HELDER HUGO DOS SANTOS

COHABITATION AND CONFLICT IN COHABITING TERMITE SPECIES

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*

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APROVADA: 29 de Fevereiro de 2016

Prof. Terezinha Maria Castro Della Lucia

Prof. Gabriel Cipriano Rocha

Prof. Og Francisco Fonseca de Souza
(Orientador)

DEDICATÓRIA

Dedico este trabalho à minha família,
por moldarem meu caráter e terem sempre acreditado em mim.
Em especial, dedico à minha mãe, não só a melhor do mundo,
mas também a pessoa mais incrível que eu conheço.

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RESUMO

SANTOS, Helder Hugo dos, M.Sc., Universidade Federal de Viçosa, fevereiro de 2016. **Coabitação e conflito em espécies de cupim coabitantes**. Orientador: Og Francisco Fonseca de Souza. Coorientador: Paulo Fellipe Cristaldo.

Apesar de seu comportamento agressivo frente a invasores de ninho, cupins são frequentemente encontrados compartilhando suas construções com outras espécies de cupins. Uma vez em um único ninho, cupins coabitantes enfrentam o desafio de lidar com os conflitos que podem surgir de eventuais encontros. Uma questão intrigante é como coabitações desse tipo são possíveis. Estudos anteriores tem sugerido que cupins inquilinos adotariam estratégias de redução da frequência de encontro com seus hospedeiros. Entretanto, uma vez que ninhos podem ser considerados limites físicos que confinam os indivíduos em um único ambiente, é plausível supor que, à longo prazo encontros entre hospedeiros e inquilinos acabariam ocorrendo e nestas circunstâncias inquilinos precisariam agir de maneira adequada evitando o conflito. Essa predição teórica, todavia, nunca foi diretamente testada e na verdade pouco se sabe sobre o que realmente acontece em encontros entre hospedeiros e inquilinos. Aqui mostramos que, uma vez expostos aos seus hospedeiros, cupins inquilinos tendem a se comportar de forma pacífica evitando o aumento de confronto, mesmo quando o contato direto com oponentes é estabelecido. Por meio de etogramas focais, observamos que inquilinos apresentam um perfil de baixa agressividade, interagindo muito pouco com hospedeiros mesmo sob condições forçadas, tais como arenas experimentais. Nossos resultados apresentam diferentes manobras evasivas executadas pelos inquilinos, sugerindo que provavelmente existe evitação ativa. O conjunto de adaptações comportamentais que descrevemos pode ter um papel importante na coabitação de espécies, uma vez que poderia incrementar as chances de partilha de ninho entre hospedeiros e inquilinos sem maiores problemas.

ABSTRACT

SANTOS, Helder Hugo dos, M.Sc., Universidade Federal de Viçosa, February, 2016. **Cohabitation and conflict in cohabiting termite species**. Advisor: Og Francisco Fonseca de Souza. Co-advisor: Paulo Fellipe Cristaldo.

In spite of behaving aggressively against nest intruders, termites are frequently found sharing their constructions with another termite species. Once in a single nest, cohabiting termites face the challenge to deal with conflicts that may arise from eventual encounters. An intriguing question is how cohabitation in such terms is even possible. Previous investigations have suggested that inquiline termites would adopt strategies to reduce frequency of encounter with their hosts. However, because nests may be considered physical boundaries confining individuals in a single environment, it is plausible to suppose that in the long term host-inquiline encounters would eventually take place and under these circumstances inquilines would be required to behave accordingly and prevent conflict. This prediction, however, has never been directly tested and little is known about what actually happens at host-inquiline encounters. Here we show that once inevitably exposed to hosts, inquiline termites tend to behave peacefully and weaken confront escalation, even when direct contact with opponents is already established. We found in focal ethograms that inquilines present low aggressiveness profile, interacting very little with hosts even under forced condition such as experimental arenas. We show different evasive manoeuvres performed by inquilines such as reverse, bypassing and an intricate mechanism using defecation to repel hosts, suggesting that active avoidance seems to be also in place. The set of behavioural adaptations we described may play an important role in cohabitation since it could improve chances of nest-sharing by hosts and inquilines without major problems.

CHAPTER 1

Cohabitation and conflict in cohabiting termite species

Helder Hugo dos Santos; Paulo Fellipe Cristaldo & Og DeSouza

1.1 Introduction

Animal diversity is worthy of notice in nature (Wilson et al., 1988; Huston, 1994; Magurran, 2013) and perhaps for this reason, species coexistence is so easily observable (Tokeshi, 2009; Gravel et al., 2011; Lankau, 2011). Examples range from animals that temporarily overlap spaces and barely interact with each other (e.g. migratory species), to organisms that establish long-term, stable relationships (e.g different kinds of symbionts)(Chesson, 2000). Among these latter are those species that permanently coexist in the same place, experiencing multiple interactions arisen from their cohabitation.

In its simplest form, cohabitation may be understood as different species sharing a common place for some reason, often a resource such as food, shelter, and the like (Tokeshi, 2009). This is exactly what frequently happens in the inside of termite mounds (Noirot, 1970; Mathews, 1977; Redford, 1984). The list of arthropods that may cohabit termite nests is extensive (Kistner 1969) including many other insects (Kistner, 1990), although in this paper we focus only on heterospecific termite-termite cohabitation.

Inquilinism is the term used to refer to cohabitation among termite species (Shellman-Reeve, 1997) and it should not be mistaken with the social parasitism seen in Hymenoptera (Nash & Boomsma, 2008). In fact, there is a bit of confusion with the term "inquiline", also used to refer to cohabiting hymenopterans (Brandt et al., 2005). The difference, however, is quite simple: while hymenopteran social parasites have close relationship with their hosts, usually taking advantage of their resources, e.g. food and brood (Hölldobler & Wilson, 1990), inquiline termites are thought to be primarily associated with the nest structure itself, regardless of their association with the host species. Inquiline termites are classified either as (i) *facultatives*, when they are still able to construct their own nests, or (ii) *obligatories*, when constructive behaviour is virtually absent and nest invasion mandatory (Collins, 1980). The number of facultative inquilines described is quite remarkable and surpasses greatly that of obligatories (Mathews, 1977). Despite this, the relationship established between host and obligatory inquiline termite species offers unrivaled opportunities to investigate coexistence and its consequences, given that species are presumably more closely associated to one another. Once in a single nest, cohabiting termites face the challenge to deal with conflicts arisen from eventual encounters. This is especially likely considering that hosts generally react aggressively in front of nest invaders (Bouillon, 1970; Noirot, 1970; Shellman-Reeve, 1997), which in turn, represents a risk for inquilines. An intriguing question is how cohabitation in such terms is even possible.

