 08:00 and 14:00. Rain often occurred in the late afternoon, so conducting trials during this time was specifically avoided.

2.1. Forager Marking and Training. A pool of 50 individually marked workers was established for each nest at least one day prior to testing to differentiate individuals and members of different nests. A sucrose-filled feeding dish atop a tripod stand was placed directly against the nest. As workers stepped onto it from the nest to feed, they were caught, placed in vials on ice until immobile, and marked on the thorax using paint pens (Decocolor, Uchida of America, Corp. Torrance, CA, USA). The markings encoded a unique number for both nest and individual. After marking, individuals were returned to the tripod stand, where they warmed up before flying back to the nest.

Before each experimental trial, foragers were trained to feed from a round, 4.5 cm-diameter tin dish that was covered by a lid. The side of each dish had an opening through which a glass microscope slide projected, providing a landing platform down which foragers could walk to access the liquid

(see [34] for details). The feeder was filled with a 2 M sucrose solution and placed against the nest in the same fashion as during the marking process. Foragers crawled down from the nest and fed from the dish. After 15–20 workers started feeding from the dish, we began moving it upwind from the nest in increments of approximately 1 m until it was 10 m from the nest. During this time, the number of foragers coming to the feeder often dropped considerably. If fewer than two marked foragers arrived, the feeder was again moved near the nest until at least two marked foragers made repeated visits. Because there is some evidence that scented solutions are less likely to be marked [30], we did not scent the solution with any extracts.

2.2. Experimental Trials. After at least two marked foragers were trained to the feeder and it was 10 m from the nest, the feeder was replaced with a clean one in the center of the tripod stand. If a resource is actively marked with a chemical signal, then few visits should be required to make a feeder more attractive. Therefore, the trained foragers were allowed to visit the new feeder (hereafter referred to as the test feeder) a total of five times (i.e., a total of five visits distributed among all the trained foragers). This process occurred very quickly, usually taking less than 2 minutes. Immediately thereafter, the test feeder was moved to one side of the stand, and an identical but unvisited feeder (hereafter referred to as the control feeder) was placed on the opposite side of the stand, 9.5 cm from the test feeder. The stand was rotated so that the line connecting the two dishes was perpendicular to wind direction.

A trial consisted of 40 choices made by a colony (distributed among 2–5 foragers, each making 1–25 choices). All choices made by arriving, marked foragers were recorded for use in the analysis until 40 visits were reached. All unmarked foragers were captured and held until the conclusion of the trial. To avoid the biasing effects of local enhancement, foragers that arrived while another forager was feeding were not counted [13, 15]. The position of the test feeder on the tray (either right or left as viewed from downwind) was determined by a coin flip and switched between visits. If the control feeder was visited, its slide was replaced, and if other portions of the control feeder were walked on, they were also replaced. An observer sat crosswind, approximately 1 m from the feeders, to minimize interference with any scent plumes left behind by foragers.

2.3. Statistical Analysis. The food-site-marking hypothesis predicts that the test feeder will receive more visits than the control feeder. If no scent marking has occurred, then both feeders should be visited equally. Therefore, we let Y_i = proportion of landings on the test feeder for each colony and conducted a t -test with $H_0: \mu = 0.5$. A 95% confidence interval for the mean was also constructed. We conducted further tests to explore the roles of previous visits and positional fidelity on feeder choice. For each individual making at least eight choices during a trial, we conducted two Fisher’s exact tests. The first tested whether there was any preference for the previously visited test feeder, and the

TABLE 1: The proportion of landings on the test feeder (40 visits per colony) for the eleven colonies tested.

Colony no.	Proportion of landings on test feeder
08-003	0.475
08-012	0.525
08-024	0.550
08-027	0.550
08-030	0.475
08-045	0.625
08-057	0.500
08-058	0.550
08-059	0.550
08-072	0.475
08-073	0.500
Mean	0.525

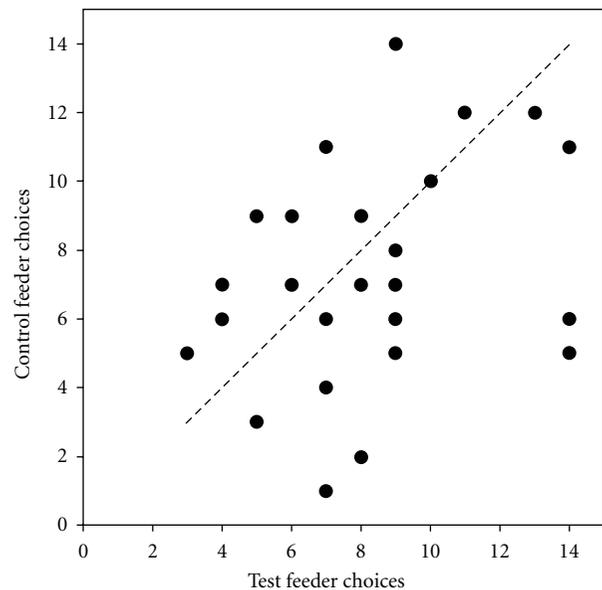


FIGURE 1: The number of choices for the test feeder and control feeder made by individuals that made at least eight choices during a trial. Each symbol represents an individual. The dotted line represents choices of 1 : 1.

second tested whether foragers showed any positional bias (i.e., chose the feeder associated with the left or right side more often during their visits).

3. Results

For the population of foragers from the 11 nests tested, the mean proportion of visits to the test feeder was 0.525, with a confidence interval that included 0.5 (95% CI: 0.494–0.556) (Table 1). A t -test revealed that colonies did not choose the test feeder any more or less often than expected by chance ($t = 1.80$, $df = 10$, $P = .102$). There were 25 foragers that made at least eight choices during the trials. Of these, none chose the test feeder significantly more often (Figure 1), but

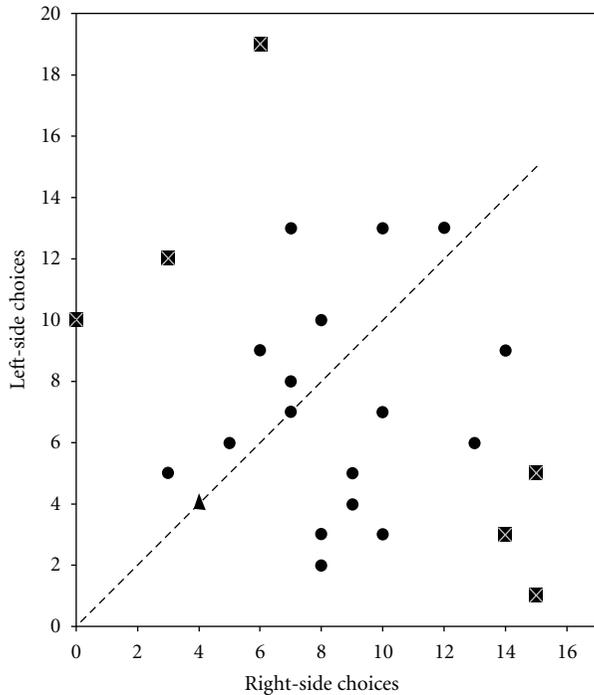


FIGURE 2: The number of choices for the feeder on the left or right side made by individuals that made at least eight choices during a trial. Each symbol represents an individual. The dotted line represents choices of 1 : 1. The triangle represents two overlapping individuals that chose the right and left equally (4 times each); the squares containing an X represent individuals that showed a significant bias for one side ($P < .05$).

six of the 25 showed a side preference (Figure 2). This is sufficient to reject the null hypothesis that no individuals had a side preference ($P = .0012$). Foragers approached the feeders from downwind but did not fly in a zig-zag pattern as they approached and did not hover before entering a feeder. Instead, foragers flew directly to a feeder before landing and entering. During the trials, no dragging of the gaster on the feeders was seen, except when foragers exited the feeders. However, because there was no evidence that exiting foragers actively wiped the gaster on any portion of the feeder as described for swarm emigration [37, 38], the dragging was likely due to the mass of the imbibed liquid weighing the gaster down. The average time between visits to the test feeder by successive foragers during testing was 1 minute 20 seconds (maximum time: 6 minutes 25 seconds). This figure is slightly inflated because the choices of foragers were scored only if no other forager was present.

4. Discussion

We found no evidence that attractive scent marks were actively applied to the food dishes, and thus we conclude that scent marking does not occur in the context of carbohydrate foraging in *P. occidentalis*. In addition, foragers were observed taking direct lines of flight from the nest to the feeders, and no hovering near the feeders occurred before

landing. This is in contrast to the behavior of wasps using olfactory cues to locate food [10, 12]. In these studies, wasps approached the feeders using zig-zag patterns and hovered before landing at the resource [10, 12]. It is unlikely that scent markings applied to the feeders would have deteriorated during the trials. Chemical signals evolved in this context should be long-lasting. Markings laid down by bumble bees and stingless bees can last for more than an hour [41–43]. Here, the maximum time between visits to the test feeder was only 6 minutes 25 seconds.

It is also unlikely that the feeders did not exceed a threshold of profitability required to elicit scent marking. The concentrations of dissolved sugar in the carbohydrate resources often exploited by wasps (floral and extrafloral nectar, fruit, and honeydew) range from approximately 0.5 M to 2.5 M [44–47]. Thus, it is likely that a 2 M solution located a mere 10 m from the nest would be perceived as highly profitable to the wasps and therefore likely to be marked if the behavior indeed did exist. However, if wasps only switch to marking at greater distances, similar to honey bees switching to waggle dances only when food sources are >100 m from the nest [2], our test would not have detected its presence.

Because we tested only for responses to signal-based, active scent marks, we cannot entirely rule out the possibility of passive footprint cues, such as those utilized by stingless bees and bumble bees, that might accumulate after many repeated visits [5, 23–26]. The number of visits required to make a feeder more attractive varies among species. A foraging honey bee need land only briefly on a feeder to leave an attractive scent mark [30]. However, in some stingless bees, 20–40 visits are required to make a visited feeder more attractive than an unvisited one [23, 43]. *Vespula germanica* foragers did not choose a feeder visited 50 or 100 times any more often than an unvisited one [34]. On the other hand, these wasps were shown to follow a trail in the nest entrance tunnel after it had been walked over by more than 200 individuals [48]. However, if such a large number of visits is required to mark a food site with a footprint cue, it would be of little value to foragers.

Because we used a carbohydrate food source, our experiment cannot rule out the possibility that scent marks are deposited on protein resources. Several swarm-founding wasps scavenge on carrion [49–51], especially those in the genera *Agelaia* and *Angiopolybia*. On the other hand, the scent of rotting meat may render active marking of a resource superfluous [8]. Indeed, recruitment was not found in *Agelaia multipicta* or *A. hamiltoni*, two species known to exhibit necrophagy [7]. In the tropics, these wasps must also contend with stingless bees and ants that feed on carrion [3, 4, 52]. Wasps may not be able to compete with these insects, especially those that can amass large numbers of foragers at these highly profitable sources using recruitment.

Foragers also did not choose the test feeder any less often than the control, suggesting that *P. occidentalis* does not leave behind repellent scent marks, either. There remains the possibility, however, that repellent scent marks may be left behind if a feeder is depleted. When repellent behavior has been found in bees, the experiments utilized real flowers or

artificial flowers that were depleted after feeding. In contrast, our feeders remained filled.

Although we found no evidence for the role of scent marks in forager resource choice, we did find an effect of feeder position for some individuals. Presumably, these wasps learned the relative position of the feeder on the tripod stand using local landmarks and subsequently returned to that same feeder more often. Because scent was weak, visual cues may have been the only reliable cues available. Indeed, visual cues are known to be used by wasps upon return if a food source has not been depleted [53–55]. However, not all individuals displayed a side preference. It is possible that these individuals encountered other foragers at the feeders during a trip, and this interaction caused them to choose a different feeder on subsequent trips. Alternatively, the strength of positional fidelity may have varied among individuals. A study addressing the phenomenon directly is needed to resolve the issue.

