

ARTÍCULO ORIGINAL

CLOSURE OF SOLENOPSIS GAYI (SPINOLA, 1851) (HYMENOPTERA: FORMICIDAE) AGAINST RETICULITERMES FLAVIPES (KOLLAR, 1837) (ISOPTERA: RHINOTERMITIDAE), IN ARTIFICIAL NESTS¹

HERMETISMO DE SOLENOPSIS GAYI (SPINOLA, 1851) (HYMENOPTERA: FORMICIDAE) SOBRE RETICULITERMES FLAVIPES (KOLLAR, 1837) (ISOPTERA: RHINOTERMITIDAE) EN NIDOS ARTIFICIALES

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RESUMEN

El presente estudio se realizó con especies *Solenopsis gayi* (Spinola, 1851) y *Reticulitermes flavipes* (Kollar, 1837) en nidos artificiales. Las especies en estudio fueron recolectadas en diferentes localidades de la Región Metropolitana. Una vez en laboratorio y después de un período de aclimatación apropiado, termitas "intrusas" fueron transferidas a colonias de hormigas residentes, y hormigas "intrusas" fueron transferidas a colonias de termitas residentes. Diferentes eventos conductuales fueron observados en cada uno de los nidos de especies residentes. Eventos conductuales de agresividad estuvieron presente en ambas especies residentes. La frecuencia de estos eventos fue más alta en hormigas residentes.

PALABRAS CLAVE: Formicidae, *Solenopsis gayi*, Rhinotermitidae, *Reticulitermes flavipes*, eventos de comportamiento, hermetismo.

ABSTRACT

In order to study closure in ants and termites on a laboratory setting, groups of *Solenopsis gayi* (Spinola, 1851) and *Reticulitermes flavipes* (Kollar, 1837) ants and termites, respectively, were collected from different locations. Once in the laboratory and after the proper acclimatization period, intruder termites were transferred into colonies of resident ants, and intruder ants were transferred into colonies of resident termites. Behavioral performances showed on each resident species were recorded. Aggressive behavioral performances were present in both resident species; frequencies of these behavioral events were higher on resident ants.

KEYWORDS: Formicidae, *Solenopsis gayi*, Rhinotermitidae, *Reticulitermes flavipes*, behavioral events, closure.

INTRODUCTION

Ants differ from most insects because they are eusocial insects, estimating that only 2% of insects live in colonies. These colonies are characterized by

being large cooperative groups, keeping a complex structure of classes or castes, where each individual performs well determinate and vital functions for the rest of the colony.

These insects are capable of differentiating between intruder individuals (homo or hetero-specifics) and nest members. This is possible because they have chemical identification mechanisms, based on cuticle hydrocarbons, which are synthesized by gland located in the abdomen, later stored in a head gland found on formicid ants called retropharyngeal or postpharyngeal gland. Through trophalaxis (interchanged regurgitated food between adults and larvae) an odour mixing takes place among remaining colony

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members, until reaching a "gestalt" or the reflex of a state of a colony in a given moment. (Ipinza-Regla *et al.*, 2004; Ipinza-Regla *et al.*, 2008a), which are interchanged among same colony members to create a characteristic colony scent. Because of this identification mechanism the intruder is discriminated and when alien to the colony it is generally rejected, attacked, and carried out of the nest (Lenoir *et al.*, 2001). Due to these behavior ants social species are considered hermetical (Ipinza-Regla *et al.*, 1991; Ipinza-Regla *et al.*, 2008a; Ipinza-Regla *et al.*, 2008b).

The underground termite *Reticulitermes flavipes* (Kollar, 1837) was for the first time detected in Chile on 1986, carried into the country on a wooden container coming from the North Hemisphere. Currently, it is distributed throughout the Metropolitan Region, most of V Region, and a small area of VI Region (Ripa & Luppichini, 2004).

This termite is found on depths from 20 cm to 80 m. They go to the surface to get wood pieces to eat. Therefore the most affected wood pieces are those located underground or that are in direct contact with it. Also, these termites have preference for some kinds of woods, especially pine (*Pinus radiata*).