Previous works have tackled such an issue, suggesting for inquiline termites mechanisms that would ultimately reduce the frequency of encounter with hosts. In other words, once settled into host nests, inquilines would be able to decrease the chance of being noticed in the first place. To do so, they would exhibit different kinds of behaviour, such as (i) avoid walking through nest galleries occupied by the host species (Mathews, 1977; Grassé, 1986; Cunha et al., 2003), (ii) exploit diets not used by hosts and so, have less contact with them while foraging (Florencio et al., 2013; Miura & Matsumoto, 1997), (iii) intercept hosts' chemical signals and prevent encounters with nonrelatives (Cristaldo et al., 2014) and (iv) even use their constructive behaviour, despite somewhat degenerated (Emerson, 1938) to seal chambers in the nest, thereby keeping themselves conveniently separated from hosts.

Mechanistic differences aside, all these behavioural strategies seem to coincide in their outcomes: while preventing direct contact with hosts, inquilines reduce the frequency of encounter. One would expect such an effect to be enough in solving conflict within the nest. After all, with minimized host-inquiline encounter, conflicts would be presumably attenuated. However, because the nest may be interpreted as a physical boundary forcing individuals to be locally restricted, none of the above strategies will always prevent contact with hosts, especially in the long term. It is plausible to suspect that host-inquiline encounters would eventually take place and under these circumstances inquilines would be required to behave accordingly, mitigating any detrimental consequences. Although logical, this theoretical prediction has never been directly tested and little is known about what actually happens when host and inquiline termite species eventually come across each other or to what extent inquiline strategies are effective enough to cope with the menace of imminent confrontation with hosts.

Here we tested the hypothesis that these inquiline termites are characterized by unchallenging behaviour towards their host. We predict that, once inevitably exposed to host individuals, such inquilines would modulate their behaviour and so appear less threatening, thereby reducing the intensity of conflicts. They would do so (i) acting lethargically , hence minimizing encounters with their host and (ii) exhibiting low aggressiveness , by avoiding to initiate or retaliate attacks. In doing so, inquilines would weaken conflict escalation.

To test this hypothesis, we observed in detail the behaviour of the obligate inquiline (*Inquilinitermes microcerus*) in the presence of its host (*Constrictotermes cyphergaster*). Termites have been exposed to each other under two scenarios: in arenas where inquilines could flee from their hosts and in arenas where no escaping route was available and interspecific encounters were unavoidable. Such an approach allowed us to compile full ethograms for the meeting events between inquilines and their hosts. Behavioral profiles thereby arisen lent support to the notion of *Inquilinitermes microcerus* as a peaceful inquiline.

1.2 Material & Methods

1.2.1 Ethical statement

Permits required for biological sampling and transportation were appropriately requested by authors and provided by the national regulatory institutions (IBAMA - The Brazilian Institute for the Environment and Renewable Resources). ODS holds a permanent collecting and transporting permit (# 33094) from IBAMA. Additionally, tacit approval from the Brazilian Federal Government is implied by hiring ODS as Scientific Researcher, by awarding research grants to PFC and HHS and by awarding ODS with a Fellowship from CNPq (The Brazilian National Council for Research). Species adopted in surveys are neither endangered nor protected ones and, thus, no specific permits were required for laboratorial experiments.

1.2.2 Terms and definitions

Except for minor adaptations, here we adopt terms used in previous investigations on cohabitation among termites (Florencio et al., 2013; Cristaldo et al., 2014): "Nest" is the physical structure built by the host, whereas "Colony" means the assemblage of all individuals of a given species; "Cohabitation" refers to simultaneous occurrence of at least two different termite species within a given nest, regardless any positive or negative reciprocal influences; "Host-inquiline encounter" is the event in which termites from both species meet each other in experimental arenas; "Host" is the builder *Constrictotermes cyphergaster* and "Inquiline" the obligatory intruder *Inquilinitermes microceus.*

1.2.3 Biological model

To test our assumptions, we investigated a biological model composed by two cohabiting termite species. As a typical case of inquilinism among termites, in this relationship a nest owner (host) lives in close relationship with a nest intruder (inquiline). The host, *Constrictotermes cyphergaster* Silvestri 1901 (Termitidae: Nasutitermitidae), is a Neotropical species widely distributed in the South America with records in Brazil, Bolivia, Paraguai and Argentina (Mathews, 1977; Krishna et al., 2013). As opposed to some other termites, this species is known to forage at night in exposed columns and without protection of covered galleries (Moura et al., 2006a). Usually, nest foundation starts on the ground with a royal couple and after reaching a certain size (Mathews, 1977), colonies migrate to the trees (Emerson 1938) where they establish arboreal nests (Noirot, 1970; Moura et al., 2006a). At this phase, it is particularly usual to find a secondary species inhabiting the nest: the termite *Inquilinitermes microcerus* Silvestri (1901) (Termitidae: Termitinae). Such a suggestive name as *Inquilinitermes* has its reasons: these termites are known to live exclusively as inquilines in *C. cyphergaster* nests (Emerson, 1938; Mathews, 1977). Although little is known about nest invasion process, it has been shown that there is a critical nest volume in which *I. microcerus* colonies are more likely to be found inside host nests, usually above 13.6 L (Cristaldo et al., 2014). In fact, being negatively related to defence rates, nest size seems to indirectly affect inquilinism in termites (DeSouza et al., 2016). As compared to *C. cyphergaster*, *I. microcerus* colonies are much smaller in size (Cunha et al., 2003) but still, once inside host nests, inquiline colonies are easily detectable. Usually, they inhabit galleries covered by a dark lining (Moura et al., 2006b; Cristaldo et al., 2012; Florencio et al., 2013), contrasting to clearer host galleries. In adittion, the presence of inquilines is mostly associated with chambers fully filled with a dark material supposed to be waste dumped by hosts (Emerson, 1938; Mathews, 1977).

1.2.4 Study site and collection

Constrictotermes cyphergaster nests containing *I. microcerus* colonies were sampled in the Brazilian Cerrado (Eiten, 1972; Ratter et al., 1997) at two different locations: Sete Lagoas-MG (19º27'57"S, 44º14'48"W) and Divinópolis-MG (20º08'20"S, 44º53'02"W). Both sites present climate resembling to savannas with average precipitation between 1300-1400 mm. The average annual temperature is around 21.5°C (Dias, 1992). To carry out the experiments, 27 *C. cyphergaster* nests containing *I. microcerus* colonies were collected from either locations (Sete Lagoas-MG, N=15 ; Divinópolis-MG, N=12). Sampling was performed in July, 2012 (Sete Lagoas-MG) and January, 2015 (Divinópolis-MG).

1.2.5 Behavioral recording

In order to access behaviors performed by individuals at eventual host-inquiline encounters, cohabitants were taken from their nests, separated in containers for acclimation (approximately 30 min.) and then gathered in arenas for video recording. Arenas consisted in plastic Petri dishes (53mm diameter) covered with Whatman®Nº1 filter paper discs at the bottom (Šobotník et al., 2008). For each arena assembled, five-minutes videos were recorded using a digital camera (model Nikon D300S™).