The apparent absence of scent marking in wasps and its presence in some bees may be related to differences in food sources utilized. Bees derive much of their carbohydrate sustenance from flowers, while wasps get theirs from a variety of sources including fruit, extrafloral nectaries, honeydew, and human refuse [39]. Repellent scent marks left by bees on depleted flowers allow subsequent visitors to discriminate between visited and unvisited flowers (i.e., each flower represents a point source). In contrast, a repellent marking on a non-point source, such as honeydew, would not be as beneficial for a foraging wasp. Yet, like most bees, wasps feed on flowers ([39, 40], B. Taylor, pers. obs.). However, because wasps are restricted by their short glossas to flowers with short corollas or cup-like morphologies, flowers likely make up a smaller portion of their diet compared to bees, and therefore, selective pressure favoring scent marking of these sources may be weak. The application of attractive scent marks to clustered food sources, such as concentrations of honeydew-producing Hemiptera or human refuse, could be beneficial for wasps. However, fruit and human refuse may be similar to rotting carrion in that they could be easily detected by means of their scents alone. Also, the overall distribution of these resources in the environment near *P. occidentalis* nests, though unknown in this study, may select for an opportunistic foraging strategy. Johnson [56] reasoned that environments with an abundance of small resources and few large, transient resources would select for opportunism, rather than recruitment and defense of resources. An opportunistic strategy may also allow foragers to find resources more quickly [56].

The seemingly anomalous presence of scent marking in *Vespa mandarinia* may be explained by this wasp's unique food [8, 36]. These hornets attack and overwhelm colonies of other social wasps and of honey bees. It is highly unlikely that a single hornet would succeed at this, but by coordinating and attacking en masse they can overcome the strong defenses of colonies that can contain up to tens of thousands of individuals. This coordination is facilitated by the scent marking [35]. For wasps such as *P. occidentalis* that do not utilize such well-defended food sources, scent marking may not be adaptive.

Despite having the machinery for scent-marking swarm emigration routes, *P. occidentalis* does not utilize it in the context of carbohydrate foraging. This suggests that the benefit-to-cost ratio of the behavior must fall heavily on the cost side. Unlike nest-based recruitment mechanisms that remain cryptic to non-nestmates, field-based mechanisms are subject to eavesdropping by non-nestmates and heterospecifics. Indeed, some foraging stingless bees, honey bees, and bumble bees use marks by individuals from other nests and even other species [28, 57]. Thus, the cost of olfactory eavesdropping may render scent-marking behavior unprofitable to most wasp species.

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Review Article

Waggle Dances and Azimuthal Windows

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Because the waggle dances of honeybees contain celestial components, modifications of the dances occur with changing celestial moves relative to a honeybee nest. Since the direction of a particular resource is static, the dances must alter to compensate for the sun's passage. The position of the sun is seasonal between the Tropics of Cancer and Capricorn so that turns at the end of waggle runs will vary with season and latitude. The bees are confronted with a new difficulty when the sun closely approaches its zenith because only slight errors in the bees' estimation of the relative positions of the sun and zenith generate very large errors. So, the sun compass loses its usefulness when at its zenith. We review experiments and observations on both foraging and absconding in relation to the azimuth. The honeybees' solution for the paradox of the azimuth includes an azimuthal lull, preferences, and time windows.

1. Foraging and the Azimuth

Navigation in honeybees is a reference system in which vector information is derived from path integration encountered en route to a specific goal [1]. The cavity nesting bees and giant honeybees perform waggle dances only in the vertical mode so that the direction and distance of a target resource are in relation to the position of the sun as a vector relative to gravity. However, the dwarf honeybees, *A. andreniformis* and *A. florea*, perform their waggle dances on a horizontal plane at the top of their nests. They dance directly relative to the sun [2, 3] so that less complex calculations are required. Whatever level of behavioural sophistication the waggle dances may represent, direct measurements now show that the system is not without the noise of statistical scatter and indeed is ameliorated by olfactory and visual cues [4]. The worker bees recruited only by the information from dances are not able to find a highly localized and unscented food sources on their own. To pinpoint those food sources, the experienced foragers provide additional cues to new recruits by circling the food source and scenting it. Similarly, the new recruits which are exposed to dances without a

precise indication of the position of the sun can overcome a navigation gap by following the buzzing flight or marked scent of experienced foragers around food source [5].

In any event, because the intricate displays of the waggle dances contain celestial components, it can be expected that modifications of the dances will occur with changing celestial moves relative to a honeybee nest somewhere on earth. Indeed, long ago von Frisch [6, 7] demonstrated that a forager which has located a desirable resource returns to its nest and communicates its distance and direction in the waggle dance. The angle between the direction of the dance and the vertical is equal to the angle between the azimuth (compass direction) of the resource and the azimuth of the sun [6, 7]. Since the direction of a particular resource remains the same, the dances must gradually alter to compensate for the sun's passage if the dances are to work at all.

If the path of the sun lies to the south, the alteration of the direction component of the dance should be anticlockwise, but clockwise to the north. The position of the sun is seasonal, north alternating with south within the Tropics of Cancer so that the axis of the waggle phase will vary with

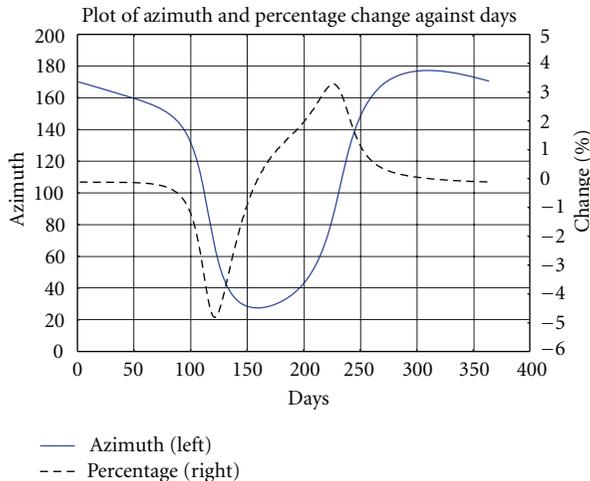


FIGURE 1: Plot of azimuth and percentage change against days of the year for Chom Bueng, Thailand (13.37N, 99.35E, altitude 86 m). Sharp peaks in the percentage change represent winter and summer solstice. The percentage change is simply the rate of increase or decrease of the azimuth from one day to the next, then multiplied by 100.

both season and latitude [2]. Anticlockwise turns switch over to clockwise as the daily path of the sun moves from south to north of the observer [8, 9]. When the sun closely approaches its zenith at noon the bees are confronted with a new difficulty because only slight errors in the bee's estimation of the relative positions of the sun and zenith generate a very large error estimation of the sun's azimuth.

2. The Azimuth Paradox

The azimuthal position of the sun provides navigation guidance to the honeybees. The particularly precise compass cue is provided by the pattern of polarized light, that is, the pattern of the electric (E -) vectors of light in the sky [10].

In the waggle dance of honeybees, the direction of the target source is indicated by performing a dance on the vertical and so is equal to the angle between the azimuth of a food source and the azimuth of the sun [9]. The dance for the same food source will alter progressively during the day to compensate for the movement of the sun across the sky. As the sun approaches near to the zenith at noonday, the bees would encounter problems in accurately reading its azimuth. In the first of such studies, Lindauer [2] observed in very nearly equatorial Ceylon (= Sri Lanka), that honeybees exhibited disoriented waggle dances when the sun was within $\pm 3^\circ$ of its zenith and that this was associated with a decline in foraging numbers. Further, von Frisch [11] confirmed that the sun compass loses its usefulness to bees at the zenith of the sun. Lindauer [12] noted that honeybees between the tropics of Cancer and Capricorn experience sun compass failures twice a year at the solstices. This is because when the sun moves through its zenith twice a year, it is impossible to deduce any direction based on the sun's position. Although outgoing bees might memorize landmarks, how could successful foragers communicate the

direction during their waggle dances if they fail to integrate precisely the azimuthal position of the sun?

Indeed, Lindauer [12] concluded from his observations that when the sun passes within 2.5° of the zenith in Sri Lanka that the dances are in fact disorientated. Moreover, the sun's azimuth at these times is altering rapidly and at a varying rate [8, 9]. This suggested to D. A. T. New and J. K. New [9] "that bees might solve the problem of communication at small zenith distances of the sun by dancing to sun positions memorized from a few days previously." They tested this idea when the sun was close to the zenith, at three different islands in the Caribbean within the tropics, respectively, at 5°N , 10°N , and 18°N , by further observations of the dances. What they observed was that when the dance angles were plotted against time, there were smooth and symmetrical curves around noon, but of smaller maximum slope than for the changing azimuth. Turns at the end of the waggle phase were often the obverse of the expected. Their final interpretation was the proposal of two possible mechanisms of control: (1) a mechanism using all information about the real azimuth limiting the possible dance angles which become increasingly wide the nearer the sun is to the zenith; (2) an ancillary mechanism based on memory such that angles are proportional to time [9].

This ancillary mechanism arises from Lindauer's observations [12] that dances performed at any time during night indicated approximately the angle between the daytime resource and the azimuth of the sun at the particular time of night. This led to the hypothesis that the bees extrapolate the complete 24 h circle of azimuth change from the part that they are able to see. The problem here is that Lindauer [12] and D. A. T. New and J. K. New [9] invoked *Zeitgedächtnis* (= finely tuned internal clocks of honeybees [13–15]) to compensate for azimuth change. *Zeitgedächtnis* enables bees to continuously modify and adjust their behaviour with respect to memory and time [2, 16, 17]; but these clocks can be modulated by external factors [1, 15] of which the sun is particularly important [11]. In reality, time and memory would be extremely hard put to calculate the actual changes in the azimuth on sequential days throughout the year (Figure 1) so that it is inescapable that honeybees require clear readings of the sun for successful communication.

3. Resolving the Paradox

Avoiding times when there would be confusion in taking the sun's angle from the zenith is probably the common cause of noonday lulls in honeybee foraging [18, 19] and is even more acute in cases of swarming or absconding. Koeniger et al. [20] demonstrated that the sun is important in the orientation of dances in *A. florea* because if a "surrogate" sun (a hand-held mirror) is reflected on the bees as a reference point for dancing, changing the angle of the mirror affects the angle at which the bees dance. This shows that the bees can use the surrogate sun in their orientation dances; therefore, if the sun is placed by a mirror at the zenith, it might be possible to increase the bees dancing at noon. The experiment with the mirror showed that even though changes in solar attitude does not influence the orientation, nonetheless, the accuracy

TABLE 1: Numbers of directional and nondirectional components in the waggle dances observed over three time periods—10:00 to 11:00 h, 12:00 to 13:00 h, and 13:00 to 14:00 h ($n = 5$ colonies).

Time	Waggle dances		χ^2 -value	df	P-value
	Directional	Nondirectional			
10:00-11:00	87	13	54.8	1	<0.0001
12:00-13:00	51	49	0.04	1	0.8415
13:00-14:00	89	11	60.8	1	<0.0001
Heterogeneity G			49.7	2	<0.0001

of the compass reading decreases with the increasing solar elevation [21]. This might effect in the noonday lull of dancers because of errors in compass reading. Interestingly, in comparisons of the precision of dances by *A. florea* for food sources and nest sites, Beekman et al. [22] showed that workers of *A. florea* dance with the same imprecision irrespective of context. Combining the above observations of Koeniger et al. [20], Beekman et al. [22], and Duangphakdee et al. [23], Duangphakdee et al. [24] performed experiments that demonstrate the importance of the position of the sun for individual waggle dancers of *A. florea* foragers at different times of day.

Gardner [25] found changes in accuracy in the dances of honeybees over the course of a day, particularly at noon (Figure 2), and subsequent studies have confirmed this finding. Foragers of five colonies of *A. florea* were observed between July 2009 and March 2010 at Chom Bueng, Thailand (13.37N, 99.35E, altitude 86 m). Waggle dancing was bimodally distributed with a pronounced lull at noontime, 12:00-13:00 h (Figure 3(a)). The angular accuracy of the deviation of all waggle phases from the mean vector (expected waggle phase) of the waggle dances over time was significantly reduced during the noon hour compared with other times (Heterogeneity G-test: $\chi^2_2 = 49.7$, $P < 0.0001$; Table 1). Typically in a waggle dance, the dancer runs straight ahead and returns in a semicircle to the starting point then runs again through the previous straight line direction and returns in a semicircle in the opposite direction [26]. The straight part of the run represents the “direction component” of the target, and the axis angle of this waggle phase relative to the vertical represents the angle of the goal in relation to the sun’s azimuth. Most of the dances observed between 10:00-11:00 h and between 13:00-14:00 h clearly had a direction component in the waggle phase, while between 12:00 h and 13:00 h about half of the dances had no direction component (Table 1). Moreover, angular accuracy between 10:00-11:00 h and between 13:00-14:00 h was significantly lower than that between 12:00-13:00 h.

