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# Use of Pheromones to Enhance Foraging Efficiency in *Atta cephalotes*

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## ABSTRACT

*Atta* (Formicidae: Myrmicinae) are important herbivores in the Neotropics, cutting between 12 and 17% of the vegetation there (Holldobler and Wilson 1990). *Atta cephalotes* uses pheromones to communicate and maximize efficiency during foraging. The presence and specificity of pheromones was studied on leaf segments, between trails in the same colony and between different colonies. All studies were completed at the Santuario Ecológico in Cerro Plano, Costa Rica. Leaves cut by ants were retrieved from the cache at a significantly faster rate than those cut by hand (p-value = <0.0001), suggesting pheromones were laid on the plant material. The leaves were also retrieved at a significantly faster rate when compared to leaves collected from ants on a different trail within the colony or from a different colony (p-value = 0.0386 and 0.0010 respectively). Individual ants also foraged at a significantly faster rate on their own trail when compared to when they were transferred to a different trail within the colony or to a trail of a different colony (both p-values = <0.0001). This suggests that the trail pheromones used for foraging are more complex and specifically recognized by workers on a certain trail. A higher specificity of pheromones may prove beneficial to the enhancement of worker specialization and colony foraging efficiency.

## RESUMEN

Las hormigas *Atta* son herbívoros muy importantes en los neotrópicos, consumiendo entre el 12 y el 17% de la vegetación. *Atta cephalotes* usa feromonas para comunicarse y forrajear eficientemente. Se estudió la presencia y especificidad de las feromonas en pedazos de hojas, entre senderos en el mismo hormiguero y entre hormigueros diferentes. Todos los experimentos se realizaron en el Santuario Ecológico en Cerro Plano, Costa Rica. Las hormigas recolectaron hojas de grupos que fueron cortadas por las hormigas a una velocidad significativamente más rápida que las de grupos de hojas cortadas a mano (p = <0.0001), sugiriendo que las feromonas fueron puestas en los pedazos de hoja. Las hojas también fueron recogidas significativamente más rápido cuando se les comparó con las hojas que fueron recolectadas de hormigas en un sendero diferente del mismo hormiguero o de un hormiguero completamente diferente (p = 0.0386 y 0.0010, respectivamente). Las hormigas individuales también forrajearon a una velocidad más rápida en su mismo sendero en comparación con hormigas que fueron trasladas a un sendero diferente del mismo hormiguero o a un sendero de un hormiguero diferente (p = <0.0001 para los dos tratamientos). Este resultado sugiere que las feromonas de los senderos usados para buscar material de plantas son más complicadas y reconocidas específicamente por las hormigas trabajando en un sendero particular. Una especificidad más alta de las feromonas puede ser beneficiosa para el mejoramiento de la especialización de las hormigas y la eficiencia de forrajeo para el hormiguero.

## INTRODUCTION

The most successful organisms are those that can forage the most efficiently; being able to obtain the maximum net yield of energy creates a higher fitness and survival rate for that organism (Rockwood and Hubbell 1987). Group foraging, division into castes and mass communication are ways that have enhanced foraging efficiency in ants.

*Atta* (Formicidae: Myrmicinae) is a genus of leafcutting ants in the Neotropics. They are the dominant herbivores in these systems and cut between 12 and 17 percent of produced vegetation (Holldobler and Wilson 1990). Organizing millions of individuals to accomplish such a feat can be complex and require specialization, motivation, and effective communication. A caste system is observed in leafcutting ants, allowing individuals to focus on one task and be proficient.

Ants have reduced or no eyes and rely on chemical, tactile and mechanical signals for effective communication (Holldobler 1995). *Atta cephalotes* find, cut and carry leaf segments or flowers back to their nest to be used as a substrate for fungal gardens. Clearing a main trail increases foraging rates as much as four to ten times when compared to foraging through the leaf litter (Rockwood and Hubbell 1987).Chemical signals are also secreted and recognized by workers in order to coordinate the recruitment of plant material. In general, the trail pheromones are volatile compounds with a high molecular weight (Holldobler and Wilson 1990). These characteristics allow them to be more complex than and not as volatile as alarm pheromones, which have a lower molecular weight and can be sometimes recognized by different species (Hughes *et al*. 2001, Robinson *et al.* 1974).

