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A free meal: Foraging behaviour and opportunistic tendencies of the termite-raiding ant *Megaponera analis* in the Dja Biosphere Reserve of Cameroon

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Abstract

In this study, the never-before-asked question of whether or not the termitophagous ant *Megaponera analis* are opportunistic foragers was investigated via the presentation of both *Macrotermes* and *Cubitermes* termites along *M. analis* raiding trails. These trials occurred in the Dja Biosphere Reserve in Cameroon during January and February of 2017. The results showed that the ants were in fact opportunistic for *Macrotermes* but not opportunistic for *Cubitermes*. Quantitative raiding behaviour data showed significant similarities in foraging behavior (including velocity and distance) gathered from other studies of different African regions. Lastly, nest-mate recognition between *M. analis* was gathered by introducing ants to foreign colonies and recording responses; the result showed a statistically significant difference in response between home-colony and foreign ants, with *M. analis* acting more hostile to foreign ants. This is the first *M. analis* study conducted in a Congolese lowland rainforest.

Keywords: *Foetens*, Congolese, *Macrotermes*, *Cubitermes*, *Pachycondyla*

1. Introduction

Megaponera analis (previously known as *Pachycondyla analis* and *Megaponera foetens*) is a sub-Saharan ant species that forages on termite nests. The social communication and foraging habits of this species have been studied in a Tanzanian coastal dry forest ^[1], a Nigerian primary savannah woodland ^[12], and a Kenyan savannah ^[17]. No *M. analis* research, however, has been completed in a Congolese lowland rainforest until now. We studied *M. analis* in the Dja Biosphere reserve, a 526,000 ha dense tropical rainforest, in Cameroon, Africa.

Megaponera analis exclusively raids termite nests. Raids tend to occur right after dawn or before dusk. It is hypothesised that the activity of the ants is influenced by temperature ^[1]. Once scout ants have located a potential food source, they return to their colony and recruit a column of workers (average of 300 ants) to begin a raid. The foraging party follows the pheromone trail previously laid by the scout to the termite nest ^[10]. Upon arrival, the major workers break through the termite tunnels, while the minor workers dig further in. Termites are then captured, stung, and placed near the tunnel entrance. Both major and minor workers then haul the prey back to their colony ^[2]. *Megaponera* foraging behaviour has been studied several times, with each successive study finding similar patterns in this behaviour ^[9].

The primary purpose of this study was to analyze the behavioural repertoire and social structure of *Megaponera analis* via series of field experiments focused on foraging habits and nestmate recognition. The goal was to determine whether: 1. *M. analis* would opportunistically predate any encountered termites whilst following a raiding trail; 2. *M. analis* would demonstrate inter-colony aggression. We hypothesised that *M. analis* does forage optimally and can distinguish between nestmates and foreigners.

2. Materials and Methods

2.1 Study Site

The present study was conducted in the Dja Biosphere Reserve in Cameroon, Africa (02°40' to 03°23'N; 12°25' to 13°35'E, elevation 400 to 800 m) during the dry season from 30 January, 2017 to 13 February 2017. The Reserve was a dense tropical rainforest surrounded by the Dja

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River and covering approximately 526,000 ha. Rainfall averages 1600 mm annually. Research was conducted near the centre of the reserve at the 25 km² study area surrounding the Bouamir Research Station (3°11'N 12°48'E) [8].

2.2 Sample Size

Data was collected on 28 total colonies found near the field station and along foot trails (Fig. 1). We gathered descriptive data and conducted experimental trials on 21 of these colonies. Purely experimental trials (termite manipulation and nest mate recognition) were carried out on the remaining seven of these colonies.

2.3 Descriptive Data Collection

Before conducting field experiments, we collected quantitative descriptive data for each colony during a raid, including: velocity of the raiding column, number of ants in total, number of ants carrying termites, distance travelled, temperature, humidity, and type of habitat. This data was collected in order to compare *Megaponera analis* raiding behaviours between different locations. We calculated both the outgoing and incoming raiding velocity of the ants. The outgoing velocity was calculated by timing how long it took a raiding ant to traverse a distance of 50 cm on its way towards a termite nest [17]. The incoming velocity was calculated by timing how long an ant took to travel the 50 cm on its way back to the home colony nest. In order to measure this distance, we laid a tape measure parallel to the pheromone trail and timed how long it took an individual ant to travel the 50 cm, both to and from a raid.