The number of foragers dancing significantly declined at noontime compared to the morning or afternoon. Most of the waggle dances performed at noontime consisted of great errors in the accuracy of angle measurement, or a direction component was absent from the waggle dance. Similarly, in *A. mellifera*, waggle dances became disoriented when the sun was within $\pm 3^\circ$ of the zenith [2, 8, 9]. There are several

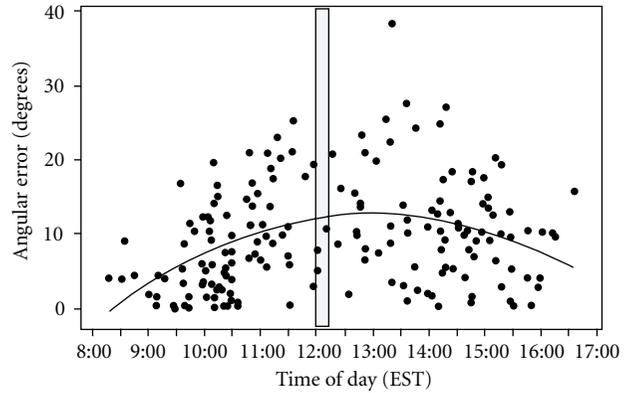


FIGURE 2: Inaccuracy in the dances of honey bees over the course of a day. The shaded area indicates the time during which the sun is at its highest altitude for the dates that dances were recorded [25].

possibilities as to why the red dwarf honeybees may have difficulty in dancing accurately and so avoid noontime. The changing azimuth is a major factor in this case. The closer the sun approaches the zenith, the more rapidly the azimuth changes at noon, particularly in the tropics. This makes it very difficult for the bees to accurately determine the azimuth because very slight errors in perception of the relative positions of the sun and zenith will lead to a very large error in estimating the azimuth of the sun.

The error of reading the sun’s azimuth also affects other activities of honeybee colonies. Consequently, between 12:00-13:00 h, the waggle dances become disoriented and less accurate and the bees take off for new nesting sites significantly less frequently at noontime [23]. This is also associated with the fact that the bees largely avoid dancing at noontime both for foraging (Figure 3(a)) and finding new nest sites (Figure 3(b)). Beekman et al. [22] showed that *A. florea* workers dance with the same imprecision irrespective of whether at a colony level seeking new nest sites or at an individual level as in foraging. To this we add that the level of imprecision in the dance language is exacerbated by the movement of the sun about high noon.

4. Abscending and the Azimuth

The movements of honeybee colonies away from the maternal nest come about in two ways: reproductive swarming and absconding/migration. Reproductive swarming is defined as the movement of at least one queen and part of a honeybee colony from the maternal nest to an entirely new site for colony reproduction. On the other hand, migration in its broadest sense is the seasonally predictable movement of many whole colonies of the same population from one region to another while absconding usually refers to abandonment of one or a few colonies away from the maternal nest site to another place, usually caused by local environmental perturbations [19, 27]. Indeed, seasonal migration and absconding are characteristic of *Apis florea*, throughout Asia, and these traits are linked to a combination of resource depletion and adverse microclimatic conditions [28–32].

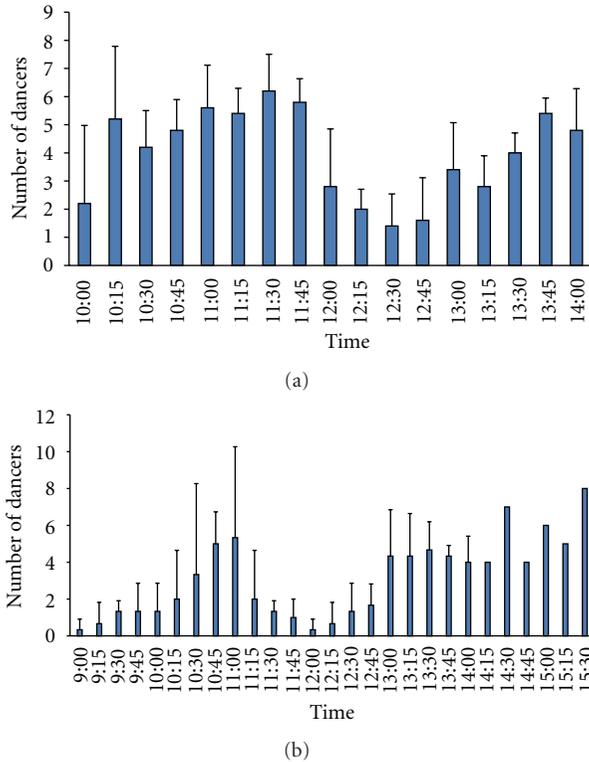


FIGURE 3: (a) The mean frequency distributions of the numbers of dancing bees for (a) food sources at Chom Bueng, Thailand (13.37N, 99.35E, altitude 86 m). (b) The mean frequency distributions of the numbers of dancing bees for new nest sites [23].

For a honeybee colony to successfully swarm or abscond requires, as in foraging, navigational skills. However in this section we are discussing the integrated behaviour of whole colonies and not just individual foragers. Given the daily rotation of the earth, honeybees have quite a range of solar angles available on each day that they choose to abscond. However, as seen with foraging, the noon hour in the tropics presents honeybee colonies with difficulties leading to a reduction in effective dances which results from inaccurately determining the sun’s position, and, as for foraging, slight errors in the perception of the relative position of the sun result in very large orientation errors [8, 9]. Similarly, Gardner [25] reported that the angular accuracy of waggle dances was correlated with the altitude of the sun even at 42°N. Because this becomes especially crucial when a colony decides to find a new nest site, Duangphakdee et al. [24] hypothesized that colonies of *A. florea* defer from absconding during that time of the day when perception errors might result in large orientation errors. Oldroyd et al. [33] suggested that dances of *Apis florea* for nest sites may only indicate a general direction, so that precise information in dances may be unnecessary because scout bees may release Nasonov pheromones to attract flying nestmates to join the cluster. However, accurate information communicated by the dances plays a role in the swarm movement by firstly advertising the target nesting site to the scout recruits, and the accuracy of dancing becomes important because

imprecise information will not lead bees to check the right site and will later influence the “voting mechanism” of the whole colony. Secondly, during swarm movements, scout bees release Nasonov pheromone for the guidance for other bees to move to a specified nesting site.

5. Azimuthal Lull

The observations of Duangphakdee et al. [23] were made on 37 separate absconding events by colonies of *A. florea*, between 2007/05/22 and 2009/02/18 at Chom Bueng, Thailand (13.37N, 99.35E, altitude 86 m). The brood comb extending below the crown was cut away and removed to induce absconding. Time was local clock time, *not* solar time. Once the absconding time data was entered into a spreadsheet, the altitude angles at which the bees absconded were calculated as was the sun’s zenith [34] for that particular day. Video recordings of each colony were made on the day of absconding from morning until the colony absconded. To assess any possible effects of daily temperature fluctuations on the temporal frequency of dancing, they also obtained hourly ambient temperature values for those days on which the colonies actually absconded.

Duangphakdee et al. [23] found that the frequency distribution of absconding with respect to local clock time was bimodal with a pronounced lull between 12:00 h and 13:00 h. Nearly 90% of absconding occurred between 09:00 h and 12:00 h (32.3%) and between 13:00 h and 16:00 h (57.1%) (Figure 4(a)). The altitude angle (sometimes referred to as the “solar elevation angle”) describes how high the sun appears in the sky. The altitude angle is measured between an imaginary line between the observer and the sun and the horizontal plane the observer is standing on, in this case, the apiary colonies at Chom Bueng. The mean (\pm SD) altitude angle corresponding to the times of absconding between 11:50 h was $56.2 \pm 6.3^\circ$ and between the absconding times between 13:05 h and 14:45 h was $66.1 \pm 9.7^\circ$. No absconding took place below an altitude angle of 38.7° , nor above an altitude angle of 81.4° . The distribution of the altitude angles averaged $60.0 \pm 10.3^\circ$. The altitude angles when the sun was at its zenith were determined for each day (Figure 4(b)). The mean angle was $71.7 \pm 11.4^\circ$, with a range from 55.0° (in November) to 88.3° (in May). The records of absconding time in 35 cases taken from other species (*A. dorsata*, *A. cerana*, *A. Mellifera*, and *A. andreniformis*) also show that only 8.5% absconded at noon (Figure 4(c)).

From Figures 4(a) and 4(c), it can be seen that the bees merely abscond in the early morning and late afternoon, and this can be explained by the bees needing time for navigation to a food source/nest site and it requires about two hours to reach a quorum. For example, if the bees had started dancing between 18:00 and 19:00 h the bees would only have an available time window for absconding from about 21:00 h by which time darkness has fallen.

The mean frequency distribution of the numbers of foragers dancing in three colonies is shown in Figure 3(a) from which it is clear that dancing was bimodally distributed with a pronounced trough between 12:00 h and 13:00 h. Until the time of actual absconding, foragers were advertising

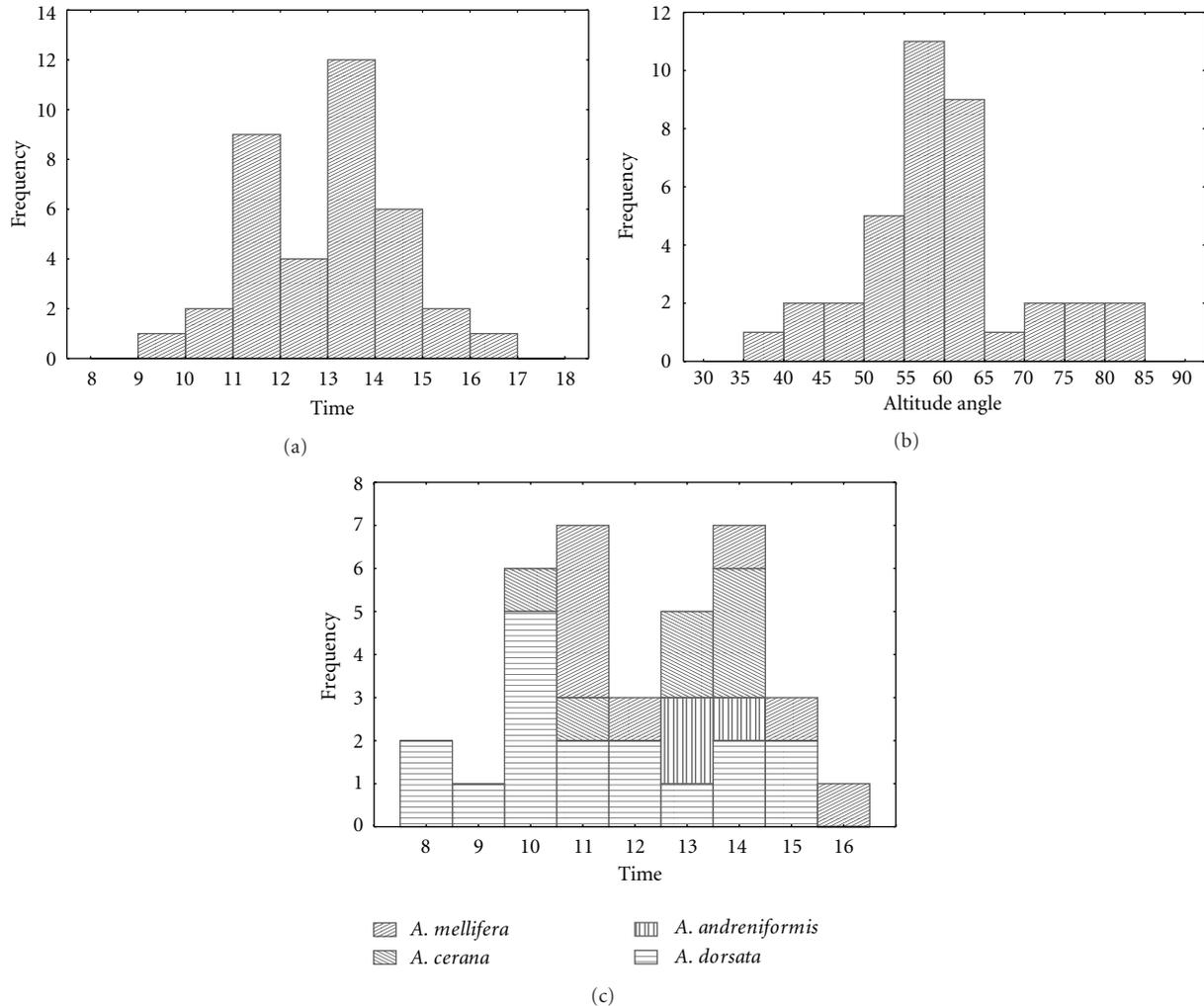


FIGURE 4: (a) Frequency distribution for local clock time of absconding by the red dwarf honeybee at Chom Bueng, Thailand (13.37N, 99.35E) during 2007–2009 ($n = 37$) [23]. (b) Frequency distribution for absconding by the red dwarf honeybee at Chom Bueng, Thailand (13.37N, 99.35E) with respect to altitude angle of the sun (in degrees) during 2007–2009 ($n = 37$) [23]. (c) Frequency distribution for local clock time of absconding by *A. dorsata* ($n = 3$ [35], $n = 14$ [36]), *A. cerana* ($n = 1$ [37], $n = 4$ Duangphakdee, O. personal observation, $n = 2$ Wongvilas, S. pers. comm.), *A. mellifera* ($n = 6$ [38], $n = 2$ [39]), and *A. andreniformis* ($n = 3$ Wongvilas, S. pers. comm.).

different directions for a new nest site. Turning to angular accuracy of the waggle dance over time, during the morning period 10:00–11:00 h, of 60 observed dances, 51 clearly had a direction component in the waggle dance and 9 did not. Between 12:00 h and 13:00 h, only 17 of 60 dances had a direction component while 43 were nondirectional. Of those dances with a direction component performed between 10:00 h and 11:00 h, the deviation in angle accuracy was 0.24° ; however between 12:00 h and 13:00 h, the deviation in angle accuracy was 10.11° [23].