It is important to determine when and how the chemical signal gets used to increase the foraging efficiency of *A. cephalotes*. It has been suggested that the ants apply pheromones to the leaf when it is being cut, as well as along the trail, nest, and trunk to orientate the foragers (Holldobler and Wilson 1990). Marking the supplies and foraging area with information may be the best way to organize group foraging. Pheromones on the plant material itself may help in recognition and retrieval if foraging is interrupted and the material dropped. Plant material selection is very specific and can be time consuming (Holldobler and Wilson 1990). Retrieving material that has already been chosen and carried a distance would therefore be more efficient than finding and retrieving new material.

The specificity of pheromones is essential for efficient communication. Many species of ants use different concentrations and mixtures of compounds in order to elicit the correct response, for example recruitment/foraging, alarm, or attack (Holldobler 1995). It may be helpful to use different pheromones for each of the different trails within a colony while foraging. Optimal foraging theory predicts that the more energy expended on acquiring food should result in a larger reward (Holldobler and Wilson 1990). Therefore, more ants may be allocated to a trail where the plant material being collected is in greater demand or of a higher quality. A trail-specific pheromone may also be useful for the workers to specialize and remain on a single trail. A high rate of trail fidelity was found among *A. cephalotes*; an average of 94% of the marked ants remained on the same trail and an average of 74% returned to the original trail after being removed for 24 hours (Romer 2004).

A colony-specific pheromone would be useful in foraging to assure allegiance as well as exclusive resource accessibility. The workers would benefit the colony and therefore themselves if they were loyal and only foraged for their original colony. It is possible that there is competition for the same resources when there are multiple nests in the same area. If each colony within the same species had an unrecognizable odor, the trails of other colonies would not be exploited. Previous research suggested that the ants distinguished between plant material collected from a different colony, and they also

recognized it after retrieving unmarked material, and discarded it (Berry 1994). A colony odor could also be helpful for protection against other conspecifics that may attack the nest (Jaffe and Marcuse 1983). If an individual from a different colony is recognized because they have a distinguishing colony pheromone, it would be easier to organize a counter attack and remove the threat.

It was hypothesized that *A. cephalotes* use specific pheromones to distinguish between plant material, different trails, and different colonies in order to increase their foraging efficiency. It may also be that there is a general recruitment pheromone used and recognized by all conspecifics. This would mean that there are other mechanisms that colonies of *A. cephalotes* have developed in order to assure successful foraging.

## METHODS

### Study Site

The study was carried out at the Santuario Ecológico in Cerro Plano, Monteverde (Fig. 1a) in the spring of 2006. Figure 1b depicts the layout of the study site, which used to be a farm and is now primarily secondary growth. Data were obtained from each of the four well established *A. cephalotes* nests located on the property. Most observations were made between 7:00 a.m. and 12:00 p.m., while the ants were still foraging at a steady rate.