Raiding ants were counted both as they left their nest and as they returned to their nest. This was done in order to determine if the number of ants in a foraging party changed over the course of a raid. Multiple counts were taken each way. We also tabulated the number of foraging ants that successfully brought termites back from a raid in order to estimate the foraging success of each raid [17]. The length of the pheromone trail was measured by recording the trail distance from an ant nest to the raided termite nest [12]. The temperature and relative humidity of the air was recorded using a Kestrel 3000 Pocket Weather Meter (MPN #0830) and the surface temperature of the ants' nest entrance were recorded using an infrared thermometer. Air temperature, relative humidity, and nest surface temperature were recorded during raids. We used a measuring tape to record nest height and also documented the type of nest. Raid duration was tabulated by recording the length of time it took from the first emergence of a raiding party to when the final ant entered the home nest after a raid. This period of time accounted for a raiding party's departure from its home nest to when it returned home from a raid. GPS coordinates were recorded and logged in a handheld Garmin GPSMap 60CSX (Unit ID: 3570101364).

2.4 Termite Manipulation

We carried out termite manipulations on a total of 21 different raiding parties to test if *Megaponera analis* is opportunistic when foraging. We did this by collecting termites and then presenting them to *M. analis* raiding parties. *M. analis* raiding parties were presented with these termites both on their way towards a raiding site as well as on their way home after a raid. We utilized two different species of termites for these manipulations. The first species was of the genus *Cubitermes*. The second species was *Macrotermes ivorensis*. We tested only one species of termite per trial to control for any potential termite preferences by the ants. Multiple termites (of

only one species at a time) were experimentally placed along the pheromone trail of the ants. We observed and recorded the ants' reaction to this termite presentation. This *M. analis* reaction was categorized into two discrete behaviour: 1. taking the termite or 2. rejecting the termite.

2.5 Nest Mate Recognition

We took several steps in order to determine whether *Megaponera analis* can distinguish between members of its own colony and those of a foreign colony. First, we collected major and minor workers from different *M. analis* colonies encountered along trails. Each of these samples were then separated by colony number and placed into separate plastic Ziplock bags. These ants were then marked on the abdomen with Decocolour paint markers (Uchida of America Corp., Torrance, CA, U.S.A.). We then designated these collected ants as "foreign colony" samples and carried them with us whilst we surveyed surrounding trails for additional traveling colonies of *M. analis*. Once we found a new traveling column, we designated it as the "home colony" and collected major and minor workers from it, placed them into a new Ziplock plastic bag, and then marked the ants with a paint marker (in a new colour). Finally, we would simultaneously introduce both the "foreign" and "home" colony ants from their respective plastic Ziplock bags to the traveling column (from which the "home" ants were collected). We introduced the pair of major ants, one "foreign" and one "home," first. We recorded the column's response to the major worker introduction. Then we introduced the pair of minors and recorded this response.

We created a scale ranked 1 to 5 to describe the behavioural response to "foreign" and "home" ants: 1 indicated no response, 2 indicated interactions lasting less than three seconds, 3 indicated contacts lasting longer than three seconds, 4 indicated aggressive interactions such as fighting and stinging, and 5 indicated interactions resulting in the death of the introduced ant. We continued to monitor the ants until both the home column had returned to its nest and the "foreign" ant had travelled a distance far away enough for us to lose sight of it. This process was applied to 12 different home colonies for a total of 12 sampling units.

2.6 Data Analysis

In regards to foraging behaviour, the data was compared to the prior literature using two-sample *t*-tests. Temperature, velocity in/out, distance, and number of ants in a raid were among the comparison variables utilized by the two-sample *t*-tests. *M. analis* foraging success rate was calculated by dividing the number of ants with termites by the total number of ants returning from a raid.

In order to analyze the data of the termite manipulations, a Fisher's exact test was used. This test was utilized in order to determine whether the ants were more likely to opportunistically forage for either species of presented termite. The average nestmate recognition rankings between the home colony and foreign colony responses were compared with a Wilcoxon paired-sample rank test. The number of attacks on nestmates versus foreign ants were compared with a Fisher's exact test.