Two experiments were developed. In the first, arenas were closed with no openings to external areas (Fig. 1.1 A) and the termites were intentionally kept together for video recording. Although unnatural, this forced condition was necessary to allow the occurrence of host-inquiline encounters and consequently the recording of interactions between cohabitants. In the second experiment, we used a different type of arena to test whether inquilines would remain quiet even when given a chance to flee from their host. These arenas were mostly identical to the ones used in the first experiment, except for the presence of an exit gate (Fig. 1.1 B). Gates consisted basically in a single opening (3.5mm diameter) made in the arena wall, giving access to an external area (88mm diameter) which encompassed the inner one (53mm diameter). Two treatments were set using these arenas: (i) open arenas containing only hosts and (ii) open arenas containing hosts and inquilines.

To enhance the quality of our observations, trials were conducted with individuals kept under optimal density (c.a., 0,12 area/area, Miramontes & DeSouza (2008)), and in worker-soldier ratios as close as possible to field conditions. Since *C. cyphergaster* workers occupy 17 mm^2 and soldiers occupy 16 mm^2, whereas *I. microcerus* workers occupy 16 mm^2 and soldiers occupy 19 mm^2, an optimal density was achieved confining 15 termites in a 53 mm diameter. Thus, to simultaneously comply with both restrictions (density and worker-soldier ratio), experimental groups contained: one soldier and four workers of *C. cyphergaster* and one soldier and nine workers of *I. microcerus.* In both experiments, individuals forming an experimental group were used only once, to prevent any interferences from prior contact between them in the behavioral patterns observed.

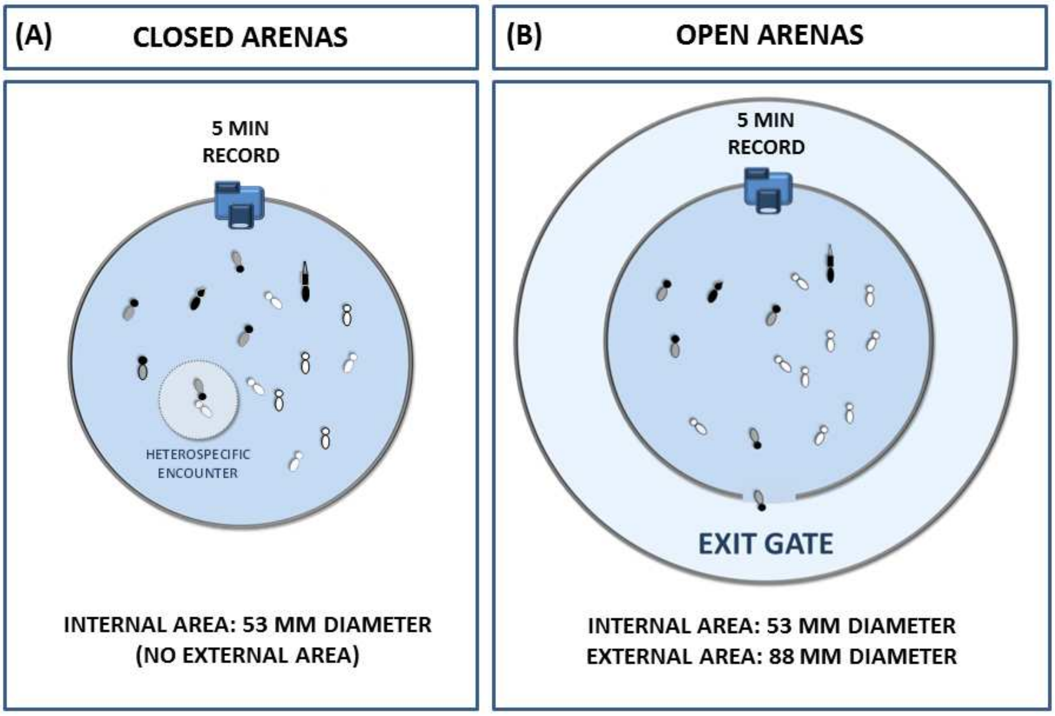

Figure 1.1: Arena settings for video recording: (A) Closed arenas, (B) Open arenas; Five-minutes videos were recorded and focal observation was carried out by a single observer (HHS) replaying the movie in a 14" LED-LCD monitor; heterospecific encounter is highlighted in dashed circle. Hosts = *C.cyphergaster*; Inquilines = *I. microcerus*; W = worker; S = soldier.

1.2.6 Observational protocol

We hypothesized that inquilines would exhibit unchallenging behaviour as an adaptation to deal with hosts inside nests. Our assumption comes from previous research on the biological model (Florencio et al., 2013; Cristaldo et al., 2014) that suggests conflict avoiding strategies on the part of inquilines, in which intruders would be able to lower the level of conflict with invaded ones.

An affordable way to accurately describe behaviours is using ethograms (Altmann, 1974). Easy to develop, the method provides useful information about the animal in focus. Once behaviour takes many forms in nature (Davies et al., 2012), ethograms can be created out of a variety of observations (usually, using a previously defined observational protocol) (Lehner, 1998). For instance, data may be collected by *scan sampling*, in which observers scan a large number of organisms for a defined period of time, recording the number of individuals displaying a given behaviour (Altmann, 1974). This protocol, however, has limitations such as the recording of behavioral sequences. As multiple individuals are observed simultaneously, it becomes difficult for observers to track behavioral change. When sequences are desirable (as in our case), scan ethograms are somewhat inappropriate and a convenient solution is to use an alternative protocol: the *focal animal sampling.* (Lehner, 1998). In this method, rather than focusing simultaneously on several animals, the observer follows a single focal individual at a time, recording all behaviors performed (Altmann, 1974).

To investigate interactions between *C. cyphergaster* and *I. microcerus*, we adopted focal animal sampling (Altmann, 1974) in which observations were taken from five-minutes videos made of experimental arenas. At total, we recorded 20 videos, ten of closed arenas and ten of open arenas (Supplementary Material, Table 2.1). Videos were watched four times in order to observe the behaviour of the following groups: host workers (HW), host soldiers (HS), inquiline workers (IW) and inquiline soldiers (IS). In each video, one individual of each group was arbitrarily selected for focal observation (hereafter, focal animal).

Using a 14" LED-LCD screen (Full HD, resolution: 1366x768), scans (three seconds observations) were taken at every ten seconds by a single observer (HHS) in each one of the videos. This provided 31 scans per focal animal in each one of movies. Behaviours observed were subsequently accounted directly onto a proper sheet. To allow the observer to visually recheck the data collected (whenever needed), focal animals had their identity saved in jpg image files (i.e print-screen of the arena with labels and markings indicating each one of the individuals observed).

As focal animals had no marks distinguishing them from others in arenas, the time scheduled to perform a scan was systematically indicated at every ten seconds by beeps (sound signals). This simple solution avoided interruptions in the screen observation and prevented mistakes in tracking (e.g. by accidentally switching individuals when not looking at them). Additionally, to avoid pseudo-replicates we never repeated the same colony in different arenas.