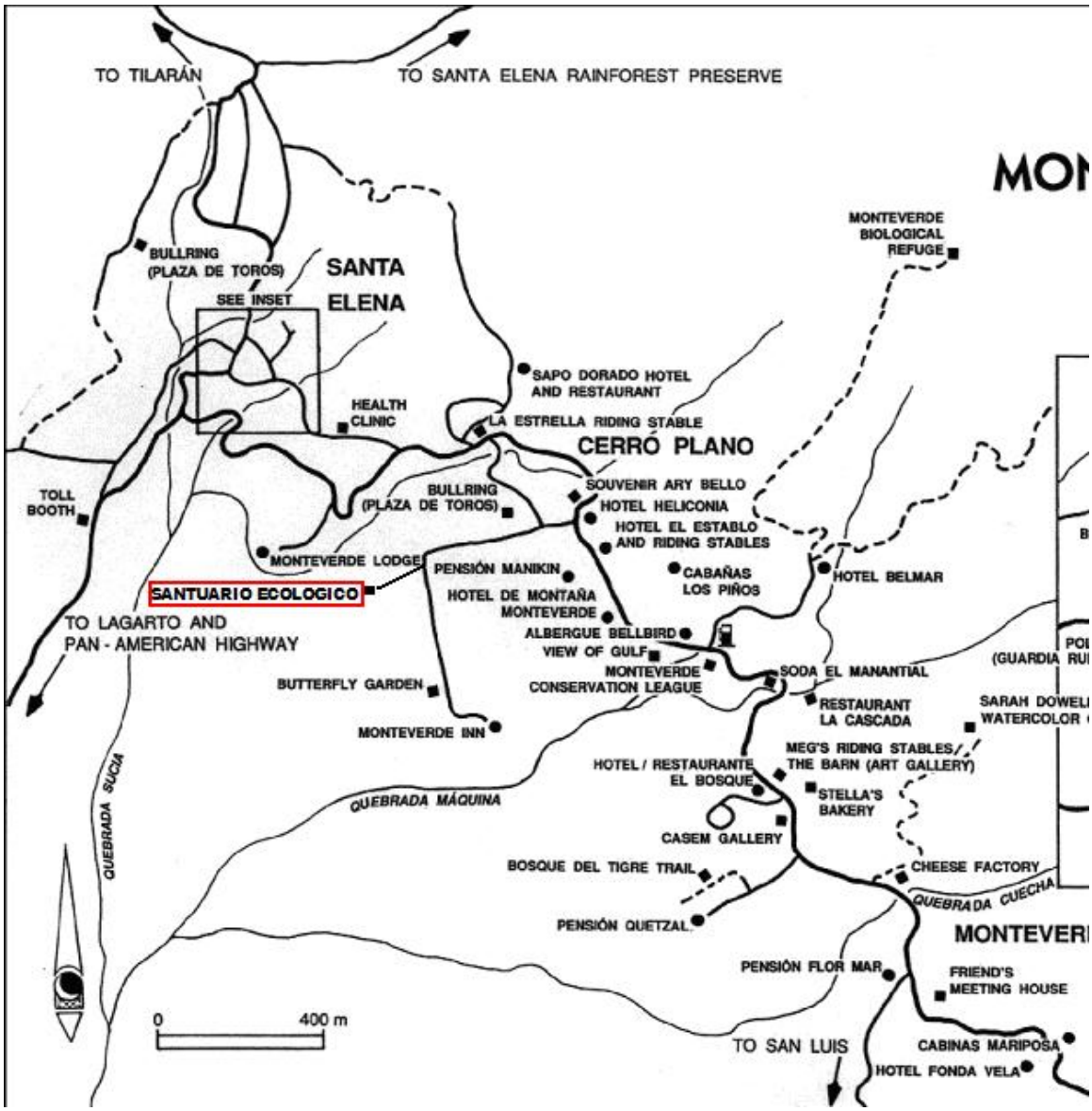


Figure 1a. Map of Monteverde, Cerro Plano and Santa Elena in northwestern Costa Rica in the Puntarenas province. All data were taken from the Santuario Ecológico (highlighted), Cerro Plano.

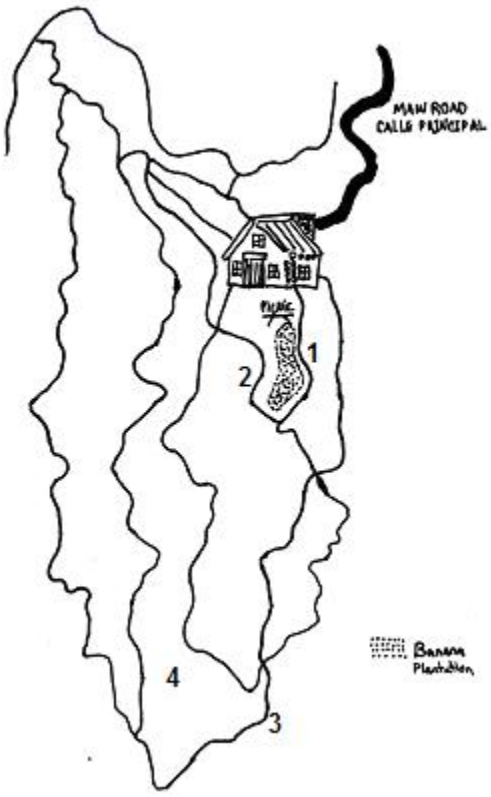


Figure 1b. Map of Santuario Ecológico located in Cerro Plano, Costa Rica. The trail system is depicted and the four *A. cephalotes* nests used for data collection labeled.