3. Results and Discussion

3.1 Descriptive Data

We determined *Megaponera analis* colonies to be primarily located in above-ground, inactive termite nests, though we also observed some colony nests that were located directly underground. These branched underground location findings were in accord with a previous study in South Africa [15]. The

surface temperature of each ant nest was always a few degrees Celsius cooler than the air temperature (Table 1). Compared to a study in the Tanzanian coastal dry forest, the air temperature at which *M. analis* conducted raids in the Dja was very similar ^[1] (Two-sample *t*-test, $P = 0.330$) (Table 3). However, raids in the Kenyan Savannah at Mpala occurred at a lower average air temperature ^[17] (two-sample test, $P < 0.001$).

In the Dja, the mean velocity of ants leaving their nest to forage was slower than that of ants (that were not carrying termites) coming back to their nest from a forage, and this trend was found in Kenyan savannah and Nigerian primary savannah woodland (Table 1) ^[17, 11]. Compared to a study in the Tanzanian coastal dry forest, these velocities were similar (Table 2) ^[11]. A study conducted in a Nigerian Guinea Savannah found going and returning velocities to be, on average, faster than those of the Dja (two-sample *t*-test, $P = 0.0027$) ^[11]. *M. analis* tended to travel much faster in the Kenyan Savannah than in the Dja as well ^[17] (two-sample *t*-test, $P < 0.001$). The velocity of the ants returning to their nest was higher in comparison to the ants leaving their nest, and this trend was also found in Kenyan savannah ^[17] and Nigerian primary savannah woodland ^[11].

Megaponera analis raiding parties travelled distances of up to 53.34 m from their colonies to termite nests. Compared to the study in the Tanzanian coastal dry forest, the mean distance travelled at the Dja was almost 10 m further (Table 2) ^[11]. Colonies that travelled more than 15 m saw a greater percentage of ants successfully return with termites than those colonies that travelled less than 15 m out to raid (Table 1). This trend is supported by another study which found distance traveled to be positively correlated with abundance of termites collected ^[4].

Interestingly, the average number of total ants in a raiding party at the Dja was similar to what the prior studies found in the Tanzanian coastal dry forest (two-sample *t*-test, $P = 0.551$), Kenyan savannah ($P = 0.646$), and Nigerian guinea savannah (0.473) ^[1, 11, 17]. Another study found column formation to be stable, with individual ants occupying the same physical spots in the column each time, and this supports the trend of raiding party size consistency ^[3]. The average number of successful ants returning from a raid was similar between the studies in the Dja, Tanzania, and Nigeria.

3.2 Termite Manipulations

In utilizing Fisher's exact test to determine if *M. analis* has foraging preference for either species of presented termite (*Cubitermes* vs *Macrotermes ivorensis*), it was found that the ants are significantly more likely to forage on *Macrotermes* ($p = 3.402 \times 10^{-5}$). Out of 10 trials, *M. analis* took no *Cubitermes* termites. The ants encountered these termites and inspected them with their antennae, sometimes even grasping the termites with their mandibles, but then always left the termites behind and continued to move along in their column. When *M. analis* raiding parties were presented with *Macrotermes ivorensis*, all but one of 11 raiding parties took them. The ants stung, grabbed, and carried these termites, behaving and rejoining their column just as they would during a normal raid. The column fixedness was supported by another study in which the column positioning was suggested to be based on individual ant roles ^[3]. In accordance with another study, the ants would grip these termites with their mandibles on their way back to their nest ^[6]. The one party that did not take the presented *Macrotermes ivorensis* behaved the same way as the original 10 parties did with the presented *Cubitermes*.

The results suggest that *Megaponera analis* is opportunistic for termites of genus *Macrotermes* but not *Cubitermes*, and these results support our hypothesis in the idea that the ants do show opportunistic tendencies in foraging, but also that the genus/species of presented termites matter. *M. analis*' preference for *Macrotermes* was supported by a prior study ^[7]. This is reflected in the fact that *M. analis* never took *Cubitermes* but took *Macrotermes ivorensis* 90% of the time. Further experimentation must be conducted to understand the preferential behaviour of the ants. A prior study found foraging decisions to be individually made, and this offers insight into why only some ants would examine the offered termites ^[4]. Based on our observations, the differing habitats of the two termite species (*Macrotermes ivorensis* and *Cubitermes*) may play into why the ants prefer *M. ivorensis*. We found *M. ivorensis* to live in the ground while the *Cubitermes* live in free-standing soil mounds that are raised off the ground, and this was supported by previous studies ^[12, 16]. We never observed *Megaponera* leaving the ground whilst on a raid. It is perhaps possible that the ants we observed do not recognize *Cubitermes* (when we offered it) because they do not raid in that type of habitat.