Many termite control systems have been exclusively based on the use of chemical agents. A significant number of these agents have proved to have secondary effects on people, domestic and wild animals, and the environment. Because of this, it has currently grown an interest to investigate alternative control methods, among others, the use of biological control.

The present work will try to determinate closure presence of *Solenopsis gayi* (Spinola, 1851) against *Reticulitermes flavipes* (Kollar, 1837) in artificial nests.

MATERIALS AND METHODS

In order to verify the presence of closure between *Solenopsis gayi* and *Reticulitermes flavipes* we worked with five *S. gayi* ant nests (S1, S2, S3, S4, and S5), that were collected from a zone in Agua del Palo, Metropolitan Region. Each nest was compound of two-hundred workers with a variable number of eggs and larvae, which were placed into artificial nests at the Universidad Mayor Zoology and Ethology Laboratory.

Rearing devices consisted of 20 x 30 x 10 cm (width x length x height) plastic boxes, kept at 20° ± 2°C and a

relative humidity of 45-50%. A 12 h/light photoperiod was used by means of fluorescent tubes (Ipinza-Regla *et al.*, 1991). Feeding consisted of bee honey, shredded apple, and animal protein (cooked chicken, ham or insect body-parts). In addition, five *R. flavipes* populations were collected (T1, T2, T3, T4, and T5) on several areas of the Metropolitan and V Regions.

Each termite population consisted of two hundred samples which were placed within twenty artificial devices, which consisted of 15 cm (diameter) by 20 cm (height) plastic boxes. Termites were kept at 27° C in complete darkness, and 90% relative humidity. Diet consisted of pine wood and cardboard pieces.

A month later, after acclimatization period of artificially reared insect nests, transfer experiences were performed to observe rejection behaviors among receptor ants. Insertion of intruder termites into receptor ants took place, while intruder ants were transferred into receptor termite nests, respectively.

Transfer Experiences

T1 nest ants (intruders) were transferred one by one until reaching a number of 10 individuals (Ipinza-Regla *et al.*, 1991, 1998a, 1998b, 2000, 2002, 2004, 2005, 2008a, 2008b) into S1 ant nest (*S. gayi*, receptor). Then, this procedure was repeated by transferring termites from T2, T3, T4, and T5 nests into ant S3, S4, and S5 nests; respectively.

Ants from S1, S2, S3, S4, and S5 nests were transferred one by one until reaching 10 individuals for each nest into termites T1, T2, T3, T4, and T5 nests, respectively.

After a six minute observation period per transfer, the following behavioral events were recorded from resident ants when confronted to intruder termites; antennal exploration (EA), mandible opening (AM), biting (Mo), abdomen ventral flexion (FVA), fight (L), intruder transportation (TI), and death (M). Besides, it was measured the latency time (in seconds), which corresponds to the mean time running from the introduction of an intruder individual into the resident nest. This time corresponds to time 0 until the occurrence of each behavioral event, occurred for the first time, by resident individuals.

Then during six minutes, behavioral events and latency period of receptor termites, when facing intruder ants, were recorded as follows: approach to foreigner and brief follow up by one or more termites (ACE), mandible opening and closing (MAN), biting (MOR), and fight (LU) (Sepúlveda, 1997).

Chi-square (χ^2) test was performed to determinate if there were significant differences among species. To assess the presentation latency time of each species event, it was performed the Mann-Whitney test to see if there is significant differences between the two species. Finally, Kruskal-Wallis test was performed to determinate if any nest was more hermetic than the others, on both species studied.

RESULTS

Because not all behavioral events observed between species being studied during transfers can be compared because they are not homologous between them it could only be done a statistical comparison between recognition events of the two

species. In this case, these events were antennal exploration (EA) for ants and approaching (ACE) for termites, and among agonistic events; biting and fight.

