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ABSTRACT

Hermetism, a behavior that isolates ant colonies, was studied in the laboratory between two ant species in Chile, *Camponotus morosus* Smith and *Linepithema humile* Mayr. Ants from five nests of *C. morosus* and *L. humile* were used, transferring ten individuals per nest of one of the colonies to receiving nests of the other. Conducts, including antennal exploration (AE), mandible opening (MO), dorsal flexion of the abdomen (DFA), biting (Bi), ventral flexion of the abdomen (VFA), fight (Fi), transport of the intruder ant (TI), moving backwards (MB), and death (De) were recorded during ten min, with ten replicates. The times of occurrence \pm SD of each event were analyzed with the Student t test. The events AE, MO, DFA, and MB had a short latency time in receiving *L. humile*, while *C. morosus* presented lesser latency for the events Bi, VFA, and Fi. When analyzing by χ^2 the frequency of occurrence of each event per species, AE and MO were similar in both species, and in aggression and rejection events like DFA, Bi, VFA, Fi, MB, TI, and De, there were greater frequencies of observations in *C. morosus* than in *L. humile*. From these results, *C. morosus* is a more hermetic ant than *L. humile*.

KEYWORDS: Behavior events, artificial nests, Chile.

RESUMEN

Se estudió en laboratorio el hermetismo, un comportamiento de aislación entre colonias entre dos especies de hormigas en Chile, *Camponotus morosus* Smith y *Linepithema humile* Mayr. Se utilizaron hormigas desde cinco nidos de *C. morosus* y *L. humile*, y se transfirieron diez individuos por nido desde una de las colonias hacia nidos receptores de la otra. Las conductas se grabaron durante 10 min, incluyendo exploración con las antenas (EA), abertura mandibular (AM), flexión dorsal del abdomen (FDA), mordedura (Mo), flexión ventral of del abdomen (FVA), vuelo (Vu), transporte de la hormiga intrusa (TI), retroceso (Re) y muerte (Mu), con diez repeticiones El tiempo de desarrollo \pm DS de cada evento se analizó con el test t de Student. Los eventos EA, AM, FDA y Re tuvieron un tiempo de latencia corto al recibir a *L. humile*, mientras que *C. morosus* presentó menor latencia en los eventos Mo, FVA y Vu. Al analizar mediante χ^2 la frecuencia de ocurrencia de cada evento, EA y AM fueron similares en ambas especies, y en los evento de agresión y rechazo como FDA, Mo, FVA, Vu, Re, TI y Mu hubo mayor frecuencia de observaciones en *C. morosus* que en *L. humile*. Con estos resultados, *C. morosus* es una hormiga más hermética que *L. humile*.

PALABRAS CLAVE: Eventos conductuales, nidos artificiales, Chile.

INTRODUCTION

In Chile, there are 62 species of ants described (Snelling & Hunt 1975, Ipinza-Regla 1984), distributed in six subfamilies, for of them more important for their number and distribution, Ponerinae, Dolichoderinae, Myrmicinae, and Formicidae (Ipinza-Regla 1984), which are found from

Arica through Tierra del Fuego, with a longitudinal transect from sea level through 2540 m elevation (Ipinza-Regla *et al.* 1983).

Social insects may recognize their sisters by means of mechanisms base don visual, sonic, and tactile/olfactory signals. In ants, recognition is based in cuticle chemical markers, hydrocarbon molecules synthesized in the colony,

that give a particular odor to each nest, which is recognized by the olfactory receptors in the antennae (Lahav *et al.* 1999; Katzav-Gozansky *et al.* 2004).

The differentiation between ants of the same species but different colonies is based in the recognition of these chemical markers (Jaisson 1985).

Ant societies are considered hermetic, that is, they do not accept individuals from another colony, either congeneric or not (Ipinza-Regla *et al.* 1996). Hermetism is recognized by violent aggressive reactions of the resident ants toward those homo- or hetero-specific intruders, which are often killed. The degree of hermetism is affected by the number of queens in the colony (monogyny or polygyny), the age of the intruder, the distance between nests and the removal of the colony (Ipinza-Regla *et al.* 1991).

Herein we evaluated in the laboratory the behavior parameters of rejection and tolerance (hermetism) toward an intruder in artificial nests of two ant species, *Camponotus morosus* Smith, 1858, and *Linepithema humile* Mayr, 1868, observing and comparing the frequency and latency time of these events.