3.3 Nest Mate Recognition

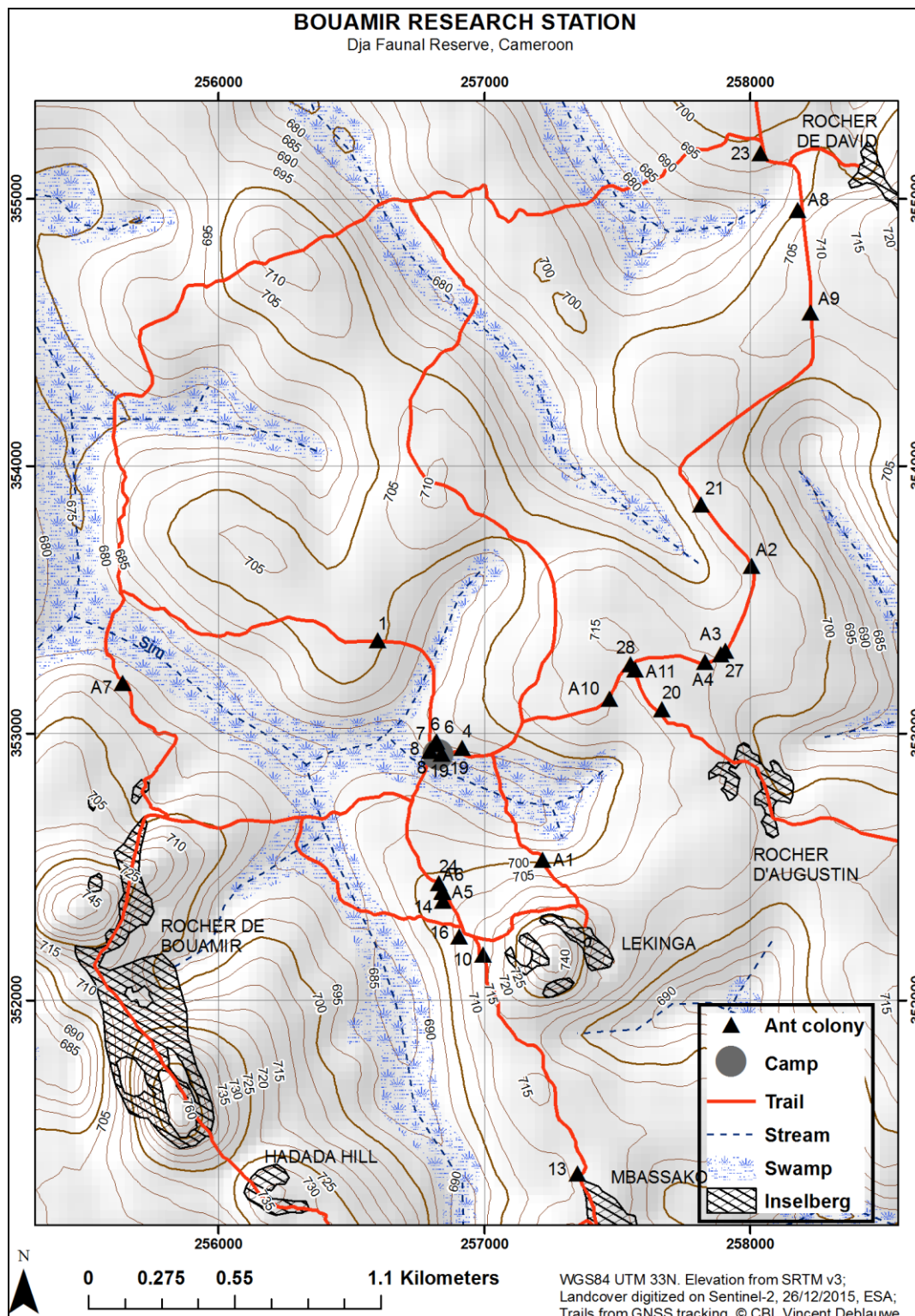
Megaponera analis reacted aggressively towards the "foreign" ants from the experimental group but they remained neutral towards their "home" nestmates from the control group (Fig. 2). The column, or main party, always recognised their nestmates and did not attack them even once in any of the 12 trials. However, foreign ants were grabbed, bitten, and stung in nine of the 12 trials. Interactions between the main party and the experimental group (foreign) were often ranked as a 4 (mean = 3.33, $n = 12$), indicating biting and stinging (Fig. 2). Interactions between the main party and the control group (home) were often ranked as a 2 (mean = 1.83, $n = 12$). The average rankings of the two groups were statistically different; colonies responded more aggressively to foreign ants than to their painted nestmates, according to a Wilcoxon paired-sample rank test ($P < 0.003$). The number of attacks on nestmates was also significantly different from the number of attacks on foreign ants, as suggested by a Fisher's exact test ($P < 0.001$).