The possibility that both the noonday lull and actual absconding time might be related to particular temperature profiles was considered. However, the temperature data from 07:00 h to 17:00 h during February 2009 at Chom Bueng, Thailand, which is shown in Table 2, indicates that the noonday lull is not associated with the highest temperatures of the day for any of the 7 days shown. Moreover, another

indication that the noonday lull is not related to the temperature is given by the fact that absconding was clearly not inhibited by high temperatures because in 7 out of 8 absconding events occurred at temperatures greater than the corresponding noonday lull [23].

6. Azimuthal Preferences

The results on the frequency distributions of absconding by the red dwarf honeybee with respect to both time (Figure 4(a)) and altitude angle of the sun (Figure 4(b)) make it evident that these bees largely avoid flying off between 12:00 h and 13:00 h on the one hand and that their preferred departure angle of the sun is between 55° and 65° , on the other. However, there is no linear correspondence or relationship between time and sun angle (Figure 1). The preferred altitude angles at which the bees absconded were

TABLE 2: Hourly temperatures at Chom Bueng, Thailand on absconding days for 8 colonies of *Apis florea* and absconding times and ambient temperatures (T) at absconding events.

Date	Colonies	Absconding		Time of day								
		Time	T (°C)	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00
2/8/2009	1	11:00	33.45	25.79	30.64	32.51	34.69	36.97	38.82	40.07	38.99	36.6
2/9/2009	2	13:40	37.20	26.01	31.41	33.13	35.32	37.6	39.09	40.19	40.16	37.27
2/12/2009	3	14:36	43.14	21.79	33.32	35.4	37.75	39.9	41.93	43.22	41.39	38.49
2/13/2009	4	13:52	41.26	24.25	32.42	34.86	37.29	39.39	40.85	41.47	42.64	39.26
2/16/2009	5	13:22	42.80	29.4	33.27	34.77	37.63	40.05	42.06	43.64	43.16	39.08
	6	13:46	42.25									
2/17/2009	7	12:25	43.24	27.33	34.06	35.9	38.37	40.91	43.34	44.53	44.59	40.91
2/18/2009	8	12:45	39.14	25.43	33.63	34.82	36.39	38.74	41.07	42.73	43.59	38.7
Average (°C)				25.71	32.68	34.48	36.78	39.08	41.02	42.26	42.07	38.66
SD				2.38	1.25	1.22	1.36	1.40	1.63	1.73	1.99	1.40

on average about $\pm 6^\circ$ on either side of the sun's zenith for that particular day, despite the fact that the visual acuity of honeybees is about 1° [40]. The mean frequency distribution of the numbers of foragers dancing in three colonies declined in the noontime lull, and likewise, the angular accuracy of the direction component declined precipitously. To understand the rationale of the nest site selection process of honeybees, it extends through group decision making processes at the end of which only one site becomes dominant in further scouting and dancing [38, 39]. A reduction of dancing and the presentation of nonconstructive dances (or disoriented dances) would have greatly disturbed the decision making process.

There are no other similar datasets for swarming, migrating, or absconding in honeybees, but there are three relevant reports with respect to foraging. First there is the report of Lindauer [2] that waggle dances became disoriented when the sun was within $\pm 3^\circ$ of the zenith. There are other reports by New et al. [8] and D. A. T. New and J. K. New [9] which noted similar difficulties for waggle dancers between $\pm 3^\circ$ and 4° at tropical latitudes similar to those of Chom Bueng. New et al. [8] and D. A. T. New and J. K. New [9] also observed that when over a few days the sun's position switched from north to south, the bees began to confuse observed sun angle with both clockwise and anticlockwise dances.

There are several possibilities to consider as to why the red dwarf honeybee may avoid the noontime period. Temperature can be excluded because the noontime lull was not associated with the highest daily temperatures (Table 2) and the bees were absconding at higher temperatures than those of the noontime lull. Alternatively, Dyer and Dickinson [41] suggested that celestial compass orientation requires the use of a time-compensated measure of the sun's azimuth, based on an innate template that can be adjusted by learning. Be that as it may, learning takes time that absconding bees lack. The time element becomes critical because the closer the sun passes to the zenith, the more rapidly the azimuth changes at noon particularly in the tropics. Before a colony will swarm or abscond, it goes through a process of reaching a consensus on where to ultimately go [38].

7. Azimuthal Windows

In *Apis mellifera*, a new nest site will be selected through the bee's decision making process. A priori, scouts will go out and find a prospective home site. Different scouts will return to the nest/swarm and communicate the location of her finding by means of waggle dances. Initially, the scouts perform dances for a number of different sites, but eventually they all dance for just one site by "reaching consensus," shortly before whole colony takes off [38, 39]. It is generally assumed that the chosen site is the best of the sites that they have discovered. The nest site selection process of *Apis florea* has been reported to be quite similar to that of *Apis mellifera* [3, 33]. One difference is that nondirectional dances occur significantly more in *Apis florea*. Nevertheless, like *Apis mellifera*, all waggle dances also eventually converge on one site shortly before becoming airborne.

In the case of the red dwarf honeybee, video recordings of absconding dances indicate that consensus takes about 2 h to achieve (Duangphakdee, unpublished observations). No days at Chom Bueng have less than 12 h sunlight, and no days have ambient temperature too low for honeybee flight, yet the red dwarf honeybees only use a time window almost exactly one half of that available to abscond. The hour from 12:00 h to 13:00 h is a definite lull period for *A. florea* (noontime laziness of von Frisch [11]). Given the difficulties of taking an accurate reading of the sun at angles $\pm 6^\circ$ of the sun's zenith (resulting in a 1 h loss around noon) and the 2 h required to reach consensus, the bees are simply left with two time windows, morning and afternoon, in which to abscond and, indeed some 90% of the red dwarf honeybee colonies do so.

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Research Article

Pollen Sources for *Melipona capixaba* Moure & Camargo: An Endangered Brazilian Stingless Bee

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Pollen samples were collected in three different periods from 11 *Melipona capixaba* Moure & Camargo hives and analyzed with melissopalynological methodology. A total of 33 pollen types were identified, of which 23 genera and 15 families were identified. The following families showed the highest pollen richness: Fabaceae (7), Myrtaceae (3), Solanaceae (3), Arecaceae (2), Asteraceae (2), Euphorbiaceae (2), Melastomataceae/Combretaceae (2), Rubiaceae (2), and Sapindaceae (2). The most frequent pollen types (>45%) were *Eucalyptus*, which generated great similarities between the samples, except one in which the *Tibouchina* was predominant. Although the majority of the pollen types showed low percentage values, the results demonstrated that *M. capixaba* has taken advantage of the polliniferous sources available in the Atlantic Rainforest as well as in the “Capoeira” (brushwood, secondary forest) and “ruderal” (field) plants, probably implying its importance as a pollinator of the native flora and of the exotic species.

1. Introduction

Melipona Illger constitutes the genus of Meliponini tribe with the biggest amount of species. It occurs in the whole neotropical region, which is the most diversified in the Amazon basin [1]. *M. capixaba* (popularly known as uruçu-preta or uruçu-capixaba) is endemic to the Atlantic Rainforest where it is restricted to the mountainous area of Espírito Santo State in the municipalities of Domingos Martins, Conceição do Castelo, Venda Nova do Imigrante, Alfredo Chaves, and Afonso Cláudio embracing Pedra Azul State Park, which is protected by the government. *M. capixaba* was described by Moure and Camargo [2] who referred it to the Meliponini group of the Amazon region and designated by Rocha and Pompolo [3] to the same karyotype group of *M. scutellaris* Latreille. Experiments proved that the lack of anatomical or behavioural isolation mechanisms allowed the crossing of these species when they were brought into the same area. These observations suggest that the two species are capable of forming fertile hybrids [4]. The fact that two

ecologically different species of stingless bees, separated by more than 300 km, could still cross when placed in the same area suggests that there has not been any pressure to develop reproductive isolation [4]. However, *M. capixaba* is mentioned on the list of Endangered Brazilian species (Normative Instructions no. 3, May 27th 2003, Ministry of Environment) because its original habitat has been almost completely fragmented to make room to pastureland, coffee (*Coffea* sp), and *Eucalyptus* sp cultivars. Currently only 10% of the original Atlantic Rainforest remains and has become a “hot spot” for this bee [4, 5].

Despite its endangered status and ecological importance as pollinator, few studies have examined the ecology and biology of *M. capixaba*. Pollen analysis may be useful to indirectly determine its food sources and help clarify its role as a vegetation pollinator [6]. Knowledge of the several pollen sources that are used by *M. capixaba* in its natural environment helps the beekeepers to manage them. Likewise, complementation of the ecological data,

TABLE 1: Data regarding origin and date of collection of the pollen samples stored in food pots of *Melipona capixaba*.

Samples	Origin (Latitude–Longitude)	Date of collection
FC0	Venda Nova do Imigrante municipality (S20° 18' 57.6"–W41° 07' 55.2")	october 2007
JV1	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	may 2008
JV2	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	may 2008
JV3	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	may 2008
EM4	Domingos Martins municipality (S20° 27' 22.8"–W41° 00' 27.8")	may 2008
JOV5	Alfredo Chaves municipality (S20° 32' 56.4"–W40° 48' 02.8")	may 2008
JOV6	Alfredo Chaves municipality (S20° 32' 56.4"–W40° 48' 02.8")	may 2008
JOV7	Alfredo Chaves municipality (S20° 32' 56.4"–W40° 48' 02.8")	may 2008
FC8	Venda Nova do Imigrante municipality (S20° 18' 57.6"–W41° 07' 55.2")	may 2008
JV9	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	march 2009
JV10	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	march 2009

obtained through melissopalynological analysis, is important for the development of the preservation programs for this bee. This may help in directing the efforts to recover the vegetation in the affected areas utilizing botanic species that guarantee its food supply. There is only one publication in the scientific literature that reports a case of the workers of *M. capixaba* carrying *pollinarium* attached to the *scutellum*, of the orchid subtribe Maxillariinae species, possibly of the genus *Maxillaria sensu lato* or *Xylobium* [7]. However, food storage in the beewax pots of *M. capixaba* has not yet been palynologically analyzed. The aim of this study was to investigate the influence of the local flora on the pollen harvest by *M. capixaba* to characterize the vegetation in which the sources were obtained in order to help in the conservation efforts of this bee.