## Natural History

*A. cephalotes* workers will follow the foraging trail to the resource, then carry the cut plant material back to the nest (Holldobler and Wilson 1990); therefore, if they are carrying plant material, they should be headed back toward the nest. *A. cephalotes* exhibits caching behavior during a disturbance such as rain, obstacles in the trail, or an attack on the nest. The ants will drop the leaf they are carrying into a pile if they are foraging, and attend to the more urgent matter. Hart and Ratnieks determined that this behavior makes it more probable that they will later retrieve the leaves (2000).

## Leaf Marking

EXPERIMENT 1: The rate at which marked and unmarked plant material was retrieved from a cache was determined in order to see if pheromones are placed on the plant material itself. Leaves being carried by the ants were collected using tweezers and assumed to have pheromones present. Then leaves from the same species of tree were hand-cut to approximately the same size (one $cm^2$), taking care not to contaminate them with other scents. Every trial presented ten pieces of plant material for each treatment on opposite sides of a main trail and observed for a maximum of twenty minutes. The rates of retrieval were then compared using a Wilcoxon sign-rank test to see if there was a significant difference between the treatments.

### Trail Marking

EXPERIMENT 2: Leaves were collected from ants on two different trails of the same colony to determine if there were trail-specific pheromones. The rate at which the leaves were retrieved from the different caches was recorded for 26 trials and compared using a Wilcoxon sign-rank test to see if there was a significant difference in recognition by the ants.

EXPERIMENT 3: Ants carrying leaves were transferred using tweezers from one trail to a different trail of the same colony to determine if there were trail-specific pheromones laid on the actual trail. Toothpicks were used to mark the replacement point and an arbitrary distance in either direction along the path (17 cm). The rate and direction that 131 individuals traveled on both trails was recorded and compared with the Wilcoxon sign-rank test.

### Colony Odor

EXPERIMENT 4: Methods and analysis from experiment 2 were performed using leaf samples collected from two different colonies for a total of 28 trials.

EXPERIMNET 5: Methods and analysis from experiment 3were performed with 123 individuals moved between two different colonies using a compartmentalized box.

## RESULTS

### Leaf Marking

EXPERIMENT 1: The leaves that were cut by the ants were retrieved from the caches at a significantly faster rate than those that were hand-cut (Fig. 2) (z-value = -4.043, p-value = <0.0001).

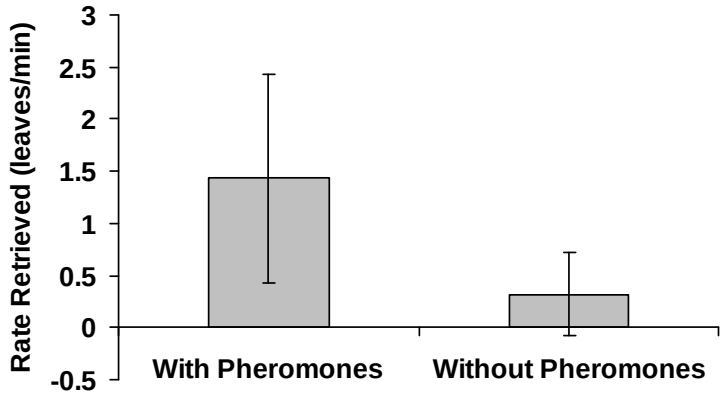


FIGURE 2. Mean rate at which *A. cephalotes* retrieved cached leaves that were cut by the ants (with pheromones) and cut by hand (without pheromones). Every treatment had a pile of ten leaves and a maximum observation time of 20 minutes. (n = 24). The bars represent ± 1 standard deviation of the means.

## Trail Marking

EXPERIMENT 2: Leaves from the original trail were retrieved at a significantly faster rate than those from a different trail in the same colony (Fig. 3) (z-value = -2.068, p-value = 0.0386).