1.2.7 Behaviour definition

As recommended, prior to the focal animal sampling we first performed *ad libitum* observations (Altmann, 1974; Lehner, 1998), in order to detect which specific behaviours could be exhibited by focal animals. At this stage, we spent efforts to capture and describe as much behaviours as we could. Rather than extensive behavioural descriptions, which could possibly lead observers to make misleading observations, we opted for simple, straightforward labels hierarchically organized (Supplementary Material, Fig. 2.1). The list containing the behavioural descriptions previously defined is presented in Table 1.1.

Table 1.1: Behavioural description based in preliminary observations. *Ad libitum* observation (Altmann, 1974; Lehner, 1998) was performed using additional 10 five-minutes videos, recorded only for preliminary observation. (Abbreviations: aCS=conspecific antennation, aHS=heterospecifc antennation, aW=wall antennation). Observations are classified as *Within-species* (w) or *Between-species* (b) and *Aggressive* (agg) or *Non-aggressive* (non-agg).

Behaviour	Description	w/b	agg/non-agg
Rest	Remains stationary in the same place	within	non-aggressive
Walk	Moves freely around the arena	within	non-aggressive
aW	Reaches arena wall and performs only antennation	within	non-aggressive
aCS	Encounters conspecific and performs only antennation	within	non-aggressive
aHS	Encounters heterospecific and performs only antennation	between	non-aggressive
Ignore	Encounters heterospecific and do not react to contact	between	non-aggressive
Reverse	Encounters heterospecific and performs u-turn manoeuvre	between	non-aggressive
Pass	Encounters heterospecific and performs bypass manoeuvre	between	non-aggressive
Attack	Encounters heterospecific and performs aggression	between	aggressive

1.2.8 Measuring aggressiveness and host-inquiline interactivity

To measure host's and inquiline's aggressiveness in closed arenas, interactions observed were classified into two mutually exclusive categories: (i) *aggressive*, in which individuals interacted with heterospecifics and performed aggression (i.e. *attack*); and (ii) *non-aggressive*, in which individuals interacted with heterospecifics but did not performed aggression (i.e. *heterospecific antennation*, *reverse*, *pass*, *ignore*)

To measure interactivity between hosts and inquilines in closed arenas, behavioral observations were classified into two mutually exclusive categories: (i) "*Within-species*", in which individuals performed actions either alone (i.e. *rest*, *walk* and *wall antennation*) or with their conspecifics (i.e. *conspecific antennation*); and (ii) "*Between-species*", in which individuals performed actions after establishing physical contact with heterospecifics in host-inquiline encounters (i.e. *heterospecific antennation*, *pass*, *ignore*, *reverse* and *attack*).

1.2.9 Assessing behavioural profiles

To verify the influence of specific behaviours in the behavioural profile of hosts and inquilines, we developed a network analysis using the free software yEd Graph Editor version 3.14.4 (available in: https://www.yworks.com/). To build the graphs (one for each caste), we performed the following procedure: First, using behavioural sequences observed in trials, we constructed adjacency matrices containing the behavioural change for each caste (Supplementary Material, Table 2.2) and then, data was imported to yEd to draw graphs. As in network analysis (Brandes & Erlebach, 2005), graphs consisted in networks of nodes linked by connecting edges (i.e. directional arrows). In our case, nodes represented specific behaviours performed by individuals whereas connecting edges represented behavioral changes occurred from source to target behaviours (i.e. if individuals changed from *rest* to *walk*, the behavioural change was rest-walk). As nine specific behaviours were defined (Table 1.1), 81 (9x9) types of behavioural change could be observed. After graphs were constructed, centrality measures (i.e. scores that provide a rough indication of the social power of a node based on how well it ”connects” the network) (Sabidussi, 1966; Freeman, 1979) were calculated for each node using the number of incoming connecting edges (Koschützki et al., 2005). Last, using calculated centrality scores, we adjusted the size of nodes to visually represent the degree of influence exerted by each behaviour upon the profiles (i.e. the bigger the size of a node, the higher its influence in the network).

1.2.10 Ethogram completeness

To test whether our ethogram achieved suitable completeness, we adopted a procedure suggested by Dias et al. (2009), in which Behavioural Accumulation Curves (BAC) are used as tools to assess the best compromise between sampling effort and ethogram completeness. The performance of our ethogram was analyzed and is shown in Figure 1.2.

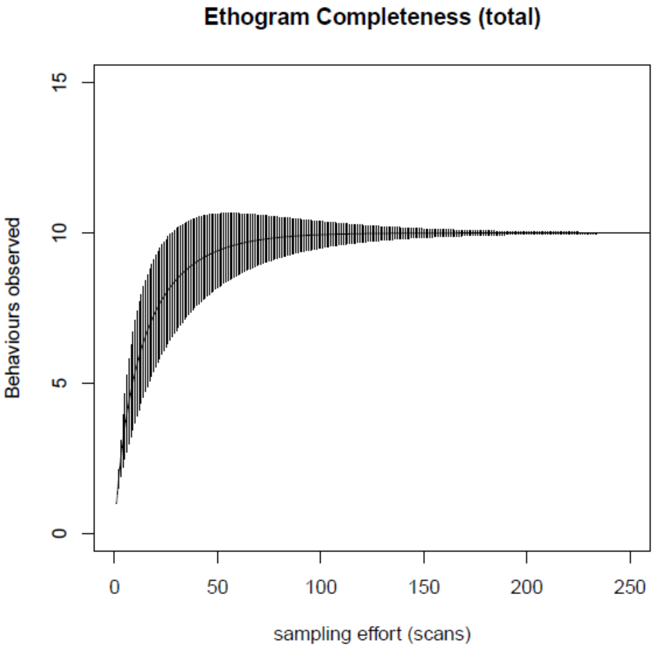

Figure 1.2: Ethogram completeness; Behavioural Accumulation Curve: x-axis represents sampling effort (i.e. the accumulative number of scans performed); y-axis represents the accumulative number of behaviours experimentally observed. The graph shows that the ten-behaviours asymptote can be reached accumulating a little more than 50 observations. In our approach, we performed 1240 observations (31 scans x 2 species x 2 castes x 10 videos = 1240).

1.2.11 Statistical analyses

Statistical analyses were performed in R version 3.0.2 (R Core Team, 2016) within R Studio version 0.99.442 (R Studio Team, 2016) using Generalized Linear Modelling (GLM) under Binomial errors with log-link. As a conservative approach, the significance of treatments was accessed using contrast analysis, in which a complex model is compared with a simpler one achieved by combining treatment levels (Crawley, 2012). If simplification did not provoke significant changes, the simpler model was accepted and the combined treatments were considered equivalent to each other. Adjusted models were then submitted to residual analyses to check normality of error distribution and suitability of the modelling equation. When necessary, error distribution was adjusted with Quasi-binomial distribution. In all tests, significance was assessed at $\alpha = 0.05$.