MATERIALS AND METHODS

Eighty to 100 ants and a variable number of eggs and larvae were collected from five nests of the ants *C. morosus* (nests A1-A5) in San Carlos de Apoquindo, a pre-Andean location in the Las Condes Commune, Santiago, Chile, and *L. humile* (nests B1-B5) on the La Pirámide mountain, Metropolitan Park, Huechuraba Commune, Santiago. This material was taken to the Zoology and Ethology Laboratory, Agronomy School, Universidad Mayor, and set in 10 x 30 x 10 cm plastic rearing boxes that were maintained at 20±2°C, 45-50% RH and a 12 h photoperiod under white fluorescent light (Ipinza-Regla *et al.* 1991). The diet was honey mixed with apple puree and some insects or small pieces of chicken meat as energy and protein sources. Water was provided in wet cotton in glass tubes.

The ants had 30 days acclimation to the artificial nests, after which individuals were transferred to observe the hermetism conducts between the resident and intruding ants, until completion of ten replications with the five nests representing each species:

- 1) Ten ants from nest A (*C. morosus* intruders) were transferred individually to nest B (*L. humile* resident), the conducts were observed during 10 min and then they were taken away. The latency time of the conduct events was registered from the introduction of each intruding ant (time 0) until the first detection of each conduct action of the resident ant.
- 2) Vice-versa, ants of nest B (*L. humile* intruders) were transferred to nest A (*C. morosus* resident). The conduct parameters registered were antennal

exploration (AE), mandible opening (MO), dorsal flexion of the abdomen (DFA), biting (Bi), ventral flexion of the abdomen (VFA), fight (Fi), transport of the intruder (TI), movement backward (MB), and death (De).

STATISTICAL ANALYSIS. The parameter results were analyzed with the Chi square test (χ^2) to determine the significance of the difference between the numbers of observations (frequency) of each event between both species. Also, for the latency time variable the means and standard deviation (SD) were calculated, and the Student t test was used to determine the significance of the differences between the mean latency (occurrence) times of each conduct event.

RESULTS

In the transferences from nests A (*C. morosus*) to the receiving nests B (*L. humile*), and vice-versa, the events AE (antennal exploration) and MO (mandible opening) occurred always (100%) in both species, either as receptors or intruders (Table 1). Both recognition events occur simultaneously (Ipinza-Regla 2008a, 2008b, Hefetz *et al.* 1996).

The dorsal flexion of the abdomen (DFA) was observed in both species. As resident, *C. morosus* had a significantly greater frequency (92.8%) than *L. humile* (73.2%), according to the Chi² test. Biting (Bi) was observed in both species as residents, with a significantly greater frequency in *C. morosus* (48.8%) than *L. humile* (15.2%).

As in the previous event, the ventral flexion of the abdomen (VFA) occurred in both species, with a significantly greater frequency in receiving *C. morosus* (34.4%) than in receiving *L. humile* (8.0%).

Fighting (Fi) was significantly more frequent in *C. morosus* (24.0%) than *L. humile* (5.6%).

The transport of the intruder (TI) occurred only in receiving *C. morosus* (26.0%), and did not occur in receiving *L. humile*.

Moving backwards (MB) was observed in both species as receptors, but significantly more frequently in *C. morosus* (33.2%) than *L. humile* (16.4%).

Lastly, the death of the intruder (De), as its transport (TI) occurred only in the receiving nests of *C. morosus*, with a frequency of 26.0%.

The order of occurrence of the conduct events for the transferences of each species (from B toward A and vice-versa), is presented in Table 2. The events of antennal exploration (AE), mandible opening (MO), and dorsal flexion of the abdomen (DFA) occurred in both transferences and in the same order of occurrence. It calls the attention that the transport of the intruder (TI) and its death (De) was not observed in receiving *L. humile*.

Antennal exploration (AE) and mandible opening (MO) were the first events to appear and they occurred jointly.

The means of the events observed in each transference were grouped for species and are presented in Table 2, and their latency times in Table 3.

Latency time of antennal exploration (AE) was significantly shorter in *L. humile* (14.01 sec) than *C. morosus* (18.23 sec) ($t = 2.494$; $p = 0.01$).

Mandible opening (MO) presented a latency time for *C. morosus* receiving of 18.53 sec, and 14.16 sec for *L. humile* receiving ($t = 2.53$; $p = 0.01$).

Latency time for dorsal flexion of the abdomen (DFA) was also significantly greater for *C. morosus* (56.36 sec) than *L. humile* receiving (37.37 sec). ($t = 2.952$; $p = 0.0033$).

Latency time for fighting (Fi) tended to occur earlier in *C. morosus* (143.9 sec) than in *L. humile* (179.07 sec), but with not a significant difference ($t = 0.779$; $p = 0.4384$).