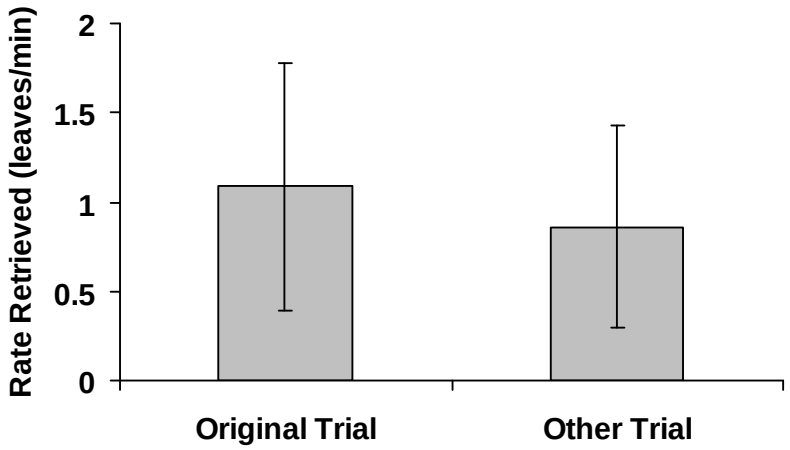


FIGURE 3. Mean rate at which *A. cephalotes* retrieved cached leaves from their trail and a different trail from the same colony. Every treatment had a pile of ten leaves and a maximum observation time of 20 minutes. (n = 26). The bars represent ± 1 standard deviation of the means.

EXPERIMENT 3: Ants foraged at a significantly slower rate when placed on a different trail in the same colony (Fig. 4) (z-value = -7.142, p-value = <0.0001). Thirty-four percent of the ants placed on the foreign trail went away from the nest, while 100% of the ants replaced on their original trail went toward the nest.

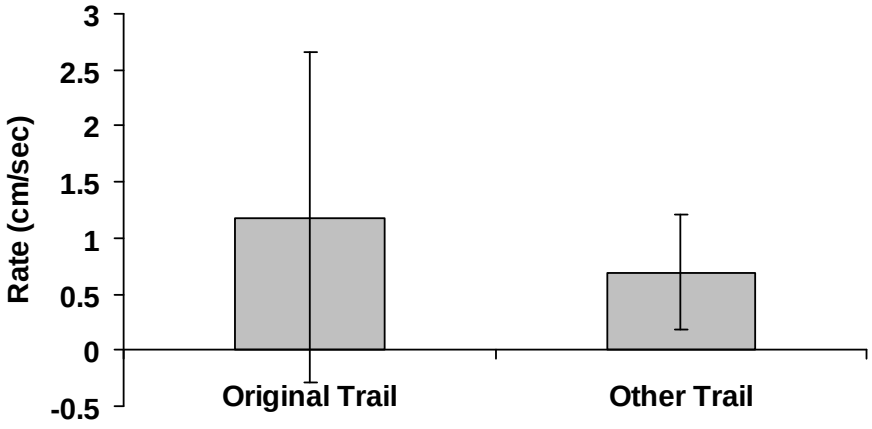


FIGURE 4. Mean rate at which *A. cephalotes* foraged on their own trail and when transferred to a different trail within the same colony. (n = 131). The bars represent ± 1 standard deviation of the means.

**Colony Marking**

EXPERIMENT 4: Leaves from the original colony were retrieved at a faster rate than those from a different colony (Fig. 5) (z-value = -3.304, p-value = 0.0010).