1.3 Results

1.3.1 Inquillines were attacked by hosts

In experimental closed arenas hosts were aggressive towards inquilines (Fig. 1.3). In host-inquiline encounters, the proportion of aggressive interactions initiated by hosts was significantly higher than the proportion of non-aggressive interactions (Fig. 1.3). Expectedly, caste type was determinant in the kind of aggression performed by individuals: host workers usually injured inquilines by biting them in several portions of their bodies, whereas host soldiers adopted agonistic display (i.e. abrupt movement with stretched antennae). We were not always able to detect whether agonistic display was followed by chemical spillage, mostly because of limitations in the screen resolution to observe such a thin structure.

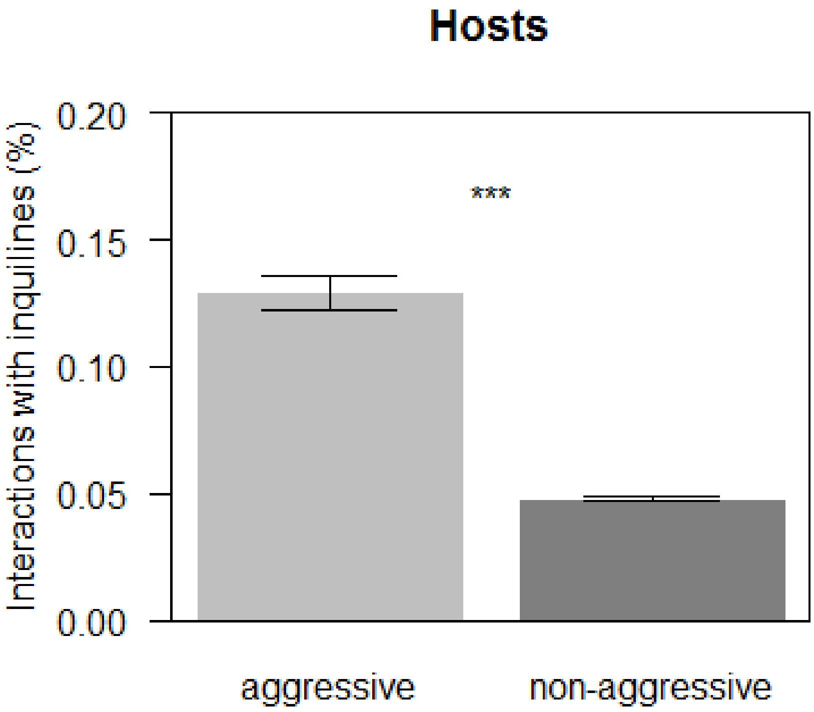

Figure 1.3: Host (*C. cyphergaster*) aggressiveness observed in experimental closed arenas. Proportions were calculated by the number of aggressive and non-aggressive interactions observed, divided by the total number of observations taken from five-minutes videos (n=10). Light bars represent aggressive interactions, whereas dark bars represent non-aggressive interactions ($F_{1,98}=16.72$, $P<0.001$).

1.3.2 When attacked, inquilines reacted with low aggressiveness

As we predicted, inquilines showed low aggressiveness when confronted by their hosts (Fig. 1.4), with inquiline workers never taking part in reactive actions (Fig. 1.5 *attack* = not observed). When threatened or even severely injured by hosts, inquiline workers never returned aggression (Fig. 1.5 and Fig. 1.8–C: *attack*; Table 1) and, instead, were more likely to perform evasive manoeuvres while quickly diverting from their aggressors: these actions occurred shortly after an effective contact with host individuals was established and included behaviours such as reversing direction and bypassing opponents (Fig. 1.5 *reverse*, *pass*; Table 1). Besides escaping from threats, inquiline workers also performed ignoring behaviour (Fig. 1.5 *ignore*; Table 1.1). In this case, individuals actively touched by hosts did not react, remaining unresponsive and stationary.

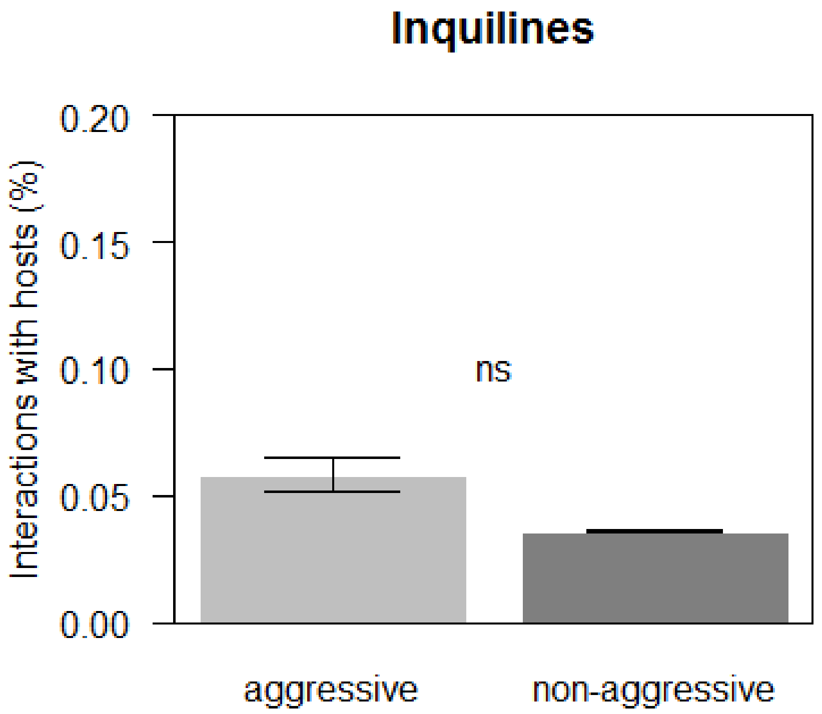

Figure 1.4: Inquiline (*I. cyphergaster*) aggressiveness observed in experimental closed arenas. Proportions were calculated by the number of aggressive and non-aggressive interactions observed, divided by the total number of observations taken from five-minutes videos (n=10). Light bars represent aggressive interactions, whereas dark bars represent non-aggressive interactions ($F_{1,98}$=1.7478, P=0.1892).

An exception to this low aggressiveness pattern was the behaviour exhibited by inquiline soldiers: as opposed to workers, inquiline soldiers eventually performed aggression in retaliation to host assaults, even though this happened in very low proportion (Fig. 1.8–D, *attack*; Table 1.1). These aggressions consisted in *snapping*, a sudden release of slender mandibles pressed against each other often producing powerful strikes over opponents (Supplementary Material, Video S1).