The transport of the intruder (TI) was observed only in *C. morosus* receiving, with a latency time of 214.0 sec.

The movement backward (MB) event tended to occur earlier in *L. humile* (137.0 sec) than *C. morosus* (174.0 sec), but the statistical analysis did not find a significant difference ($t = 1.151$; $p = 0.2519$).

The death of the intruder (De) occurred only in *C. morosus* receiving, with a latency time of 188.0 sec.

TABLE 1. Occurrence frequencies of the conduct events in the transfereces of *C. morosus* (A) toward *L. humile* (B), and vice-versa. / Frecuencias de eventos conductuales en las transferecias de *C. morosus* (A) hacia *L. humile* (B) y viceversa.

Events ¹	A toward B		B toward A		Chi square (χ^2)	p ³
	<i>L. humile</i> receiving		<i>C. morosus</i> receiving			
	N ²	%	N ²	%		
AE	250.0	100.0	250.0	100.0	---	---
MO	250.0	100.0	250.0	100.0	---	---
DFA	183.0	73.2	232.0	92.8	20.22	<0.05
Bi	38.0	15.2	117.0	46.8	56.88	<0.05
VFA	20.0	8.0	86.0	34.4	50.58	<0.05
Fi	14.0	5.6	60.0	24.0	32.12	<0.05
TI	0.0	0.0	43.0	17.2	44.88	<0.05
MB	41.0	16.4	83.0	33.2	18.03	<0.05
De	0.0	0.0	65.0	26.0	72.43	<0.05

¹Conduct events, AE: antennal exploration; MO: mandible opening; DFA: dorsal flexion of the abdomen; Bi: biting; VFA: ventral flexion of the abdomen; Fi: fight; TI: transport of the intruder; MB: moving back; De: death.

²Number of events observed in a total of 250 transfereces.

³Probability.

TABLE 2. Order of occurrence of the conduct events in each receiving ant (*C. morosus*, A, or *L. humile*, B). / Orden de desarrollo de los eventos conductuales en cada especie de hormiga receptora (*C. morosus*, A, o *L. humile*, B).

Transfereces	Conduct events ¹								
	AE	MO	DFA	Bi	VFA	Fi	TI	MB	De
B toward A	1°	2°	3°	4°	6°	5°	9°	7°	8°
A toward B	1°	2°	3°	5°	6°	7°	---	4°	---

¹Conduct events, AE: antennal exploration; MO: mandible opening; DFA: dorsal flexion of the abdomen; Bi: biting; VFA: ventral flexion of the abdomen; Fi: fight; TI: transport of the intruder; MB: moving back; De: death.

TABLE 3. Latency times (sec) of the conduct events in the transferences from *L. humile* (B) toward *C. morosus* (A), and vice-versa. / Tiempos de latencia times (seg) de los eventos conductuales en las transferencias de *L. humile* (B) hacia *C. morosus* (A) y vice-versa.

Conduct events ¹	Transferences of		Student t test
	B toward A	A toward B	
AE	n = 250 x = 18.23 sec SD = 24.22 sec	n = 250 x = 14.01 sec SD = 11.37 sec	t = 2.494 p = 0.0130
MO	n = 250 x = 18.53 sec SD = 24.45 sec	n = 250 x = 14.16 sec SD = 11.42 sec	t = 2.557 p = 0.0108
DFA	n = 232 x = 56.36 sec SD = 81.12 sec	n = 183 x = 37.37 sec SD = 35.38 sec	t = 2.952 p = 0.0033
Bi	n = 117 x = 138.85 sec SD = 139.61 sec	n = 38 x = 156.42 sec SD = 70.11 sec	t = 0.745 p = 0.4575
VFA	n = 86 x = 161.12 sec SD = 150.95 sec	n = 20 x = 163.45 sec SD = 122.01 sec	t = 0.064 p = 0.9488
Fi	n = 60 x = 143.90 sec SD = 157.13 sec	n = 14 x = 179.07 sec SD = 126.68 sec	t = 0.779 p = 0.4384
TI	n = 43 x = 214.14 sec SD = 180.56 sec	n = 0 x = 0.00 sec SD = 0.00 sec	t = --- p = ---
MB	n = 83 x = 174.07 sec SD = 174.65 sec	n = 41 x = 137.83 sec SD = 142.98 sec	t = 1.151 p = 0.2519
De	n = 65 x = 188.57 sec SD = 147.75 sec	n = 0 x = 0.00 sec SD = 0.00 sec	t = --- p = ---

¹Conduct events, AE: antennal exploration; MO: mandible opening; DFA: dorsal flexion of the abdomen; Bi: biting; VFA: ventral flexion of the abdomen; Fi: fight; TI: transport of the intruder; MB: moving back; De: death. n: number of events observed in a total of 250 transferences.