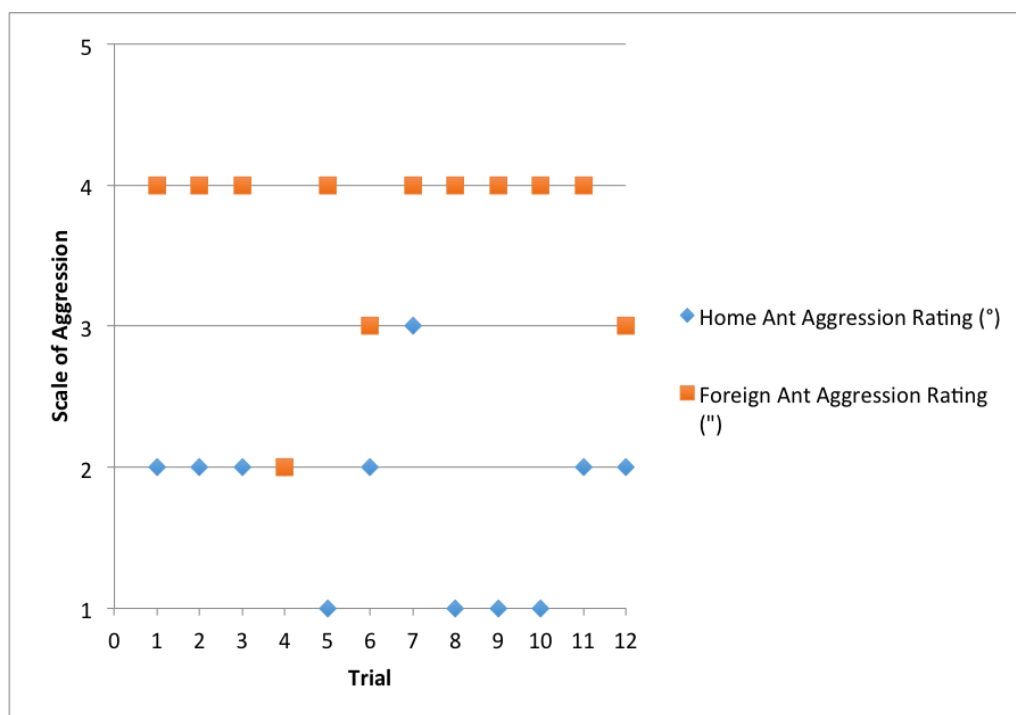
Our results support our hypothesis that colonies of *Megaponera* can differentiate between their own nestmates and ants from other colonies. This is similar to a prior study, in which aggression was demonstrated between home colony and foreign ants ^[18]. In our observations, we noticed several common behaviours between home colony ants and foreign-introduced ants. Each time we introduced an ant, whether home or foreign, it was always first inspected by the home column. This observed behavior has been found in another study, in which *M. analis* would inspect each other in assessing potential injury ^[6]. This behaviour involved the ants using their antennae to brush and their mandibles to grasp the newly introduced ant. The use of mandibles in interacting with a fellow ant was also found in another study, in which ants could physically pick up and carry each other if need be ^[5]. This redundant inspection-behaviour offers further insights into how the ants assess their surroundings.