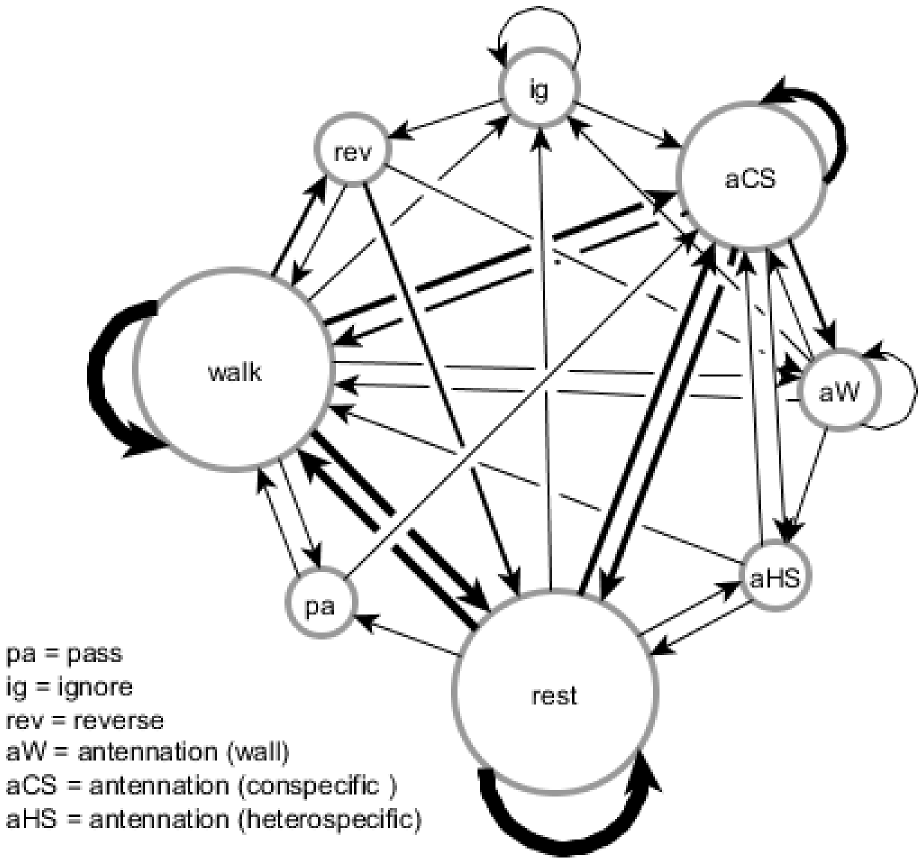

Figure 1.5: Behavioural change of inquiline workers in experimental arenas: Nodes represent specific behaviours performed by individuals whereas connecting edges (arrows) represent behavioral changes occurred from source to target behaviours. Size of nodes was adjusted using behavioral frequencies observed. Width of edges indicate how often a behavioral change happened (i.e. the larger the width of arrows, the higher the frequency).

1.3.3 Inquilines interacted little with hosts

Inquilines exhibited low interactivity with host individuals (Fig. 1.6), even when not having a chance to flee, as in closed arenas. The proportion of between-species observations was considerably lower than the proportion of within-species observations (Fig. 1.6). The absolute number of between- and within-species observations taken for each caste is presented in Table 1.2.

Table 1.2: Number of between- and within-species observations taken from five-minutes videos of closed arenas for hosts and inquiline species and respective castes. (n=number of focal animals observed; HS=heterospecific, CS=conspecific, NE=no encounter, HOSTS= *C. cyphergaster*, INQUILINES=*I. microcerus*

Individual	n	Between-species (HS)	Within-species (CS+NE)
HOSTS	10	199	79+342
Host workers	5	106	38+166
Host soldiers	5	93	41+176
INQUILINES	10	125	85+440
Inquiline workers	5	25	39+246
Inquiline soldiers	5	100	46+164
TOTAL	20	324	164+752

1.3.4 Hosts went out to external areas in open arenas

In closed arenas host individuals performed wall antennation more frequently than inquilines (Fig. 1.7), indicating that hosts could be attempting to broaden their patrolled area. This suspicion was later confirmed in the second experiment: when placed in open arenas, host individuals quickly moved to the external area passing through the gate as soon as they found it. In both treatments, that is, in the presence or absence of inquilines, there was no difference in the mean±SD time spent for the first host to leave the internal area (19.93±3.56 seconds) ($F_{1,9}$=0.3442, P=0.5719), which indicates that inquilines themselves did not threaten the hosts. Inquilines, in turn, were more prone to remain stationary and never left the internal area. This confirms the lethargic general behaviour initially supposed for the inquiline species.

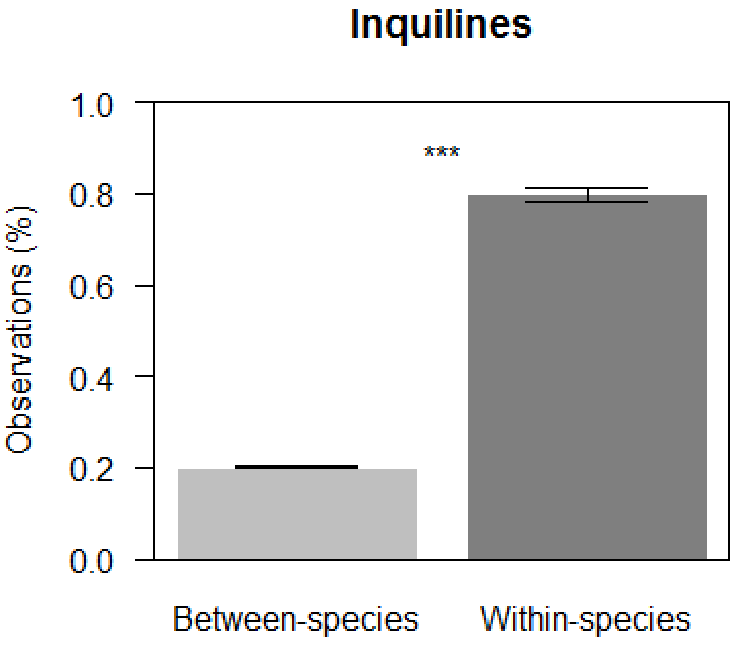

Figure 1.6: Inquilines interaction: Number of between-species and within species observations taken from five-minutes videos of closed arenas. Light bars represent Between-species observations whereas dark bars represent Within-species observations ($F_{1,178}$= 71.73, P<0.001).

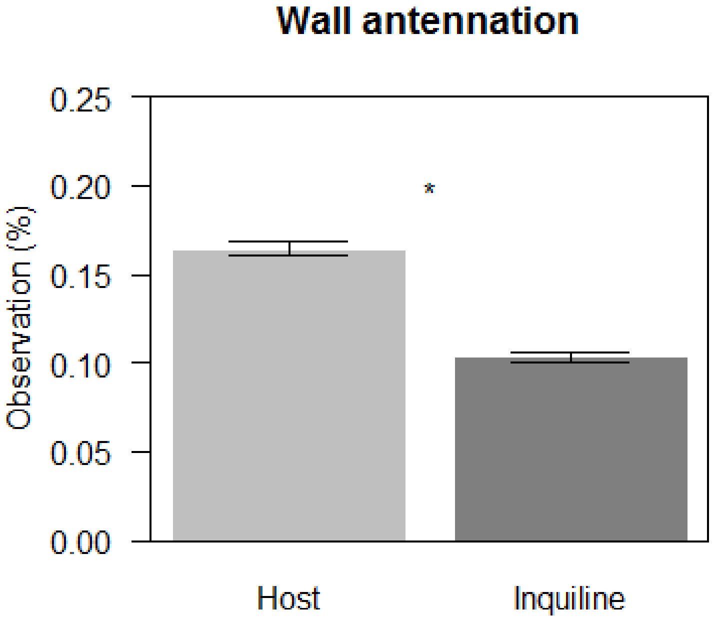

Figure 1.7: Wall antennation performed by hosts and inquilines in closed arenas. Light bars represent host antennation whereas dark bars represent inquiline antennation ($F_{1,78}$= 4.73, P<0.005).