2. Materials and Methods

Pollen samples were collected from 11 hives of *Melipona capixaba* found inside the tree-trunks and maintained by beekeepers of three different regions. The pollen samples were collected in different periods (October 2007, May 2008, and March 2009) directly from their food storage in the beewax pollen pots. Six samples originated from Domingos Martins municipality were defined as JV1, JV2, JV3, EM4, JV9, and JV10; three from Alfredo Chaves (JOV5, JOV6, and JOV7), and two from Venda Nova do Imigrante (FC0, FC8), all of them from Espírito Santo State, Brazil (Figure 1, Table 1). The meliponary located in Venda Nova do Imigrante municipality (FC) stays near

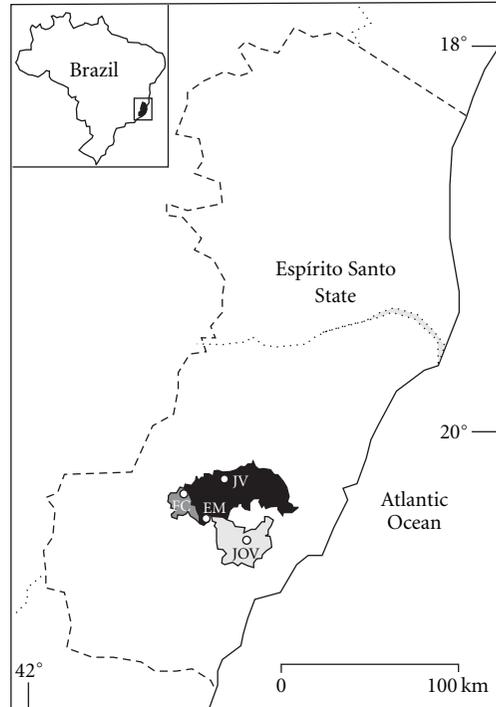


FIGURE 1: Location of the municipalities where the pollen samples of the food pots of *M. capixaba* were collected. Black = Domingos Martins municipality; dark grey = Venda Nova do Imigrante municipality, and light grey = Alfredo Chaves municipality.

to the urban area, being a small farm where they raise chicken and cultivate orchids, besides raising stingless bees. The bees collect floral resources in small forest fragments nearby, and there are some *Eucalyptus* cultivars a few meters far from the meliponary. The meliponary from Alfredo Chaves municipality (JOV) is located within a Particular Reserve of the Natural Patrimony (a private land), an area with secondary forest that has been acquired by the land owner for ecological tourism. There are a lot of coffee and *Eucalyptus* cultivars in the region. The meliponary located north Domingos Martins municipality (JV) is inside a small farm near small forest fragments that are permanent forest reserves inside private properties in the neighborhood. There are fruit, vegetable, and *Eucalyptus* cultivars. The meliponary south Domingos Martins municipality (EM) is inside a small farm near great areas of primary or secondary native forests in high stages of succession, within a permanent forest reserve in “Hotel Monte Verde”, occupying an area of about

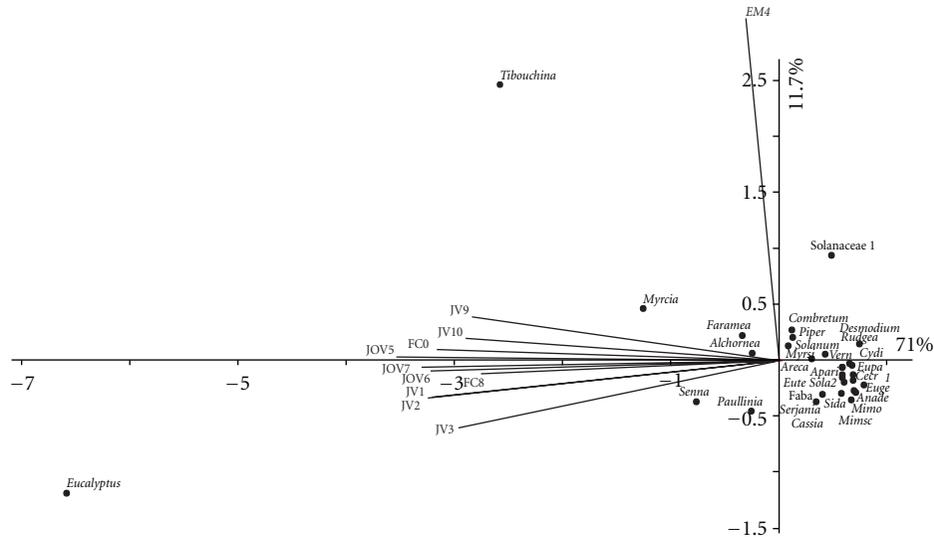


FIGURE 2: Analysis of the main components (PCA) of pollen samples, using the absolute value variables per sample. Pollen samples = FC0, JV1, JV2, JV3, EM4, JOV5, JOV6, JOV7, FC8, JV9, JV10. • = Pollen types.

3,000 hectares, and that uses the place for ecological tourism. Besides that, this meliponary is close to two State Parks, State Park of Forno Grande and State Park of Pedra Azul. There are many family farms in the region that cultivate flowers, fruits, and vegetables, besides agro-ecotourism, without *Eucalyptus* cultivars.

All the sediments of one pollen pot were analyzed as a single samples of one hive. The samples were acetolysed [8]. Microscope slides were prepared using jelly glycerine and sealed with paraffin. All samples were observed under traditional light microscopy. Pollen identification was done using literature data [9, 10] and the reference pollen slide collection of the Palynology Research Center, Instituto de Botânica, from the Environment Department of São Paulo State. A total of 500 pollen grains of each sample were counted for the frequency calculations, as shown in Table 2. Percentage values above 2% of the total count are specified. Interpretation of the data takes in account all the pollen grains of plant taxa. The term “pollen type” means a single plant species or a group of species, or higher taxa, presenting similar pollen morphology. “Monofloral” means originating mostly from a unique plant species (pollen type with >90% of the total) [11].

The multivariate analysis was performed through Principal Component Analysis (PCA) in order to verify the pollen grain occurrence in the samples. The matrix comprised the absolute value of all taxa found in each sample. The absolute numerical variables were transformed into natural logarithms $[\log(x+1)]$ using the FITOPAC program [12] and the ordination was done through covariance matrix using PC-ORD 4.0 [13].

3. Results

Thirty-three pollen types were identified with regards to family, genus, and species taxa (Table 2). The families that

showed the highest richness of pollen types were Fabaceae (7), Myrtaceae (3), Solanaceae (3), Arecaceae (2), Asteraceae (2), Euphorbiaceae (2), Melastomataceae/Combretaceae (2), Rubiaceae (2), and Sapindaceae (2). The variability between the samples of pollen comprised 82.7% on the two first axis in the Principal Component Analysis (PCA) (Figure 2). Axis 1 alone comprised 71.0%. Regarding correlation among the pollen types, the pollen samples showed great similarity related to the high occurrence of the *Eucalyptus* (Figure 3(e)), except for the EM4 sample, from Domingos Martins, in which it was absent. Further *Tibouchina* (Figure 3(d)) was common in the samples, absent only in the JV3 sample, but predominant in the EM4 sample (Table 2). *Myrcia* (Figure 3(f)), even though is also a main characteristic component, occurred in small percentages in all samples. *Paullinia* (Figure 3(h)) was observed in most samples, however in small quantities. *Senna* (Figure 3(b)) occurred in 5 samples with varying percentages. Other pollen types that were expressive, although present in few samples, were *Alchornea* (Figure 3(a)), *Combretum* (Figure 3(c)), *Euterpe/Syagrus*, *Faramaea*, *Piper* (Figure 3(g)), and *Solanaceae* type 2 (Figure 3(i)) (Table 2).

4. Discussion

The species found in the State Park of Pedra Azul forest [14] include *Alchornea triplinervia*, *Andira* sp, *Annona* sp, *Astronium graveolens*, *Cariniana estrellensis*, *Carpotroche brasiliensis*, *Cedrela* sp, *Didymopanax morototoni*, *Erythroxylum subsessilis*, *Euterpe edulis*, *Faramaea* sp, *Fuchsia regia*, *Geonoma schottiana*, *Guapira opposita*, *Melanoxylon* sp, *Miconia inaequidens*, *M. latecrenata*, *Myrsine coriacea*, *M. parvifolia*, *M. umbellata*, *Nectandra* sp, *Ocotea* sp, *Rollinia* sp, *Senna* sp, *Serjania* sp, *Schizolobium* sp, *Solanum* sp, *Solanum capsicoides*, *Sorocea ilicifolia*, *Tabebuia* sp, *Tibouchina* sp, and several species of the Myrtaceae family (mainly *Myrcia*

TABLE 2: Pollen types observed in the pollen sediment storage in the beewax pollen pots of *Melipona capixaba*.

Samples	Total of pollen types	Main pollen types	Pollen types with minor importance (<5%)	Classification of the sample
FC0	11	<i>Eucalyptus</i> (85.3%) and <i>Tibouchina</i> (9.4%)	Arecaceae type 1, <i>Faramea</i> , <i>Myrcia</i> , <i>Myrsine</i> , <i>Senna</i> , <i>Serjania</i> , <i>Solanum</i> , Solanaceae type 1 and Solanaceae type 2	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Tibouchina</i>
JV1	6	<i>Eucalyptus</i> (96.1%)	Fabaceae type 1, <i>Faramea</i> , <i>Tibouchina</i> , <i>Myrcia</i> and <i>Paullinia</i>	Monofloral
JV2	9	<i>Eucalyptus</i> (95.7%)	<i>Alchornea</i> , <i>Faramea</i> , <i>Tibouchina</i> , <i>Mimosa</i> , <i>Myrcia</i> , <i>Myrsine</i> <i>Paullinia</i> and <i>Sida</i>	Monofloral
JV3	6	<i>Eucalyptus</i> (96.6%)	<i>Faramea</i> , <i>Mimosa scabrella</i> , <i>Myrcia</i> , <i>Paullinia</i> and not identified type 1	Monofloral
EM4	8	<i>Tibouchina</i> (87.8%) and Solanaceae type 2 (8.4%)	<i>Eupatorium</i> , <i>Faramea</i> , <i>Myrcia</i> , <i>Paullinia</i> , <i>Desmodium</i> and <i>Vernonia</i>	Heterofloral with great contribution of <i>Tibouchina</i> and Solanaceae
JOV5	5	<i>Eucalyptus</i> (87.9%) and <i>Tibouchina</i> (10.3%)	Arecaceae type 1, Fabaceae type 1 and <i>Myrcia</i>	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Tibouchina</i>
JOV6	8	<i>Eucalyptus</i> (87.2%) and <i>Senna</i> (8.9%)	<i>Alchornea</i> , <i>Crotalaria</i> , <i>Tibouchina</i> , <i>Myrcia</i> , <i>Paullinia</i> and <i>Vernonia</i>	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Senna</i>
JOV7	8	<i>Eucalyptus</i> (91.4%)	<i>Alchornea</i> , <i>Cassia</i> , <i>Tibouchina</i> , <i>Paullinia</i> , <i>Myrcia</i> , Fabaceae type 1, and not identified type 2	Monofloral
FC8	13	<i>Eucalyptus</i> (75.4%), <i>Senna</i> (11.9%) and <i>Myrcia</i> (6.6%)	<i>Euterpe/Syagrus</i> (2.4%), <i>Eupatorium</i> , <i>Paullinia</i> , <i>Tibouchina</i> , <i>Combretum</i> , <i>Anadenanthera</i> , <i>Alchornea</i> , <i>Solanum</i> , <i>Piper</i> and not identified type 3	Heterofloral with great contribution of <i>Eucalyptus</i> , <i>Senna</i> and <i>Myrcia</i>
JV9	16	<i>Eucalyptus</i> (53.4%) and <i>Tibouchina</i> (28.6%)	<i>Piper</i> (4.7%), <i>Alchornea</i> (3.1%), <i>Myrcia</i> (2.8%), <i>Paullinia</i> , <i>Aparisthmium</i> , <i>Faramea</i> , <i>Rudgea jasminoides</i> , <i>Sida</i> , <i>Myrsine</i> , <i>Senna</i> , <i>Cecropia</i> , <i>Solanum</i> , <i>Combretum</i> and <i>Cydista heterophylla</i>	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Tibouchina</i>
JV10	14	<i>Eucalyptus</i> (59.5%), <i>Tibouchina</i> (13.9%), <i>Combretum</i> (8.2%) and <i>Myrcia</i> (6.5%)	<i>Senna</i> (3.0%), <i>Alchornea</i> (2.1%), <i>Faramea</i> (2.1%), <i>Aparisthmium</i> , <i>Piper</i> , <i>Cydista heterophylla</i> , <i>Euterpe/Syagrus</i> , <i>Solanum</i> , <i>Myrsine</i> and <i>Rudgea jasminoides</i>	Heterofloral with great contribution of <i>Eucalyptus</i> , <i>Tibouchina</i> , <i>Combretum</i> and <i>Myrcia</i>

