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ARTÍCULO ORIGINAL

CLOSURE OF *CAMPONOTUS CHILENSIS* (SPINOLA, 1851) (HYMENOPTERA: FORMICIDAE) AGAINST *RETICULITERMES FLAVIPES* (KOLLAR, 1837) (ISOPTERA: RHINOTERMITIDAE), UNDER LABORATORY CONDITIONS.

HERMETISMO DE *CAMPONOTUS CHILENSIS* (SPINOLA, 1851) (HYMENOPTERA: FORMICIDAE) SOBRE *RETICULITERMES FLAVIPES* (KOLLAR, 1837) (ISOPTERA: RHINOTERMITIDAE), EN LABORATORIO.

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Resumen

El presente estudio se realizó con especies de *Camponotus chilensis* (Spinola, 1851) y *Reticulitermes flavipes* (Kollar, 1837), en condiciones de laboratorio. El propósito de esta investigación fue determinar la presencia de acciones de *C. chilensis* sobre *R. flavipes* y *R. flavipes* sobre *C. chilensis*. Para tal efecto se colectaron individuos de cada especie desde cinco nidos respectivamente, los cuales fueron confinados en nidos artificiales por un período de treinta días para su aclimatación, previo a las observaciones. Se analizarán eventos conductuales entre las especies residentes versus especies intrusas. Los resultados muestran que ambas especies en calidad de residentes, son herméticas y agresivas frente a las especies intrusas. Ambas especies presentaron similitud en el reconocimiento de las especies intrusas, ya que los periodos de latencia fueron similares. En relación a los eventos de agresión, *C. chilensis* presentó una mayor frecuencia de dicho evento en comparación a lo observado en *R. flavipes*.

Palabras clave: Formicidae, *Camponotus chilensis*, Rhinotermitidae, *Reticulitermes flavipes*, eventos de comportamiento, hermetismo.

Abstract

The present study was developed with individuals of the *Camponotus chilensis* (Spinola, 1851) species and the *Reticulitermes flavipes* (Kollar, 1837) species under laboratory conditions. The main purpose was to determine the presence of confinement of *C. chilensis* over *R. flavipes* and *R. flavipes* over *C. chilensis*. To this effect five nests of each species were collected, and they were placed during a one month period of adjustment. After that, the behavior of each the species mentioned above was observed in relation to the presence of invaders of the other species and vice versa. The results obtained were as follows: both species are hermetic and aggressive in relation to invaders of the other one. Both species recognize invaders in the same manner and react at similar time. In relation to the events of aggression, *C. chilensis* presents a greater frequency of this conduct, and also it does it in less time than the *R. flavipes* species.

Key words: Formicidae, *Camponotus chilensis*, Rhinotermitidae, *Reticulitermes flavipes*, behavioral events, closure.

Introduction

In social insects territory defense is manifested through colonial closure, which is established based on an odour elaboration and a common use of it by all colony members. This colonial scent is in part made of cuticle hydrocarbons, which are synthesized by a gland located in the abdomen, later stored in a head gland found on formicid ants called retropharyngeal or postpharyngeal gland. Through trofalaxis (inter-changed regurgitated food between adults and larvae) an odour mixing takes place among remaining colony members, until reaching a "gestalt" or the reflex of a state of a colony in a given moment.

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The colony odour allows the members of a society to discriminate between same colony members and a intruder. The intruder is usually rejected with a variable degree of aggression, ultimately being carried out from the colony (Lenoir *et al.*, 2001).

There are 11,600 ant species around the world approximately; and in Chile there are 62 species, with 53 endemic and 9 exotic species (Ipinza, 1984).

There are more than 2600 termite species in the world and although they are beneficial in natural environments, in cities they can cause great lost of material goods. From this number, 150 species approximately are harmful and just half of it are considered serious pests (Abe *et al.*, 2000).

In Chile, the first underground termite detection of the genera *Reticulitermes* sp, was reported at the end of the year 1986, on the Metropolitan Region. Nowadays it is distributed throughout most of the V Region and some zones of the IV Region as well. (Ripa & Luppichini, 2004).

According to Cammousseight (1997), the family Rhinotermitidae would be represented in Chile by the species *Reticulitermes hesperus* Banks, 1920, from Nearctic origin. This statement contradicts the fact that this species probably entered our country from the east coast of the United States (Su, 2004, in Ripa & Luppichini, 2004). The termite that inhabits this area would belong to the species *Reticulitermes flavipes* (Kollar, 1837) and its morphology therefore would correspond to another species.