DISCUSSION

Although the conduct events of antennal exploration (AE), mandible opening (MO), dorsal flexion of the abdomen (DFA), and moving backward (MB) appeared in less seconds in *L. humile* than *C. morosus*, the first two events are recognition expressions, that is, they are not agonistic type conducts (e.g., Ipinza-Regla et al. 2005, 2010, 2013).

When two ants meet, they recognize each other at short distance (1.2 cm), but generally they need physical contact through antennal exploration of any part of the body for recognition, as the signal is distributed broadly in the epicuticle (Lenoir *et al.* 2001). The events of dorsal flexion of the abdomen (DFA) and moving backward (MB) are considered aggressive conducts, and moving backward is a strategy to avoid homo or heteroespecific contact with the

intruder species. The events of biting, ventral flexion of the abdomen, and fighting tended to present shorter latency times in *C. morosus* than in *L. humile*. Despite the absence of statistical differences, our results agree with the relatively low levels of aggression of *C. morosus* among three species of ants towards homo- or heterospecific intruders obtained by Ipinza-Regla *et al.* (1996).

The degree of tolerance of *C. morosus* varies toward the introduction of individuals foreign to the nest. For example, *C. morosus* is more tolerant to the transference of *Brachymyrmex giardii* Emery when this comes from a monospecific nest (formed only by *B. giardii*). This could indicate that the presence of ants of several species in the same nest could have a protecting effect on the intruder, which is less rejected when coming from a mixed site (Orivel *et al.* 1997).

Generally, the members of a nest of social insects are relatives (Crozier & Pamilo 1996). In most social insects, the individuals recognize their relatives in the nest, and reject the conspecific intruders, maintaining the integrity of the colony and ensuring that altruism is directed toward the relatives (Chapuizat *et al.* 2004). Discrimination between the members of a nest versus intruders occurs by comparing the phenotype of the olfactory signature of each individual that is met by an ant, with a custom olfactory reference, or template, the chemical signature of the ant that initiates the recognition of the intruder. The recognition of the members of the nest is a comparison of the signatures of other individuals with that of their nest (Boulay & Lenoir 2001). The standard method to study this process in ants is through aggression bioassays (Chapuizat *et al.* 2004).

The epicuticular hydrocarbons are considered the main chemical signs responsible of recognition among the nest members of ants and wasps (Lenoir *et al.* 2001, Soroker *et al.* 2001, Ipinza-Regla *et al.* 2004), although their role remains in debate (Lenoir *et al.* 2001). Numerous studies in ants have found correlations between the patterns of epicuticle hydrocarbons and the recognition process among the members of a nest, but few have purified these compounds, like in the desert ant *Cataglyphis niger* (André), where only the hydrocarbon fraction present in the epicuticle lipids is responsible for recognition (Lahav *et al.* 1999, Boulay *et al.* 2000, Hefetz *et al.* 2000; Lenoir *et al.* 2001). These hydrocarbons are mainly alkanes of low volatility, sometimes accompanied by alkenes and alkynes (Jaffe *et al.* 2002, Katzav-Gozansky *et al.* 2004).

The odour recognition hydrocarbons may come from the environment and have been carried to the nest by foraging ants, or be genetically determined odors produced by the queen, workers, or other nest members (Soroker *et al.* 1998), or may also come directly from an individual or from interactions with other individuals (Lahav *et al.* 1999, Hefetz *et al.* 2000, 2001). Thus, in any given source of odor acquisition, the large colonies have a uniform and y

unique odor (Soroker *et al.* 2001, Hefetz *et al.* 2001, Lenoir *et al.* 2001, Boulay *et al.* 2004). According to this model, the members of a colony share and exchange recognition signs and the resulting odor of this colony is an average of the individual odors of each ant in the colony (Soroker *et al.* 1998).

CONCLUSIONS

Both ant species presented hermetism, which was stronger in *C. morosus* than in *L. humile*. Also, both species presented similar conducts of antennal exploration and mandible opening.

The conducts of aggression and rejection presented significant differences in the frequency of occurrence of the conduct parameters dorsal and/or ventral flexion of the abdomen, biting, fighting, transport of the intruder, moving backward and eventual death, and occurred earlier in *C. morosus* than in *L. humile*.

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