and *Eugenia* genera), among others. In the forest outskirts one can frequently observe *Guapira opposita*, *M. latecrenata*, *Erythroxylum ovalifolium*, *Psychotria hancornifolia*, and *M. coriacea*. The areas of rocky outcrops present a singular herbaceous-shrubby stratum with *Aechmea* sp, *Alcantarea imperialis*, *Baccharis* sp, *Epidendrum denticulatum*, *Fuchsia regia*, *Leandra* sp, *Melinis minutiflora*, *M. inaequidens*, *M. latecrenata*, *Poliavana* sp, *Polypodium* sp, *Pseudolaelia vellozicola*, *Tibouchina* sp, *Vellozia* sp, *Vernonia* sp, and *Vriesea carinata*. The glades are characterized by *Cecropia* sp, *Mimosa* sp, *Solanum* sp, and *Solanum capsicoides*, with significant populations of the pteridophyta *Pteridium aquilinum* and *Melinis minutiflora* grass. In the urban gardens are found bromeliads and orchards (with guava, lime, orange, etc.). Pasture lands are dominated by *Baccharis dracunculifolia*, *Bidens pilosa*, *Borreria verticilata*, *Chamaesyce prostrata*, *Chamaecrista* sp, *Crotalaria claussemi*, *Emilia sonchifolia*, *Mellines minutiflora*, *Panicum maximum*, and *Sida* sp, among others. Based on this, it is confirmed that *M. capixaba* harvested the polliniferous resources from natives trees,

shrubs, and vines (*Alchornea*, Arecaceae, *Cassia*, *Combretum*, *Eugenia*, *Euterpe/Syagrus*, *Faramea*, *Myrcia*, *Myrsine*, *Paullinia*, *Senna*, *Serjania*, several Solanaceae, and *Tibouchina*), as well as pollen types from native trees not present in the referred list (*Anadenanthera*, *Aparisthmium*, and *Cydista heterophylla*). The altered vegetation “Capoeira” (brushwood, secondary forest) and ruderal (field) plants were represented by the pollen types *Cecropia*, *Combretum*, *Crotalaria*, *Desmodium*, *Eupatorium*, *Mimosa scabrella*, *Piper*, *Sida*, several Solanaceae, *Tibouchina* and *Vernonia*, apart from the exotic species of *Eucalyptus*, and *Rudgea jasminoides*. In spite of the high richness of pollen types, the results demonstrated a similarity between the hives regarding the preferences of pollen of *Eucalyptus*, a widely cultivated tree in the region, and, with less intensity, pollen of *Tibouchina*, a common plant in the native forest. The EM4 sample from the meliponary south Domingos Martins was the only one that did not show *Eucalyptus* pollen grains, because it is located in one of the most well-preserved areas of the region, without cultivars of this plant in the surroundings. The other areas

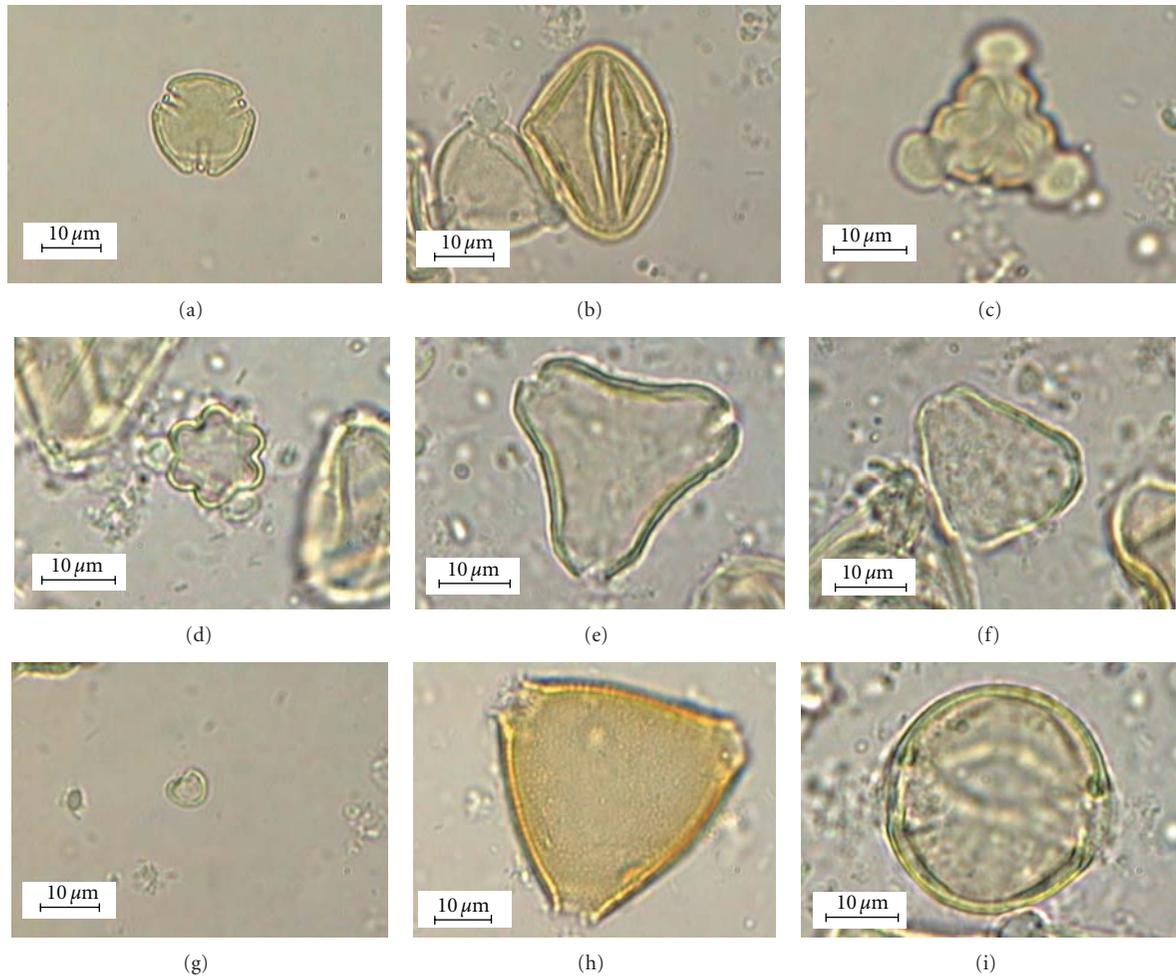


FIGURE 3: Most frequent pollen types observed in pollen samples from *Melipona capixaba* food pots. (a) Euphorbiaceae, *Alchornea*, polar view; (b) Fabaceae, *Senna*, equatorial view; (c) Melastomataceae, *Combretum*, polar view; (d) Melastomataceae, *Tibouchina*, polar view; (e) Myrtaceae, *Eucalyptus*, polar view, optical section; (f) Myrtaceae, *Myrcia*, polar view, optical section; (g) Piperaceae, *Piper*; (h) Sapindaceae, *Paullinia*, polar view, surface; (i) Solanaceae, type 2, equatorial view, optical section; Bar = 10 μ .

of the meliponaries (JOV, JV, and FC) are more deforested and replaced by pastures, and are near *Eucalyptus* cultivars. The palynological results from this sample showed that in the presence of native floral resources *M. capixaba* efficiently visits and harvest those resources, indicating their original pollen sources.

Studies based on the visitation of the pollinators of the flowers of the Atlantic Rainforest concluded that stingless bees are not specialized visitors in certain plant species. Only 7% of the plants in the Atlantic Rainforest are intensely visited by native bees; 77% of the plants are visited with less frequency, and 16% are not visited at all [15]. Analysis of palynological results in Brazil point to a great variety of trophic resources and generalized habits of pollen harvesting by the Meliponini that commonly visit a larger number of plant species [16]. However, regional studies show that the Meliponini frequently concentrate the pollen harvest to a few floral sources [17, 18]. Several studies under natural conditions in the Tropical Atlantic Domain concluded that colonies of different species of *Melipona* frequently searched floral sources of the trees of families Melastomataceae,

Mimosaceae, Myrtaceae, and Solanaceae, apart from the genus *Cassia* of family Caesalpiniaceae [17, 19–21]. The present study corroborates in great part this tendency as the families Melastomataceae and Myrtaceae were the most procured families by *M. capixaba* for pollen harvest while families Mimosaceae, Solanaceae, and plants of the genus *Cassia* (or *Senna*), although utilized, were visited to a significantly lesser extent.

Selectivity or floral preferences is a behaviour that is frequently observed among *M. scutellaris* [22]. Field research in the northeast Brazil confirmed that it prefers trees of the Atlantic Rainforest, as well as “Capoeira” vegetation, to herbs, and that it is rather selective with reference to the food source [23]. Palynological studies on pollen pellets of *M. scutellaris* in forest areas showed that the floral source preferences depended on the plant species, demonstrating harvesting selectivity as follows, with decreasing order of importance: Myrtaceae, Mimosaceae, Anacardiaceae, Sapindaceae, Caesalpiniaceae, and Fabaceae [6]. In our analyses, the selectivity also occurred in relation to the *M. capixaba* with the Myrtaceae and the Melastomataceae families as the

most popular pollen suppliers. Four pollen samples were monofloral and seven samples were heterofloral with a major percentage contribution of pollen types from these families. During the analyzed periods (March, May, and October) there was a preference of *Eucalyptus* and *Tibouchina*, in spite of other pollen sources in the region. *Combretum* (in one sample), *Myrcia* (two samples), *Senna* (two samples), and Solanaceae (one sample), also contributed with high pollen percentages. Other plants, including herbs, were sporadically visited by *M. capixaba*.

The search for *M. capixaba* in *Eucalyptus* is not a new fact for the Meliponini as the occurrence of its nectar and its pollen has been reported dominant or important in the pollen spectrum of honey and pollen pellets of other species of *Melipona* [18, 19, 21, 24, 25]. However, the almost exclusive usage of only one floral resource by *M. capixaba* can put this species in danger on the absence of this resource, besides the fact that it cannot offer all the nutritional necessities needed by the bees. Other research demonstrated that the pollen nutritional composition harvest by *Apis mellifera* did not show a correlation with the pollen type diversity, but showed a correlation with the predominance of specific pollen types [26, 27]. These authors pointed as well that the different floristic compositions have influenced on pollen pellet quality, and the harvest in different food sources by the bees is important for obtaining a well-balanced diet. The fact that the predominant polliniferous source for *M. capixaba* was *Eucalyptus* demonstrates as well an important economic issue. Nowadays there is some interest in local *Eucalyptus* cultivars, but this is not constant, because it depends on market interest. If in the future *Eucalyptus* cultivars would be replaced by any other reason (environmental or economic importance) and enough native pollen sources to feed the colonies would be absent it would have a significant prejudice for the bees, because *M. capixaba* is restricted to this montane region within the Espírito Santo's Atlantic Forest. Its restriction to this small occurrence area can be evolutionary related to the local biological characteristics, as local native flora. The palynological analysis showed the main pollen sources of native plants for *M. capixaba* and thus provided important information for future researches on the pollination biology, allowing decision making on the creation of sustainable pastures to these bee.

In conclusion, *M. capixaba* took advantage of the polliniferous sources in the forest, especially *Combretum*, *Euterpe/Syagrus*, *Faramea*, *Myrcia*, *Senna*, and several species of Solanaceae and *Tibouchina*, indicating its importance as a pollinator of the native flora, as well as in ruderal plants ("Capoeira"), even though the characteristic pollen types of these environments occurred in low percentages. The main pollen harvest was in *Eucalyptus* cultivars. The mechanisms that increased the frequency of utilization of *Eucalyptus*, even in natural forest areas, still have to be better understood in order to clarify the influence of the nutritional quality of this pollen on its diet. Physiochemical analysis of the pollen from the Myrtaceae family showed a positive and highly significant relation of crude proteins in *Eucalyptus* and, consequently, a complementary negative relation for total carbohydrates [26, 27]. Studies should be undertaken to verify the influence

that a diet almost exclusively consisting of *Eucalyptus* has on *M. capixaba*.

In order to guarantee a varied supply of *M. capixaba* pollen, it is recommended that the beekeepers and others interested in its preservation recover affected areas, reforesting with polliniferous native plants that now are known. The foraging behaviour of the *M. capixaba* explains important ecological consequences in terms of persistence capacity of the species in the environment, as in cases of local extinction of their preferential native floral sources.