1.3.5 Inquiline's defecation prevented host aggressions

While observing inquiline workers, a singular response was also observed: when threatened by hosts, inquiline workers deposited faecal pellets always towards the same direction from which they suffered the threat (Supplementary Material, Video S2). Rather than usual defecations, this behaviour seemed to be elicited by host aggressions in a very specific way. For instance, after receiving aggressions from backwards, individuals simply placed faecal pellets in front of aggressors and escaped forward. Threats received from any other direction, however, triggered a slightly different response: prior to defecations, individuals first adjusted their position accordingly, placing themselves in a way that faecal pellets could be dropped in front of the aggressors. Only after performing such a movement, inquiline workers defecated and escaped forward. In 11 of 13 video recordings, this behaviour was observed at least 33 times and in all occurrences the presence of faecal pellets immediately prevented inquilines of being chased by aggressors and consequently of receiving further attacks. Additionally, although we did not measure whether inquiline feces have repellent effect over hosts, it was clear in the recordings that the areas containing feces were less visited by host individuals (Supplementary Material, Video S3).

1.3.6 Hosts and inquilines exhibited different behavioral profiles

We found striking differences in the behavioural profile of each caste analyzed (Fig. 1.8). However, for all caste profiles, *walk* was the most influential behaviour with the highest centrality score (Fig. 1.8; A, HW_{walk}=1.00; B, HS_{walk}=1.00; C, IW_{walk}=1.00, D, IS_{walk}=1.00). The only caste to present a second equally influential behaviour was that of inquiline workers, in which *conspecific antennation* (aCS) also reached the highest centrality score (Fig. 1.8–C; IW_{aCS}=1.00). As mentioned before, *attack* was never performed by inquiline workers and so, this behaviour presented the lowest centrally score (Fig. 1.8–C; HS_{att}=0.00).

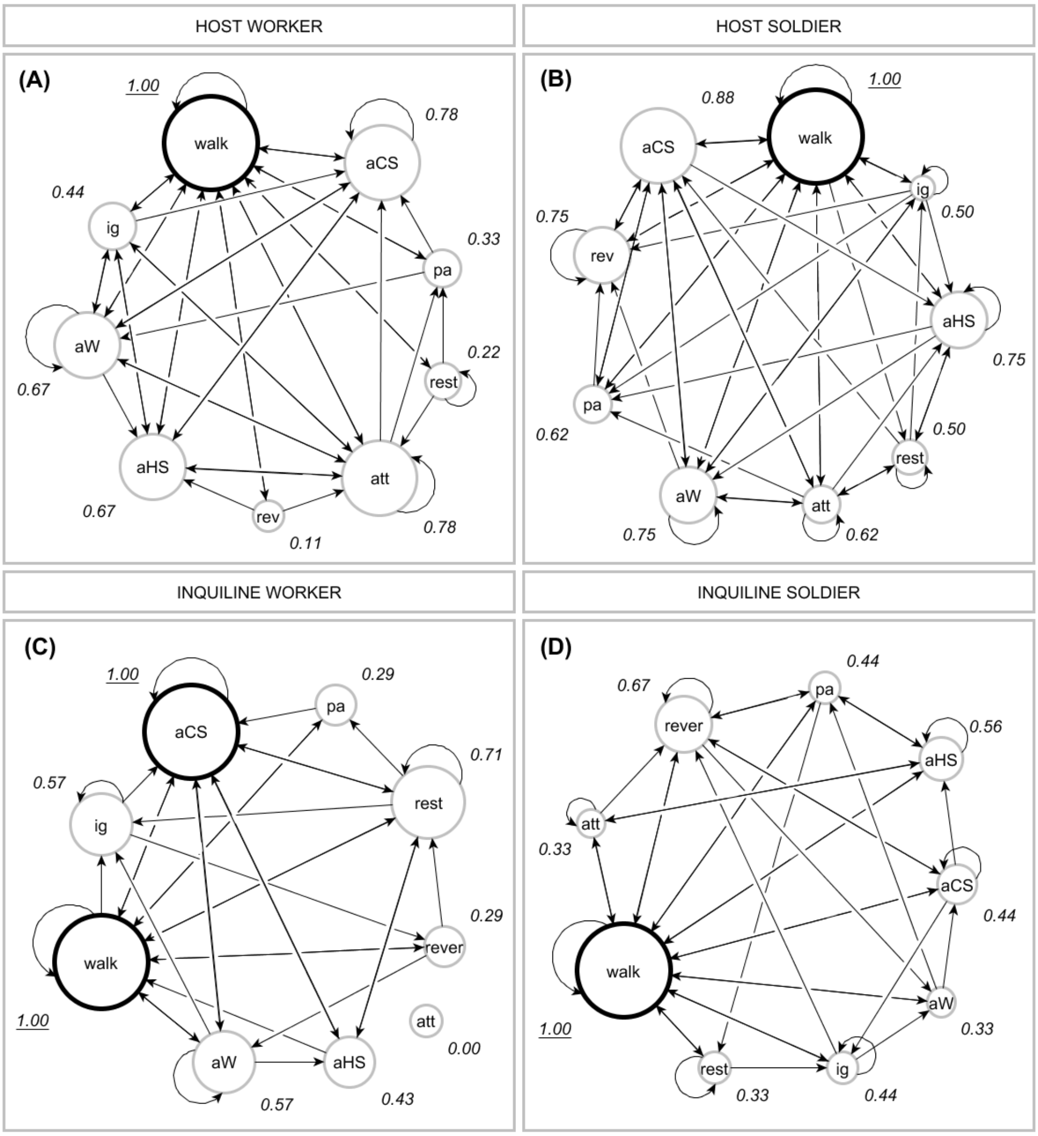

Figure 1.8: Behavioural profiles observed: (A) *host worker profile*; (B) *host soldier profile*; (C) *inquiline worker profile*; (D) *inquiline soldier profile*. Nodes represent specific behaviours performed by individuals whereas connecting edges represent behavioral changes occurred from source to target behaviours. Numbers indicate centrality measure scores calculated for each node and underlined values indicate the most influential behaviours. Size of nodes was adjusted using centrality scores to visually represent the degree of influence exerted by each behaviour upon the profiles (i.e. the bigger the size of a node, the higher its influence in the network); Abbreviations: aW=*wall antennation*, aHS=*heterospecific antennation*, aCS=*conspecific antennation*, att=*attack*, ig=*ignore*, pa=*pass*, rever=*reverse*.