Due to the great lost these termites are causing, housing damages, and increase of complaints of this pest on the Metropolitan Region, it is pivotal to look for a control of these isopteran termites. Ants represent a plausible alternative as a biological control, because these ants are termites natural predators.

The present work will allow us to evaluate if the ant *Camponotus chilensis*'s agonistic and predatory behavior could be used as a possible control of the termite *Reticulitermes flavipes*, in laboratory conditions.

Materials and methods

Five nests of *C. chilensis* including queens and workers were collected from a shrub found at the Precordillera, on the Metropolitan zone (Agua del Palo). These were carried to the Universidad Mayor Zoology and Ethology Laboratory where they were placed into artificial nests (nests A1, A2, A3, A4, and A5). Nests corresponded to a 9 x 9 x 9 cm clear plastic box (height x length x width). Artificial feeding for specimens consisted of a blend made of apple sauce, bee honey, and chicken carcass (Ipinza-Regla *et al.*, 1991). Nest were kept at 20° C ± 2° C and a relative humidity of 45-50%.

Termites were collected from five different nests coming from dwelling homes at the comuna of Quinta Normal. Just like ants, they were carried to the already mentioned Laboratory and placed into rearing devices, which consisted of artificial nests (nests T1, T2, T3, T4, and T5). Artificial nests were made of 34 x 24 x 12 cm (height x length x width) clear boxes, with their lids. Feeding consisted of pine wood and cardboard pieces, kept at 27 °C temperature and 90% relative humidity.

After three weeks of laboratory insect captivity, we proceeded to perform transfer experiences. We could observe presentation of aggressive behaviors between intruder ants placed into receptor or resident termite nests, and intruder termites placed into receptor or resident ant nests, respectively.

Transfer Experiences

Termites coming from "intruder" *R. flavipes*, nests (nests T1 to T5), were placed one by one, until reaching a number of ten termites per nest, into "resident" *C. chilensis* nests.

Similarly, *C. chilensis* ants from "intruder" A1, A2, A3, A4, and A5 nests, were transferred one by one, until reaching ten ants per nest, into corresponding "resident" termite nests, nests T1 to T5 (the size of ten individuals in a population was determined, according to experiences performed by Ipinza-Regla *et al.* 1991, 1998, 2000, 2002, 2004, and 2005, and they were statistically significant).

During six minutes behaviors of receptor ant and termite nests were recorded, respectively. Latency time records were performed; time interval taking place from the individual insertion into the other's nest, or time 0, until the occurrence of each behavioral action (calculated in seconds).

Receptor ant nest behavioral events described by De Vroey & Pasteels (1978), Ipinza-Regla *et al.*, (1991) were recorded: antennal exploration (EA), mandible opening (AM), Biting (Mo), abdomen ventral flexion (FVA), abdomen dorsal flexion (FDA), fight (L), intruder transport (TI), death (M), against intruder termites.

In relation to behavioral events of receptor "resident" termites against "intruder" ant presence, we recorded the following behaviors described by Sepúlveda (1997): approach to alien and brief follow up by one or more termites (ACE), mandible opening and closing (MAN), biting (MOR), and fight (LU).

Chi-square test was performed to observe if differences were significant in order to frequency of presentation in behavioral events. In order to evaluate presentation latency time of each event per species, the Mann-Whitney test was performed to determinate if there are significant differences between both species using the latency time. Finally, the Kruskal-Wallis test was done to determinate if there was a difference in intraspecific closure.

Results

Nevertheless most behavior events shown between ants and termites respectively are not homologous, comparisons of behaviors feasible of being homologous were performed (mandible opening (AM) - mandible closing (MAN), both being recognition events; and biting events (Mo) - biting (MOR); fight (L) - fight (Lu); being these last ones considered as aggressive behaviors).

The most observed events were AM –MAN, presented in a 100% on both species transfers, therefore it was not possible to perform the Chi-square test.

Table 1 shows that comparison between Mo-MOR was the second most repeated event. The higher frequency was shown in A1 and A3 nests, corresponding to *C. chilensis* as receptor. An overall of 100% transfers, comparing T1 and T3 *R. flavipes* nests as receptor (90% transfer total). According to the Chi-square test on both cases frequency difference of these event presentation was significant being more agressive the ants than termites.

According to table 2 frequency and L-Lu comparison of ants and termites respectively- the mentioned events were observed on both species, being higher the presentation frequency of *C. chilensis* in A1 and A4 nests, as receptors. Whereas presentation frequency of *R. flavipes* as receptor was higher between T2 and T5 nests. According to the Chi-square test, the difference in event presentation frequency between both species was significant for all nests, with the only exception of A2-T2 and A3-T3 nests comparison. As a result this event is not comparable between both species.