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Research Article

Environmental Factors Influencing Foraging Activity in the Social Wasp *Polybia paulista* (Hymenoptera: Vespidae: Epiponini)

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Foraging behavior in social wasps is important in the development of the colony and reflects an important ecological interaction between the colony and the environment. Although the social traits of the colony play a role in the foraging activities, the conditions that establish the space and time limits are mainly physical. Here, we evaluate colonies of *Polybia paulista* throughout one year in order to verify the foraging activities and the items collected, as well as the importance of temperature, relative humidity, and solar radiation on motivating foraging. Collection of liquids was always higher than that of solids; preys were collected all year long, and nests showed two annual episodic expansions. The linear mixed effects (LME) model used to analyze which weather factors influence the foraging showed temperature as the most influencing factor on the collection of materials.

1. Introduction

Polybia is the genus of Neotropical swarm-founding Polistinae (Epiponini) with the highest number of species. The swarm-founding polistines are largely tropical in distribution, and although they comprise a relatively small group in terms of species number, they exceed all other eusocial wasp groups in terms of taxonomic diversity at the generic level, diversity of nest architecture, and range of colony size [1].

Although the Epiponini are highly social bearing complex societies, the morphological caste differentiation is often weak [2]. Bourke [3] suggested that complex societies have complex division of labor, caste differentiation, and a large number of individuals. Conversely, Jeanne [4] suggested that complexity in epiponines is more related to the number of behavioral acts performed by the worker caste than to morphological caste differences.

Sociality extends the possibilities of obtaining resources and provisioning the colony since it shows behaviors such as recruitment and division of labor [5]. Different patterns of social organization among wasps correspond to different possibilities for partitioning location, collecting, transporting, or storing of resources [5].

In Epiponini, workers perform basic tasks related to nest building, foraging, brood feeding, and defense [1]. Such tasks are usually quite complex and highly organized [1]. Division of labor is based on worker's age, and it is very well developed in epiponines even in species with very small colonies [6, 7]. Workers pass through three discrete temporal castes: younger workers perform low-risk activities inside the nest [7]; midage workers perform external nest activities, such as construction, cooling, reception of loads from incoming foragers, and defense against predator and parasites [7]; finally, older workers perform high-risk activities away from the nest, such as foraging [6–10].

Foraging behavior in social wasps is important in the development of the colony and provides a significant understanding on the involvement of the castes in the division of labor [5, 7]. Furthermore, it reflects an important ecological interaction between the colony and the environment, since the colony needs water, plant fibers (pulp), protein, and carbohydrates [11]. The water is required for temperature control [12, 13], nest construction [14], and metabolic processes [5]. Plant fibers are essential for construction and repair of cells, peduncle, and envelope [15]. Animal protein is

used mainly for feeding the larvae [16]. Carbohydrates serve as an important energy source for both adult individuals and brood, so they represent a critical supply for the growth of a wasp colony [5, 13].

Many studies have been done with the purpose of verifying several aspects related to the flight and foraging activity of social wasps, such as daily and seasonal activity of searching for resources, items collected, influence of colony and environment factors on the foraging, and foraging activity behavior patterns [17–33]. The foraging activity of these wasps varies throughout a day and throughout the seasons, but also according to the place where the study is carried out [26].

Although the social behavior of the colony plays an important role in the process of foraging activities, the conditions that establish the limits where and when foraging is feasible are mainly physical, such as light and temperature intensity [34]. Temperature and relative humidity seem to have a great influence on the foraging activity of Neotropical social wasps [19, 20, 24, 26, 30, 31], even luminosity is also recognized as an important factor [18, 23, 30]. Moreover, other factors such as atmospheric pressure and winds may affect foraging rhythm of wasps, with longer days being more favorable for flight [17].

Even though physical conditions are important to determine foraging activity, few authors have tried to access worker behavior throughout a complete colony cycle and to correlate it with ecological aspects that may influence foraging activities. Furthermore, understanding the factors that influence insect activities, such as foraging, is primary for all kinds of posterior studies. So, annual studies such as the presented here bring a valuable contribution to our knowledge on social insect life histories. Here, we show that foraging behavior in *Polybia paulista* is mainly influenced by temperature.

2. Material and Methods

2.1. Study Site. The study was carried out on Universidade Estadual Paulista “Júlio de Mesquita Filho” campus IBILCE/UNESP (20° 49' 11" W, 49° 22' 46" S), city of São José do Rio Preto, São Paulo State, Brazil, from December 2007 to August 2008 and September 2009 to November 2009.

The study area is characterized by dry winter and wet summer [35] with annual mean temperature of 26.4°C and relative humidity of 68% [36]. The annual distribution of rainfall includes a rainy season with 85% of the total annual precipitation and a dry season with only 15% of the total annual precipitation [36]. The months of the year were divided in two separated seasons, the cold and dry season (CDS, mean temperature: 24.5°C, mean humidity: 63%) from April to September and the hot and humid season (HHS, mean temperature: 26.9°C, mean humidity: 71%) from October to March [36].

2.2. Data Collection. Two to four observations were performed monthly, 14 observations were carried out during the HHS, and 12 during the CDS in five different colonies (Table 1). All colonies were in postemergence stage according to the classification proposed by Jeanne [37] and had nearly

the same size, approximately 1,500 wasps. The exact amount of wasps was not censused because our primary goal was to accomplish as much observations as possible in the same nest.

The nests were observed for 15 minutes every interval of two hours, from 10:00 to 16:00 h, totaling one hour of daily observations. We counted the number of wasps returning to the nest and the type of material carried by them (pulp, prey, or liquid material).

In order to identify the material brought by the wasps, the behavior “landing on the nest” was taken into account as described by Prezoto et al. [38] and complemented by Pereira [27]. If she landed with a prey, she had a solid material in her mandibles and walked directly into the nest or, if the prey was very big, she divided this material with another wasp on the nest surface. After that, both entered the nest. When she returned with pulp, she had a dark and rounded solid material in her mandibles, and she passed or divided the material with other wasps. When the wasp returned with liquid (it was not possible to distinguish among wasps bringing nectar or water), she could enter in the nest without any visible material in her mouthparts or she could perform trophallaxis. In this case, one could see the droplets being transferred and her abdomen getting smaller. When she went back and did nothing for about 5 seconds, it was because she returned without any material. In some situations, it was not possible to identify successfully the type of material collected by foragers. In this case, the material was treated as unidentified (un).

We measured the weather conditions right below the nests at the end of each observation. To measure temperature and relative humidity, a thermohygrometer Incoterm was used under the tree where the nests were and light intensity was measured by using a digital luximeter ITLD-240 in an open area 1 m aside the nest.

2.3. Data Analyses. Since we are comparing data temporally correlated with one another, statistical methods that assume independence of observations could overstate the significance of any predictive factor. Linear mixed effects (LME) models offer a useful alternative to traditional univariate or multivariate repeated-measures ANOVA models [39]. Therefore, LME models were used to test for statistically significant influence of weather conditions on the number of wasps foraging.

In the first step, the basic model was compared to a model containing the same fixed effects and different random effects for seasonal adjustment (Table 2(a)). In the second step, the seasonally adjusted model without correction for serial correlation was compared to a seasonally adjusted model with serial correlation incorporated as a first-order auto-regressive (AR1) covariance structure (Table 2(b)). Finally, the optimal model in terms of the residual correlation structure was used to test which variables (temperature, humidity, luminosity, season, month, and hour) best explain the number of wasps foraging (Table 2(c)). We applied data dredge statistics (dredge-MuMIn R package, <http://r-forge.r-project.org/>) to automatically generate the best four models combining the fixed terms of the global

TABLE 1: Months in which each nest was sampled and the number of days that each nest was observed, as well as the substrates, growth habit, and height where the nests were found in the social wasp *Polybia paulista*.

Colony	Month(s) sampled	Number of observations	Substrate	Growth habit	Height
N1	Dec (2007), Jan, Feb, Mar, Apr (2008)	8	<i>Caesalpinia echinata</i> (Fabaceae)	Tree	1.5 m
N2	May, Jun (2008)	3	<i>Copernicia</i> sp. (Arecaceae)	Palm-tree	2.0 m
N3	Jul, Aug (2008)	4	<i>Licania tomentosa</i> (Chrysobalanaceae)	Tree	2.0 m
N4	Sep, Oct (2009)	7	<i>Licania tomentosa</i> (Chrysobalanaceae)	Tree	2.5 m
N5	Nov (2009)	4	<i>Copernicia</i> sp. (Arecaceae)	Palm-tree	1.8 m

TABLE 2: Linear mixed effects (LME) models performed between the number of wasps foraging in *Polybia paulista* and six explanatory variables (temperature, humidity, luminosity, season, month, and hour). Tested models comparison: k = degree of freedom, ΔAIC_C = difference in Akaike's Information Criterion for each model from the most parsimonious model, and $wAIC_C$ = AIC_C weight.

(a) Random effects			
	k	ΔAIC_C	$wAIC_C$
Nest + month	10	0.00	0.779
Nest + month + hour	11	2.5	0.221
Nest	9	21.7	<0.001
(b) Correlation structure			
	k	ΔAIC_C	$wAIC_C$
corAR1	11	0.00	0.541
corARMA	11	0.50	0.431
Without correlation	10	6.4	0.022
corCompSymm	11	8.9	0.006
(c) Fixed effects			
	k	ΔAIC_C	$wAIC_C$
Season + temp	7	0.00	0.41
Season + temp + humidity	8	1.15	0.23
Temp	6	1.18	0.22
Season + hour + temp	8	2.11	0.14

model. The Akaike information criterion corrected for small sample size (AIC_C) was used to select the best fitting LME models [40]. Akaike weights were used to evaluate model-selection uncertainty. A model's Akaike weight ($wAIC_C$) expresses the weight of evidence favoring that model as the best of all those in the model set; the weights of all models together sum to 1 [40]. Data analyses were performed using the program R 2.12.2 [41] (<http://www.r-project.org/>), with the package nlme [42].

3. Results

3.1. Foraging Activity throughout the Year. Foraging rate was apparently higher in some months along the year. In February, April, June, August, and September, more than 250 wasps were seen foraging in one day, which was sampled only one hour (Figure 1). Collection of liquids (water and

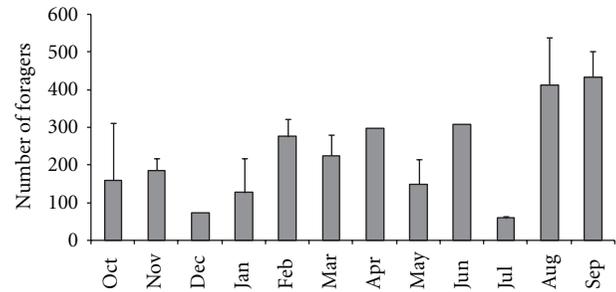


FIGURE 1: Mean number of foragers of *Polybia paulista* observed returning to the nest per day for each month of observation. Each day was sampled four times, during 15 minutes each. Thin bars are standard deviations.

nectar) was always higher than other materials collected (55% to 80% of the collected materials, Figure 2). Collection of solids was small and occurred in all months. In June, we registered the highest prey collection, in which 21% of the foragers returned with this material. In contrast, in July, September, October, November, and December, the lowest rate of prey collection was registered, *ca.* 6.25%. In January, February, March, April, May, and August, prey collection was intermediate (*ca.* 12.2%, Figure 2). Pulp collection occurred in six months, from August to January, and it was made up from 0.2% to 12% of the material collected by the foragers. However, in October and November, only three wasps were recorded collecting pulp. On the other hand, December, January, August, and September presented the highest number of wasps collecting pulp (Figure 2). Construction was observed in three different nests, one in December and January, another in August, and a different one in September.

A small percentage of foragers arrived without any material in almost all months (Figure 2).

3.2. Environmental Factors. The maximum temperatures recorded in both seasons were the same; the minimum temperatures and the means differed by about two degrees Celsius (Table 3), which shows a small variation along the year [36]. The amplitude was approximately 10.5°C for the HHS and 12.5°C for the CDS (Table 3). However, the daily amplitude was different for each season, 7.25°C on average for the HHS and 4.6°C on average for the CDS. During the HHS, the maximum humidity recorded was 92% and the minimum was 48%, the mean was 71%, and the median

TABLE 3: Weather conditions recorded throughout the year for the foraging study in the social wasp *Polybia paulista*. Maximum (max) and minimum (min) absolute values and mean values of temperature ($^{\circ}\text{C}$), relative humidity (%), and light intensity ($\text{lux} \times 100$) for each month and the entire season. HHS: hot and humid season. CDS: cold and dry season.