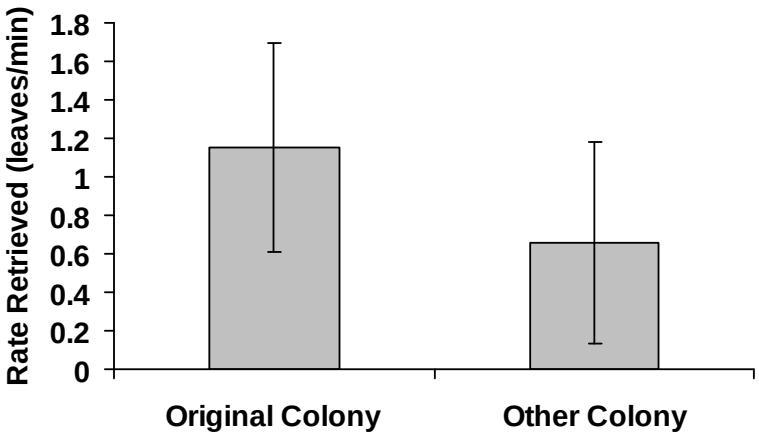


FIGURE 5. Mean rate at which *A. cephalotes* retrieved cached leaves from their own colony and those taken from a different colony. Every treatment had a pile of ten leaves and a maximum observation time of 20 minutes. (n = 28). The bars represent ± 1 standard deviation of the means.

EXPERIMENT 5: The ants foraged at a significantly faster rate within their own colony than when placed on the trail of a different colony (Fig 6) (z-value = -9.511, p-value = <0.0001). Forty-six percent of the ants placed in a different colony went in the opposite direction while 100% of the individuals went toward their own colony.

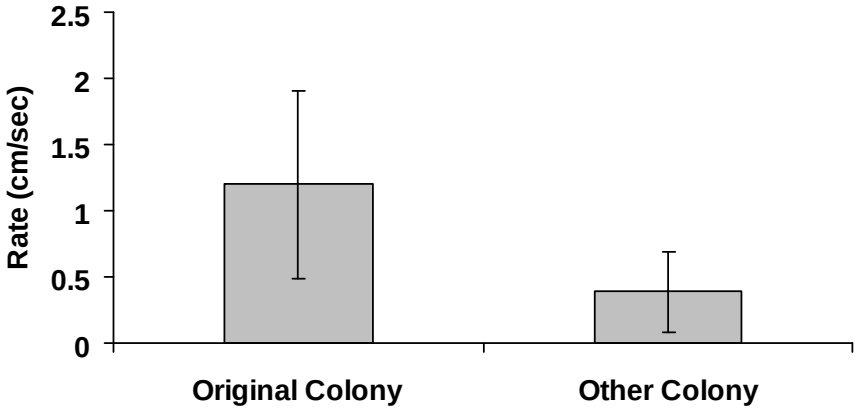


FIGURE 6. Mean rate at which *A. cephalotes* foraged on their own trail as well as when transferred to a trail in a different colony. (n = 123). The bars represent ± 1 standard deviation of the means.

## DISCUSSION

This study was important in determining if there were specific pheromones used to mark the plant material, different trails within a colony, and different colonies. This behavior would serve as effective mass communication between the workers in *A. cephalotes* to increase their foraging efficiency. It was found that plant material did get marked with pheromones because they were retrieved from caches at faster rates. The recognition of previously selected and partially transported plant material increases overall foraging efficiency.

There was a distinction between leaves being carried on different trails within the same colony. However, this difference was not as significant as the experiment comparing leaves taken from different colonies. The foragers retrieved the leaves taken from their trail at a faster rate in both instances, which supports the idea that the workers can specialize on specific trails and recognize the material being foraged. This specificity would help to distinguish pre-selected plant material from leaf litter around the trails.

The data also suggest that there are specific pheromones laid on different trails within the same colony as well as between different colonies. The ants removed from and replaced back onto their original trail recognized almost immediately which direction and where to bring their plant material. Although the individual ants foraged at a significantly slower rate on different trails and in other colonies, this was more than likely an underestimate of their effective foraging rate. There was visibly more uncertainty when an ant was transferred to a different colony, because many seconds were spent with either no movement or little net movement. Ants on foreign trails would sometimes end up going away from the nest and occasionally straight off the trail. This reduced foraging rate may also be partially due to the fact that the ants from the colony would sometimes attack the foreign ant, occasionally seizing the leaf and debilitating the ant. This specificity in trail pheromones maintains exclusive resource accessibility and assures that workers specialize on one trail to maximize efficiency.

More specific and therefore complex pheromones are used while *A. cephalotes* is group foraging in order to enhance communication and increase efficiency. A future study could focus on the directionality and specificity of trail pheromones on a single trail. It would be interesting to determine how ants know which direction to travel along the trail according to their current task.

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