For the comparative analysis of behavioral events between homospecific nests, it was used the Kruskal-Wallis test and it was calculated each observed event probability.

Table 3 gives the average times (in seconds) of observed event presentation of intruder termites in *C. chilensis* receptor nests.

Observed latency times for EA, AM events were considered significant. Both events are considerate as recognition behavioral and usually have similar values in ants. Whereas Mo, FVA, L, T, and M events were not significant because all nest are similar in aggressive behaviors.

Table 4 gives us the average (median) time analysis of observed event presentation (seconds) of intruder ants in nests of *R. flavipes* receptor.

ACE and MOR events were the only ones considered as significant with values of KW=9.75 and p=0.005 and KW=14.84 and p=0.005, respectively. Whereas MAN and Lu were not significant.

TABLE N° 1

FREQUENCY (%) AND COMPARISON OF BEHAVIORAL EVENT PRESENTATION: BITING (MO) AND BITING (MOR) OF *CAMPONOTUS CHILENSIS* ANTS AGAINST *RETICULITERMES FLAVIPES* INTRUDER TERMITES AND OF TERMITES AGAINST INTRUDER ANTS.

Observation frequency for Mo-MOR events

NEST	A (%)	T (%)	Chi-square value	p Value
1	100	90	8,53	*0,004
2	100	100		
3	100	90	8,53	*0,004
4	100	100		
5	100	100		

Mo: Biting event observed in *Camponotus chilensis* ants; MOR: Biting event observed in *Reticulitermes flavipes* termites; A: Nest of *Camponotus chilensis* ants; T: Nests of *Reticulitermes flavipes* nests; p: Probability; *: Significant values.

TABLE N° 2

FREQUENCY (%) AND COMPARISON OF BEHAVIORAL EVENT PRESENTATION: FIGHT (L) AND FIGHT (LU) OF *CAMPONOTUS CHILENSIS* ANTS AGAINST *RETICULITERMES FLAVIPES* INTRUDER TERMITES AND OF TERMITES AGAINST INTRUDER ANTS.

Observation frequency for L-Lu events

NEST	A (%)	T (%)	Chi-square value	p Value
1	100	70	32,98	*<0,0001
2	30	40	1,78	0,18
3	20	20	0	1
4	80	60	8,6	*0,003
5	50	70	7,52	*0,006

L: Fight event observed in *Camponotus chilensis* ants; Lu: Fight event observed in *Reticulitermes flavipes* termites; A: Nest of *Camponotus chilensis* ants; T: Nests of *Reticulitermes flavipes* nests; p: Probability; *: Significant values.

TABLE N° 3

AVERAGE TIME OF OBSERVED EVENT PRESENTATION (SECONDS) OF INTRUDER TERMITES INTO *C. CHILENSIS* RECEPTOR NESTS

Ants	Nest							
Events		A1	A2	A3	A4	A5	**KW**	**p**
EA	**N**	10	10	10	10	10	11,18	*0,02
	M	11	20,5	3	2	6,5		
AM	**N**	10	10	10	10	10	11,71	*0,02
	M	11,5	20,5	3	2	4		
Mo	**N**	10	10	10	10	10	7,98	0,09
	M	27,5	27,5	7,5	11	9,5		
FVA	**N**	9	7	9	9	9	3,65	0,46
	M	28	42	20	50	55		
L	**N**	10	3	2	8	5	6,36	0,17
	M	38	32	10	36	96		
T	**N**	2	3	1	1	4	2,72	0,74
	M	92	49	9	44	56,5		
M	**N**	3	4	3	4	2	4,21	0,38
	M	147	117,5	38	92	76,5		

EA: Antennal Exploration; AM: Mandible Opening; Mo: Biting; FVA: Abdomen ventral flexion; L: Fight; T: Intruder Transport; M: Death: A: Ant nest; **N** : Event presentation number; **M:** Median of presentation time; **KW:** Kruskal-Wallis; **p:** Probabilty; *: Significant values.

Table 5, shows the average (median) times of presentation (in seconds), of AM-MAN, Mo-MOR, and L-Lu events between ant and termite nests respectively, as receptors.

When performing latency time comparisons of each behavioral event we used the Mann- Whitney test. We obtained U and p values for each observed event:

Given values for AM-MAN comparison events, in A2 –T2, A4-T4 nests, gave significant p values, whereas comparisons of other nests did not give significant values. Latency times for A1-T1, A4 –T4 nests, when comparing Mo-MOR event values, were significant. However, A2-T2, A3-T3, and A5-T5 nests did not show significant values.