		Temperature ($^{\circ}\text{C}$)			Humidity (%)			Light intensity ($\text{Lux} \times 100$)		
		Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
HHS	Oct	31.0	22.0	24.4	91	48	69	1248	50	569
	Nov	32.0	24.0	26.1	91	50	70	1487	124	1108
	Dec	26.0	24.0	28.2	79	66	74	498	258	386
	Jan	27.0	21.5	24.6	91	70	80	994	227	494
	Feb	30.0	22.0	24.9	91	65	77	1547	27	888
	Mar	31.5	25.5	26.4	92	52	63	1404	82	910
	Season	32.0	21.5	26.9	92	48	71	1547	27	790
CDS	Apr	32.0	29.0	29.2	67	54	61	1213	64	637
	May	26.5	19.5	30.6	100	59	79	900	16	353
	Jun	27.0	23.0	23.3	83	63	74	933	53	643
	Jul	23.0	20.0	24.8	64	43	51	1031	479	828
	Aug	28.0	23.5	21.6	67	33	52	1087	237	672
	Sep	31.0	20.0	25.8	83	42	67	1253	159	685
	Season	32.0	19.5	24.5	100	33	63	1253	16	644

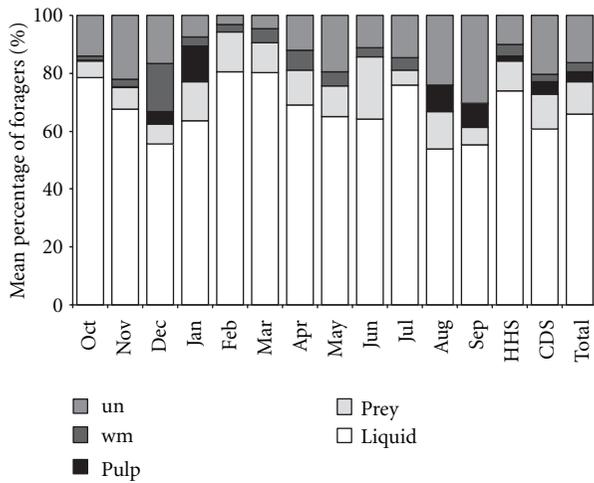


FIGURE 2: Mean percentage of foragers of *Polybia paulista* returning to the nest for each day of observation bringing liquid, prey, pulp, nothing (wm), and unidentified material (un) for each month, season, and the entire year. HHS: hot and humid season. CDS: cold and dry season.

was 71.5% (Table 3). On the other hand, during CDS, the maximum humidity was 100% in a day that was raining, while the minimum was 33%, the mean was 63%, and the median was 62% (Table 3). The amplitudes were large, 49 percentage points throughout HHS and even larger, 67 percentage points during CDS (Table 3). The maximums, minimums, and means of light intensity were very similar in both seasons and varied widely throughout the year (Table 3). According to the climatic data for the last 20 years [36], the period in which our observations were performed

presented a typical weather condition to that geographic region.

Since more than one sampling was done in the same nest and the samplings closer in the time are more likely to be under the same biological effects, the use of LME models is imperative. The model explaining the number of foraging wasps that included only season and temperature was the best supported (Table 2(c), Figure 3).

4. Discussion

4.1. Foraging Activity throughout the Year. Liquid material was widely collected (Figure 2), and this might be due to its importance in wasps metabolism (water and nectar), maintenance of nest temperature (water), and nest construction (water). Water is a limiting factor for the development of wasps [43]. On the other hand, nectar is extremely important in adults' diet, and several authors pointed out that it is the most collected material in different periods in the year [17, 19, 20, 29, 30, 33, 44].

Pulp collection, used for nest construction and repair, was observed only in six months, two months during the CDS and four months in HHS. However, construction was in fact observed only in four months when the greatest amounts of pulp were collected (August-September and December-January, Figure 2). According to Jeanne [1], as brood reproduction in swarm-founding polistines occurs in pulses, nest expansion is also episodic. Thus, when nest expansion occurs, it is typically a discrete event, lasting several days. In contrast, after a reproductive event, addition of cells to the nest may not occur for months.

Collection of preys occurred all year long (Figure 2). Since preys provide animal protein for brood development, the amount of preys captured by foragers is an indirect

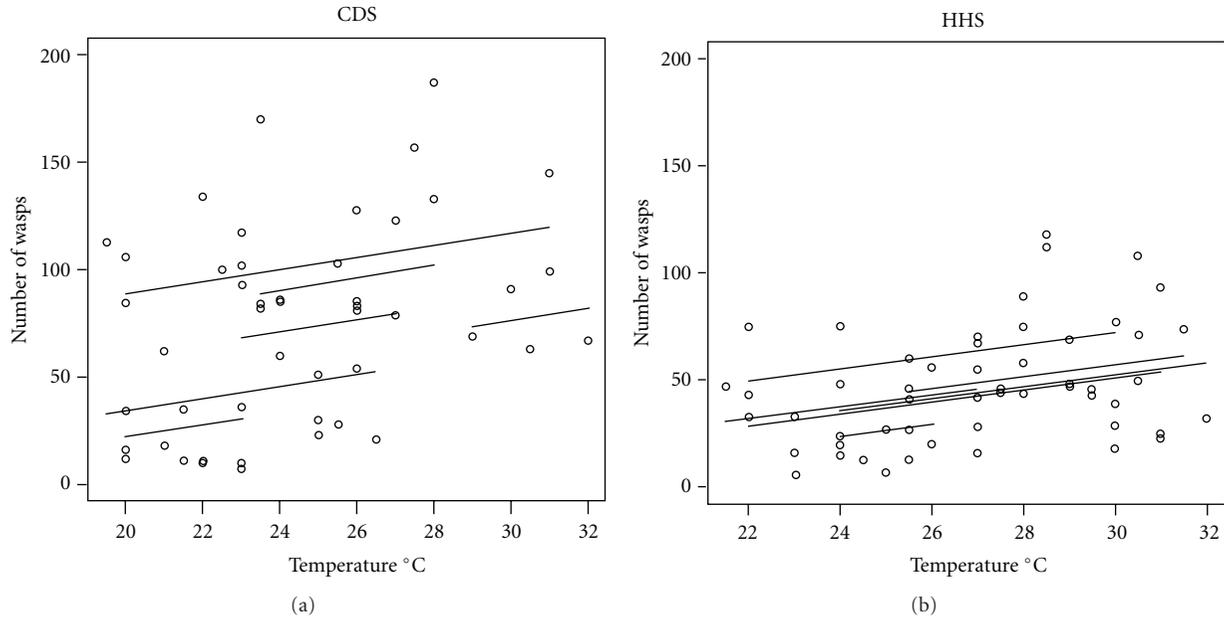


FIGURE 3: Number of foragers that arrived at the nest every 15 minutes in relation to season and temperature. There is a clear influence of temperature on foraging for both. Lines were estimated by linear mixed effect (LME) model, according to the equation: Number of wasps foraging = $-29.98 + 28.79 \times \text{Season} + 2.82 \times \text{Temperature} + \text{Random effect} + \text{Residual}$, where Random effect $\sim N(0, 23.04)$ and Residual $\sim N(0, 28.06)$. CDS: cold and dry season. HHS: hot and humid season.

measure of the number of immatures and, consequently, indicates colony reproduction. According to Machado [45], colonies of *Polybia paulista* produce reproductives once a year in the rainy months (HHS) at the ending and beginning of the year. However, the production of workers occurs in the intermediate period. So, a colony can exist for up to three years at the same place, but swarms occur periodically.

The fact that prey was collected all year long suggests that production of workers happens all over the year, depending on the necessity of each nest. It was noted by Jeanne [1] that in regions with unfavorable season (like a harsh wet or dry season), colonies face several reproductive constraints and do not reproduce all the year. The wasps seem to “consider” that both seasons are adequate to raise brood in the place of study.

Moreover, we found some evidences about a greater activity during CDS. It is known that besides an ideal temperature, water is another important requirement for egg development. When it is available in insufficient amounts, the embryogenesis becomes quiescent or the embryo remains in diapauses [46]. However, the ideal humidity quantity necessary for that is not known in any neotropical swarm-founding wasp. So, maybe more activity was recorded in CDS because more water needed to be carried to the nest to increase humidity. Hence, there is probably more to be done to reach the same success reached in HHS for raising brood. Thus, more studies concerning these aspects should be done to improve our understanding in this area. However, it is important to point out that despite of being careful to choose nests with the same size and developmental stage, our data are temporally correlated and the results might have suffered some kind of influence due to the nests chosen.

4.2. Environmental Factors

4.2.1. Temperature. A variety of studies has demonstrated the influence of weather conditions on social wasp foraging and nearly all of them point to temperature as the key factor influencing foraging [17–33, 38]. We found a great influence of temperature on foraging, since there was an increase in the number of foragers simultaneously to the increase of temperature (Figure 3). This increase in the activities can be partially explained by the wasps’ necessity for water (it is used for nest construction [14], metabolic processes [5], and nest cooling [12, 13]). Generally, when temperature increases also does their water collection once the need for nest cooling is more evident [27]. According to Jeanne [1] when the nest overheats, workers on the envelope fan their wings steadily to ventilate the nest. If that is inadequate, foragers begin to bring water to the nest, where it is spread on the surface of the combs and envelope, bringing about cooling by evaporation [8].

There are lower and upper thresholds of temperature beyond which wasps do not forage. Previous studies suggested a very low limit of 2°C or 5°C for vespine wasps [47, 48]. So, flight will not occur until a certain thoracic temperature is reached [49]. Flying insects can increase their body temperature by producing heat with their flight muscles [50]. Thus, they do this until they reach the ideal temperature for the flight. Concerning higher temperatures, Kasper et al. [28] noted in *Vespula germanica* that foraging activity increased with temperature until 20°C and it was kept practically constant between 20°C, and 35°C. Above 40°C, the foraging activity decreased and virtually ceased, probably because the heating caused by flight can overheat the

wasps [28]. These extreme temperatures were not found in this study, once the minimum was 19.5°C and the maximum was 32°C. Differently of Kasper et al. [28], the foraging increased along with temperature even up to 20°C (Figure 3), probably because these species bear strong biological differences. For example, Resende et al. [20] studying *Polybia occidentalis*, a closer species, noted that foraging increases until 34°C, the maximum temperature recorded.

4.2.2. Relative Humidity. Several works on flight activity pointed out that relative humidity can be an important weather factor influencing foraging activity in wasps [18–21, 23, 24, 26, 30–32]. We found no influence of humidity on foraging, possibly because its influence on foraging is smaller than temperature.

For instance, Silva and Noda [18] studying the external activity of *Mischocyttarus cerberus styx* and Resende et al. [20] studying *Polybia occidentalis* noted that foraging increased with temperature and decreased when humidity increased. They affirmed that higher humidity has a major influence on the foraging than moderate or lower humidity, and although humidity has certain influence, it is lower than temperature influence. It is important to say that the most part of such works did not analyze the relative humidity along several months in the year, and depending on the month that is analyzed, an overestimated or underestimated importance of this weather factor may occur. Furthermore, these authors point out that the humidity influence is greater on higher values, and the place of study presents moderate and low humidity during almost all the year. Thus, the lack of influence of this weather factor may be due to its low values recorded.

In addition, other authors such as Kasper et al. [28] did not find any correlation with humidity, and Lima and Prezoto [22], on the other hand, found correlation between these variables on *Polybia platycephala sylvestris* only in hot and humid season. However, even with the separation of the year into HHS and CDS, there are some climatic differences in the localities where our study and those by Lima and Prezoto were performed. Our locality is hotter (23.5°C versus 19.3°C for annual mean [51]) and drier (1,240 mm versus 1,644 mm for annual pluviosity [51]).

4.2.3. Light Intensity. The light intensity influence is probably the most controversial factor influencing foraging activity. Some authors found a strong influence of the luminosity while others did not. For instance, Kasper et al. [28] detected strong correlation between these variables. On the other hand, Elisei et al. [23] did not find any correlation in *Synoeca cyanea*.

Osgood [52] and Lerer [53] suggested that the foraging activities of the stingless bee *Trigona hyalinata* begin in the morning by temperature influence, and in the end of the day, there is luminosity influence. It is important to point out that our observations were accomplished until 4 p.m., a period with a great luminosity in the most part of the year, and this might have had an effect on the lack of influence of this variable on the foraging.

As a conclusion, in climates like the one presented here, with a strong wet-dry seasonality and low cold-hot seasonality, the main factor influencing foraging in colonies of *Polybia paulista* in both seasons is temperature. Although there is a great variability in humidity and solar radiation along the year and even along the day, their influence on foraging seems to be small.

Acknowledgments

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