Table 1 shows the presentation frequency (%) of EA and ACE behavioral events of *S. gayi* (S) against *R. flavipes* intruder termites (T), and vice versa. The highest chi square value (82.881) was observed comparing the EA/ACE values from S3 and T3, respectively. On the other hand, the lower value was observed on two comparisons, between S1-T1 and S5-T5, respectively; with a chi square value of 8.526. All calculated values were significant.

On table 2 can be observed the presentation frequency (%) of biting behavioral event between *S. gayi* (S) and *R. flavipes* intruder termites (T) and inversely, of termites confronted to intruder ants.

TABLE N° 1: FREQUENCY (%) OF PRESENTATION OF ANTENNAL EXPLORATION AND APPROACH BEHAVIORAL EVENTS FROM *SOLENOPSIS GAYI* (S) AGAINST *RETICULITERMES FLAVIPES* (T) INTRUDER TERMITES AND FROM TERMITES AGAINST INTRUDER ANTS.

	Receptor nest	%	χ^2	p
EA/ACE	S1	100	8.526	* 0,0035
	T1	90		
EA/ACE	S2	100	47,531	* < 0,0001
	T2	60		
EA/ACE	S3	100	82,881	* < 0,0001
	T3	40		
EA/ACE	S4	100	47,531	* < 0,0001
	T4	60		
EA/ACE	S5	100	8.526	* 0,0035
	T5	90		

*: Significant; EA: Mandible opening event observed in *Solenopsis gayi* ants; ACE: Approach event observed in *Reticulitermes flavipes* termites; S: Nest of *Solenopsis gayi* receptor ants; T: Nest of *Reticulitermes flavipes* receptor termites; p: Probability; %: percentage of presentation event total of 10 transfers; χ^2 : chi square test.

TABLE N° 2: FREQUENCY (%) OF PRESENTATION OF BITING BEHAVIORAL EVENT FROM *SOLENOPSIS GAYI* (S) AGAINST *RETICULITERMES FLAVIPES* (T) INTRUDER TERMITES AND FROM TERMITES AGAINST INTRUDER ANTS.

	Nest	%	χ^2	p
MO/MOR	S1	100	47,531	* < 0,0001
	T1	60		
MO/MOR	S2	100	104,640	* < 0,0001
	T2	30		
MO/MOR	S3	100	20,056	* < 0,0001
	T3	80		
MO/MOR	S4	100	20,056	* < 0,0001
	T4	80		
MO/MOR	S5	100	20,056	* < 0,0001
	T5	80		

*: Significant; MO: Biting event in *Solenopsis gayi* ants; MOR: Biting event in *Reticulitermes flavipes* termites; S: Nests of *Solenopsis gayi* receptor ants; T: Nest of *Reticulitermes flavipes* receptor termites; p: Probability; %: percentage of presentation event total of 10 transfers; χ^2 : chi square test.

The highest chi square value was observed on S2 and T2 comparison (104, 640). Whereas a low Chi-square value (20.056) was found on three comparisons: S3/T3, S4/T4 and S5/T5, respectively. All values were statistically significant.

Table 3 shows frequency values (%) from the fight behavioral event presentation between ants and termites being studied, respectively. The higher Chi-square value number (130.030) was observed on S3/T3 comparison, respectively. On the other hand, the lowest values (20.056) were observed on S4/T4 and S5/T5, respectively. Values were statistically significant.

For presentation latency time analysis of behavioral events between *S. gayi* and *R. flavipes* nests

being studied we have given x values, standard deviation, and U values, according to Mann-Whitney U-test and its corresponding statistical significance.

Table 4 shows the mean time analysis in seconds, from EA and ACE event presentations, as much for *S. gayi* (S) as for *R. flavipes* (T), both in the capacity of receptors. Values show that latency periods are later on termites with a x varying from 39.888 to 106.750, respectively. on ants such event was manifested earlier with x values fluctuating from 5.3 to 8.1 seconds, respectively. Lastly, to determinate the existence of significant difference Mann-Whitney U-test gave values between 2 to 14. Values were statistically significant except for EA/ACE comparisons from S2/T2 nests, with a not significant U value of 14.