TABLE N° 4
AVERAGE TIME OF OBSERVED EVENT PRESENTATION (SECONDS)
OF INTRUDER ANTS INTO *R. FLAVIPES* RECEPTOR NESTS

Termites	Nest							
Events		T1	T2	T3	T4	T5	**KW**	**p**
ACE	**N**	9	6	5	7	7	9,75	*0,05
	M	64	44	7	11	7		
MAN	**N**	10	10	10	10	10	6,65	0,16
	M	4	2,5	6	16	5		
MOR	**N**	9	10	9	10	10	14,84	*0,005
	M	74	6	22	32	18		
Lu	**N**	7	4	2	6	7	5,39	0,25
	M	155	10	140,5	61,5	56		

ACE: Approach to alien and brief follow up by one or more termites; MAN: Mandible opening and closing; MOR: Biting; L: Fight; T: Termite nest; **N:** Event presentation number; **M:** Median of Latency time; **KW:** Kruskal-Wallis; **p:** Probability: *: *: Significant values.

TABLE N° 5
AVERAGE TIME OF PRESENTATION (SEC.) OF BEHAVIORAL EVENTS BETWEEN NESTS
WHEN *CAMPONOTUS CHILENSIS* (A) ANTS IS RECEPTOR AND WHEN *RETICULITERMES FLAVIPES* (T) IS RECEPTOR

Ants/ Termites		Nests									
Events		A1	T1	A2	T2	A3	T3	A4	T4	A5	T5
AM-MAN	**N**	10	10	10	10	10	10	10	10	10	10
	Me	16,6	24,2	19,1	7,4	6,5	12,4	5,1	18	8,6	8,1
	DE	17,33	39,46	13,93	9,91	9,45	13,55	6,008	12,18	11,49	7,22
	U	44,5		20		34,5		13,5		44	
	p	0,68		*0,02		0,25		*0,004		0,68	
Mo-MOR	**N**	10	9	10	10	10	9	10	10	10	10
	Me	36,2	79,66	27,1	23,8	10,5	37,33	18,5	38,8	17,2	22,4
	DE	38,53	50,24	19,49	43,382	9,91	45,93	18,95	28,84	19,73	19,27
	U	19		29		22		23		39,5	
	p	*0,04		0,12		0,07		*0,04		0,44	
L-Lu	**N**	10	7	3	4	2	2	8	6	5	7
	Me	69,1	136,14	43,66	78,25	10	140,5	39,25	87,5	95,6	57,85
	DE	74,09	48,75	26,50	138,51	11,31	126,57	28,85	108,06	69,04	65,11
	U	13		3		0		19,5		8,5	
	p	*0,03		0,4		*0		0,57		0,15	

AM: Mandible opening; MAN: Mandible opening and closing; Mo: Biting; MOR: Biting; L: Fight; Lu: Fight, in *Camponotus chilensis* nests and in *Reticulitermes flavipes* nests, respectively; A:Nests of *Camponotus chilensis* ants; T: Nests of *Reticulitermes flavipes* termites; **N:** Number of times that the event was presented; **Me:** Average; **DE:** Standard deviation; **U:** Mann-Whitney; **p:** Probability; *: Significant values.

Finally, latency time values observed for L ant event compared to Lu termite event between A1-T1 and A3-T3 nests were not significant. Comparative values between A2-T2, A4-T4 y A5-T5 nests, were not significant.

Discussion and conclusions

Our results show that *C. chilensis* shows a higher degree of closure against *R. flavipes* termite but there is not a higher degree of closure in opposite sense This result agrees with Shelton & Grace (1996) who probed that *Coptotermes formosanus* termite is not aggressive towards other colony individuals from the same species, when reared in laboratory conditions. On the other hand, Ipinza-Regla *et al.,* (1998, 2000, 2002) showed that -in experiences performed in laboratory conditions- the two ant species *Camponotus morosus* and *Linepithema humile* behaved hermetically against *Reticulitermes hesperus* termite. The same authors showed that the *C. morosus* ant was more hermetic than *L. humile* against the mentioned above termite.

Costa-Leonardo & Barsotti (1998), in a research performed in Brazil, showed that ants from *Pheidole megacephala* species turned to be great predators of wing individuals from *Coptotermes havilandi* termite species.

In conclusion, we can mention that the *C. chilensis* ant is hermetic against *R. flavipes,* whereas the latter showed a lower degree of aggression against the intruder ant. The death event was even described only for *C. chilensis,* because it is capable of causing death to *R. flavipes,* whereas the latter could not caused it to *C. chilensis.*

The *C. chilensis* species could be considered as an eventual biological control of *R. flavipes*, al least in experiences performed under laboratory conditions.

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