The foreign ant always dispersed after being attacked (we never observed a "foreign" ant enter a "home" nest). On a few occasions, we watched a "foreign" introduced ant initially follow a "home" colony pheromone trail only to quickly disperse after coming into contact with the native ant column. Previous studies have detailed that ants instinctively follow a

pheromone trail and that perhaps *Megaponera* cannot differentiate between pheromone trails of different colonies [13]. However, one study found pheromones to be universally used as a distress signal among *M. analis*, with dimethyl disulfide and dimethyl trisulfide produced in the mandibular glands, which begs further research into possible slight chemical differences between ants of different colonies, given our recognition results [7]. Pheromone trails are able to be relocated, so it is also possible that ants may sometimes

accidentally follow the wrong trail, which could result in a potential fight [14]. No observed fight resulted in death (level 5), and the longest fight we observed lasted about 4.5 minutes. When an ant from the home colony was reintroduced to its native column, the native ants would typically inspect the reintroduced individual, but rarely followed this up with an aggressive behaviour. Only in one trial did we observe a level 3 reaction to a home colony ant, while the 11 other trials were primarily level 2 (Fig. 2).





Intercolony Aggression Scale	
1	No Interaction
2	Interaction<3s
3	Interaction>3s
4	Fighting
5	Death

Fig 2: Scale of Aggression in *M. analis*. Level of intercolony aggression between nestmate ants and foreign ants based on 12 trials conducted in the Dja.

Table 1: Average and standard deviations of quantitative behavioural data collected on raiding columns from 20 different colonies.

	<i>M. analis</i> Raid Data	
	Means	Standard Deviation
Ant Nest Temp. (°C)	22.61	1.251
Air Temp. (°C)	25.39	1.774
% RH	82.00	4.430
Velocity Out (cm/s)	2.960	1.083
Velocity In W/o Termites (cm/s)	3.394	1.174
Velocity In W/ Termites (cm/s)	2.647	0.3998
Distance (m)	22.43	12.08
Duration of Raid (min)	15.33	9.708
# Raid Ants	237.2	206.4
# Successful Ants	75.80	186.0
% Ants with Termites Overall	20.14	21.62
% Ants with Termites (Raid Distance < 15m)	9.26	16.57
% Ants with Termites (Raid Distance > 15m)	24.80	22.08

Table 2: Mean, standard deviation, and sample size comparison of quantitative raid data between the Dja, a Tanzanian coastal dry forest ^[1], a Nigerian primary savannah woodland ^[12], and a Kenyan savannah ^[17].

	The Dja	Tanzanian Coastal Dry Forest	Kenyan Savannah	Nigerian Primary Savannah Woodland
Temperature (°C)	25.40 (1.77) n = 21	24.87 (3.35) n = 79	23.10 (0.21) n = 330	N/A
Velocity Out (cm/s)	2.60 (1.08) n = 12	3.2 (0.77) n = 18	6.70 (2.30) n = 330	3.80 (N/A) n = 220
Velocity In (cm/s)	3.10 (1.17) n = 17	2.98 (0.72) n = 21	13.03 (6.40) n = 330	4.60 (N/A) n = 220
Distance (m)	22.43 (12.07) n = 20	13.03 (8.22) n = 97	N/A	N/A
# Ants in Raiding Party	237.2 (206.4) n = 20	266.6 (159.6) n = 113	259.0 (138.3) n = 330	203.0 (116) n = 67
# Ants Carrying Termites	75.80 (186.0) n = 20	88.98 (65.54) n = 113	64.62 (22.03) n = 330	N/A

Table 3: Comparison between the Dja and a Tanzanian coastal dry forest ^[1], and a Kenyan savannah ^[17].

Two-Sample T-Test: P-Value	The Dja vs. Tanzanian Coastal Dry Forest	The Dja vs. Kenyan Savannah
Temperature (°C)	0.330	<0.001
Velocity Out (cm/s)	0.113	<0.001
Velocity In (cm/s)	0.714	<0.001
Distance (m)	0.003	N/A
# Ants in Raiding Party	0.551	0.646
# Ants Carrying Termites	0.757	0.791

4. Conclusion

This study found that *Megaponera analis* are opportunistic foragers, but only for one particular species of termites (*Macrotermes ivorensis*). It was found that the foraging behavior of these ants (including velocity, raid temperature, and distance/success ratio) was similar between ants of the Dja Biosphere Reserve and other African regions. *M. analis* exhibit nest-mate recognition and show hostility to foreign-nest ants. These data support prior studies and invite further research into the exact mechanisms of opportunistic foraging and inter-colony recognition.

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