TABLE Nº 3: FREQUENCY (%) OF PRESENTATION OF FIGHT BEHAVIORAL EVENT FROM *SOLENOPSIS GAYI* (S) AGAINST *RETICULITERMES FLAVIPES* (T) INTRUDER TERMITES AND FROM TERMITES AGAINST INTRUDER ANTS.

	nest	present %	χ^2	p
L/LU	S1	100	82.881	* < 0,0001
	T1	40		
L/LU	S2	100	104,640	* < 0,0001
	T2	30		
L/LU	S3	100	130,030	* < 0,0001
	T3	20		
L/LU	S4	100	20,056	* < 0,0001
	T4	80		
L/LU	S5	100	20,056	* < 0,0001
	T5	80		

*: Significant; L: Fight event in *Solenopsis gayi* ants; LU: Fight event in *Reticulitermes flavipes* termites; S: Nest of *Solenopsis gayi* receptor ants; T: Nest of *Reticulitermes flavipes* receptor termites; p: Probability; %: percentage of presentation event total of 10 transfers; χ^2 : chi square test.

TABLE Nº 4: MEAN TIME ANALYSIS (SEC.) OF ANTENNAL EXPLORATION (EA) AND APPROACH (ACE) BEHAVIORAL EVENT PRESENTATION WHEN *SOLENOPSIS GAYI* (S) IS RECEPTOR AND WHEN *RETICULITERMES FLAVIPES* (T) IS RECEPTOR.

event	N	Me (sec.)	DS	U	p
EA S1	10	5,300	6	11	0,0041 *
ACE T1	9	60,111	51,508		
EA S2	10	26,100	40,162	14	0,0934
ACE T2	6	81,500	95		
EA S3	10	8,100	11,239	3	0,0140 *
ACE T3	4	108,75	123		
EA S4	10	6,800	5,181	2	0,0010 *
ACE T4	6	67,500	66		
EA S5	10	5,600	4,402	2	0,0001 *
ACE T5	9	39,888	30		

*: Significant; EA: event of antennal exploration in *Solenopsis gayi* ants; ACE: approach event in *Reticulitermes flavipes* termites; S: Nest of *Solenopsis gayi* receptor ants; T: Nest of *Reticulitermes flavipes* receptor termites; N: Number of times of showed event; Me: Presentation mean time of an event; DS: Standard deviation; U: L Mann-Whitney test; p: Probability.

Table 5 gives time values of latency period on seconds, for MO/MOR events of ants and termites as receptors, respectively. The highest values (in seconds) we find them in termites, with values between 41.875 and 110.333. On the other hand, ants that reacted earlier presented values between 6.8 and 39.0 seconds, with an U value that fluctuated from 9.5 to 19.0. All these values were significant, with the only exception of S1/T1 with the highest U value, which was not significant.

On presentation time analysis of Fight event, observed on both species as receptors respectively, x observed values (Table 6) for termites are higher than in ants. These values were as follows: 3, 78.8, 8.3, and 5.7; respectively. Mann-Whitney U-test gave values between 1 and 22.5. It calls our attention that only S1/T1 and S5/T5 events showed significant values.

When comparing recognition Antennal Exploration (EA)/Approach (ACE) events, latency time (calculated in seconds) was always higher in the case of nest termites as receptors. This difference was considered statistically significant in all cases, with the only exception of S2 nest transfer, as receptor.

In the case of Biting event, latency time was higher when termites were the receptors. This difference was considered significant on most cases, with the exception of T1/S1 nest transfers.

When analyzing the fight event, latency time was lower in three cases of T2/S2, T3/S3 and T4/S4 receptor termite nests. However, the difference gave no significant values. Latency time was lower on T1/S1 and T5/S5 receptor ant nests, and in this case difference was considered significant.

TABLE N° 5: ANALYSIS OF MEAN TIME ANALYSIS (SEC.) OF BITING (MO) BEHAVIORAL EVENT PRESENTATION WHEN *SOLENOPSIS GAYI* (S) IS RECEPTOR AND WHEN *RETICULITERMES FLAVIPES* (T) IS RECEPTOR.

event	N	Me (sec.)	DS	U	p
MO S1	10	6,800	5,959	19,0	0,2635
MOR T1	6	93,000	92,595		
MO S2	10	39,000	41,641	12,5	0,0117 *
MOR T2	3	110,333	120,238		
MO S3	10	17,500	24,518	14,0	0,0205 *
MOR T3	8	92,375	98,311		
MO S4	10	19,100	16,782	14,0	0,0205 *
MOR T4	8	86,875	72,135		
MO S4	10	9,4000	8,342	9,5	0,0044 *
MOR T4	8	41,875	37,157		

*: Significant; **MO**: Event of biting in *Solenopsis gayi* ants; **MOR**: Event of biting in *Reticulitermes flavipes* termites; **S**: Nest of *Solenopsis gayi* receptor ants; **T**: Nest of *Reticulitermes flavipes* receptor termites; **N**: Number of times of showed event; **Me**: Presentation mean time of an event; **DS**: Standard deviation; **U**: L Mann-Whitney test; **p**: Probability.

TABLE N° 6: ANALYSIS OF MEAN TIME (SEC.) OF FIGHT (L) BEHAVIORAL EVENT PRESENTATION WHEN *SOLENOPSIS GAYI* (S) IS RECEPTOR AND WHEN *RETICULITERMES FLAVIPES* (T) IS RECEPTOR.

event	N	Me (sec.)	DS	U	p
L S1	10	8,300	5,735	1,0	0,0040*
LU T1	4	78,800	61,556		
L S2	10	54,700	70,047	13,5	0,8112
LU T2	3	32,666	24,583		
L S3	10	25,300	32,673	22,5	0,1220
LU T3	8	3,000	0		
L S4	10	26,100	29,975	22,5	0,1220
LU T4	8	8,500	5,707		
L S5	10	15,700	13,482	7,0	0,002 1*
LU T5	8	76,375	83,819		

*: Significant; **L**: Event of fight in *Solenopsis gayi* ants; **LU**: Event of fight in *Reticulitermes flavipes* termites; **S**: Nest of *Solenopsis gayi* receptor ants; **T**: Nest of *Reticulitermes flavipes* receptor termites; **N**: Number of times of showed event; **Me**: Presentation mean time of an event; **DS**: Standard deviation; **U**: L Mann-Whitney test; **p**: Probability.

Table 7 gives analysis values of presentation time (seconds) from observed behavioral events per species and nests, when *S. gayi* was an intruder (T) termite receptor. For this analysis, for calculating the specie/nest effect, we used the Kruskal-Wallis test and therefore assessing each analyzed event within the same species.

Antennal Exploration (EA) gave a value of 3.76 under the Kruskal-Wallis test, with no significant p (0.4395). Kruskal-Wallis test for Mandible opening (AM) event gave a 4.256 value and a $p=0.3725$, no significant. Biting event gave a 6.571 Kruskal-Wallis test value and a p of 0.1604, statistically no significant. Abdomen Ventral Flexion (FVA) KW test value, between different nests, was 6.888, not significant ($p=0.1419$) also. When analyzing data through KW test for fight (L) event the total nest number

gave a value of 7.003 with a $p=0.1357$, considered no significant. Intruder transport (TI) event analysis gave a KW test value of 3.6 with a $p=1$ value, no significant. Finally, when analyzing death event data through KW test (total nest number) the value was 4.256 with a no significant p (0.3725).

DISCUSSION AND CONCLUSIONS

The results for these events agree with the ones observed by Ipinza-Regla *et al.* (1991, 1993, 1998b, 2008b) in *Camponotus morosus* ant, and *Camponotus chilensis* ant (Ipinza-Regla *et al.*, 2008a) that show, according to the authors, a considerable closure. This

TABLE N° 7: ANALYSIS OF PRESENTATION TIME (SEC.) PER SPECIES AND NEST OF OBSERVED BEHAVIORAL EVENTS WHEN *SOLENOPTIS GAYI* (S) IS RECEPTOR OF INTRUDER TERMITES (T).

		S1	S2	S3	S4	S5	KW	p
EA	N	10	10	10	10	10	3,760	0,4395
	M	5,3	26,1	8,1	6,8	5,6		
AM	N	10	10	10	10	10	4,256	0,3725
	M	5,8	26,3	11,7	11,3	6,1		
MO	N	10	10	10	10	10	6,571	0,1604
	M	6,8	39	17,5	19,1	9,4		
FVA	N	10	10	10	10	10	6,888	0,1419
	M	7,6	41,6	17,9	25,5	11,3		
L	N	10	10	10	10	10	7,003	0,1357
	M	8,3	54,7	25,3	26,1	15,7		
TI	N	0	2	2	1	0	3,600	1
	M		239	308,5	352			
M	N	5	6	7	8	5	4,256	0,3725
	M	248,6	234,166	246	243,375	243,2		

Analyzed events: EA: Antennal Exploration; AM: Mandible Opening; Mo: Biting; FVA: Abdomen Ventral Flexion; L: Fight; TI: Intruder Transport; M: Death; S: Ant nest; N: Event presentation number; Me: Mean time in seconds; KW: Kruskal-Wallis test; p: Probability.

TABLE N° 8: ANALYSIS OF PRESENTATION MEAN TIME (IN SECONDS) PER SPECIES AND NEST OF OBSERVED BEHAVIORAL EVENTS WHEN *RETICULITERMES FLAVIPES* (T) IS RECEPTOR OF INTRUDER ANTS (S).

		T1	T2	T3	T4	T5	KW	p
ACE	N	9	6	4	6	9	0,984	0,9122
	M	60,111	81,5	108,75	67,5	39,888		
MAN	N	8	7	8	8	3	5,285	0,2593
	M	55,25	154,571	65,25	74,25	44,333		
MOR	N	6	3	8	8	8	1,993	0,7370
	M	93	110,333	92,375	86,875	41,875		
LU	N	4	32	2	8	8	14,996	0,0047 *
	M	78,8	32,666	3	8,5	76,375		

Analyzed events: ACE: Alien approach and brief follow up by one or more termites, MAN: Mandible opening and closing; MOR: Biting; L: Fight; *: Significant; T: Nest of receptor termites; N: Number of times of event presentation; M: Mean time of event presentation; KW: Kruskal-Wallis test; p: Probability.

is because latency periods, calculated in seconds, are reduced for the different studied events.

Other studied performed by Ipinza-Regla *et al.* (2005) related to closure between *Solenopsis gayi* and *Brachymyrmex giardii* demonstrated that both ants were hermetic, with short latency periods.

Hefetz *et al.*, (1996), described for the ant *Manica rubida* most of the events found on our observations, giving to the postpharyngeal gland secretions the attribution of being the regulator of aggressive behavior in this ant species.

Lenoir *et al.*, (1999) observed that when two ants are confronted, they can recognize each other from a very short distance (1-2 cm). In order for this to happen is necessary a physical contact through antennal exploration, which can be done in any body part from the "ant about to recognize", because the sign is amply scattered over the cuticle.

In conclusion, *Solenopsis gayi* ant is hermetic when acting as receptor after *Reticulitermes flavipes* species intruder termite, in laboratory conditions. Receptor ants presented a higher frequency for the analyzed events. On the other hand, *R. flavipes* termite is also hermetic after *S. gayi*, but in a lower degree, because the ant showed a higher closure after the termite. On receptor termites a higher presentation time was observed, as much as recognition as in rejection behaviors. Finally, when analyzing the data by species it was possible to determinate that there are no nest effects that may influence the event presentation on both species.

After the results obtained in the laboratory; could *Solenopsis gayi* have an important role as a biological control of this underground termite.

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