1.4 Discussion

Our results seem to indicate that upon encountering their hosts, inquiline termites tend to adopt non-aggressive behaviour and prevent confront escalation. Previous works (Florencio et al., 2013; Cristaldo et al., 2014) have shown clear evidence that, once inside nests, inquilines exhibit strategies to lower the frequency of encounter with hosts. Here, we not only provide substantial behavioural data supporting this pattern, but also show that once inevitably exposed to hosts, inquilines are able to modulate their behaviour accordingly and therefore, circumvent eventual conflicts. This suggests some degree of adaptation towards a peaceful behaviour, which could in turn strongly favour cohabitation.

1.4.1 Inquiline's peaceful strategy

A set of behaviours seem to contribute for the harmless behaviour observed among inquilines. Firstly, when encountered by hosts in arenas, inquilines suffered several attacks (Fig. 1.3) but even though, reacted with low aggressiveness (Fig. 1.8 C–D). Lack of aggression was markedly evident among inquiline workers, which not only never performed a single *attack* but also exhibited consistent evasive manoeuvres (Fig. 1.8–C, *reverse* and *pass*). The same was not true for inquiline soldiers, who did retaliate performing *snapping*, although not too frequently (Fig. 1.8–D, *attack*). It is likely that in natural conditions *snapping* should also occurs at very low rates, once in inquiline colonies soldiers are known to be greatly outnumbered by workers (Cunha et al., 2003) and sometimes, even absent.

A second aspect that affected the amount of aggression in arenas was defecation by inquilines. The presence of faecal pellets seems to have shortened host-inquiline contact in virtually all occasions. As a consequence, host attacks were less frequently suffered by inquilines. This indicates that feces may improve evasion while preventing host aggressions. Defecation as evasive mechanism is not exclusive of *I. microcerus* and, in fact, was firstly described in *Skatitermes*, in which termites are able to bend their abdomen so as to place faecal pellets on the heads of attacking ants (Coaton, 1971). Also, it has been shown that termites may, indeed, execute elaborated manoeuvres while manipulating faecal pellets, for instance, constructing defensive walls (Stuart, 1967). The usage of feces by inquilines as defense may have important implications for cohabitation: if hosts are indeed repelled by feces, a faecal pellet placed in an narrow gallery could be sufficient to prevent host contact. Accordingly, repellent odor reducing host attacks has also been described in the cuckoo bumblebee (*Bombus vestalis*) (Lhomme et al., 2012). Also, it is possible that while defecating, *I. microcerus* would be spreading their scent throughout the entire nest, making harder for the host to detect its point of origin.

A third factor that seems to have contributed for the low aggressiveness in arenas was the reduced inquiline mobility and consequent reduced rate of host-inquiline interactivity (Fig. 1.6), a result in line with Florencio et al. (2013) and Cristaldo et al. (2014). In opened arenas, hosts were the only ones to move to the outside, whereas inquilines remained quiet in the inside. This result could be interpreted in at least two ways. Firstly, it could be simply a direct consequence of the host behavioural profile. As we have shown, *walk* is a relevant behaviour for all castes but the higher frequencies were observed among hosts (Fig. 1.8 C–D). Once they walk more intensively, as consequence, gates would be more readily found. In fact, the time spent by hosts to leave arenas was not affected by the presence of inquilines, indicating that walk behaviour is consistent and not triggered by the inquiline. In itself, this latter observation would confirm that inquilines do not threaten the host. An alternative explanation would be that inquiline's idle behaviour would pose selective pressures leading hosts to increase walking. That is, to the extent which inquilines would become more efficient in bypassing host detection system, hosts would need to be more efficient in finding invaders and hence, exploratory behaviours would be improved. Although mutual behavioral adjustments are known to happen between species highly related (Kilner & Langmore, 2011; Thorogood & Davies, 2013), this theoretical prediction remains to be tested for inquilinism in termites.

1.4.2 The threat behind host-inquiline encounters

Given the peaceful pattern observed, a relevant question is whether such a strategy could be useful for inquilines. After all, are encounters with the host real threats for the inquiline? Our results provide evidence that there are, indeed, detrimental consequences in encountering hosts. Host aggression was consistent, being performed not only by soldiers, but also host workers. (Fig. 1.8 A–B, *attack*). This seems to indicate that, as opposed to inquilines, host defense is integrated between castes: while soldiers attack chemically (Cristaldo et al., 2015), workers are the ones who inflict the physical damage. In fact, termite workers have been shown to exhibit complementary defensive actions in different scenarios (Thorne, 1982; Ishikawa & Miura, 2012). Thus, as inquilines may suffer aggression from both host castes, it seems plausible to assume that inside the nest they would be surrounded by potential aggressors. In such a harsh environment, is likely that a peaceful behaviour could be a simpler solution.

In fact, for other animals, behavioural strategies preventing confront escalation seems to be broadly worthwhile. For instance, when suffering attacks by host ant workers, parasite ant queens did not react aggressively and instead quickly moved into the fungus garden, remaining quiet and possibly not disturbing the host (Nehring et al., 2015). Another example is that of Lycaenidae larvae, in which individuals are apparently able to suppress ant's aggression by mimicking aspects of the pheromones of ant brood (Akino et al. (1999); for a review, see Pierce et al. (2002)). Among bees, peaceful strategies have been also reported. In the presence of cuckoo bumblebees (*Bombus vestalis*), host colonies decreased worker aggressiveness towards alien individuals, possibly due to changes in host worker discrimination (Lhomme et al., 2012). Obviously, conflict management is not exclusive of social insects or nest invaders and examples also extend to vertebrates, including free-ranging wolves (Baan et al., 2014), primates (Petit & Thierry, 1994; Thierry et al., 2008), elephants (Gobush & Wasser, 2009) and many other gregarious animals (Aureli et al., 2002).

1.4.3 Aggressiveness in termites

Many studies have used experimental data to access agonism in termites (Nel, 1968; Levings & Adams, 1984; Adams & Levings, 1987; Binder, 1987; Haverty & Thorne, 1989; Thorne & Haverty, 1991; Polizzi & Forschler, 1998; Ishikawa & Miura, 2012). Agonistic behaviour was originally coined by Scott & Fredericson (1951) as a set of actions (either aggressive or defensive) performed by individuals during a contest. In termites, aggressiveness is highly variable and may depend on different factors such as diet (Florane et al., 2004), caste ratios (Clément, 1952), nestmate recognition (Marins & DeSouza, 2008; Haverty & Thorne, 1989; Delphia et al., 2003) group composition (Haverty & Thorne, 1989) and territoriality (Levings & Adams, 1984; Adams & Levings, 1987). Even inter-colony aggression, which would be expected to be more predictable due to relatedness, is not always consistent (Binder, 1987) and species may exhibit behavioural plasticity, responding aggressively in some cases (Su & Haverty, 1991) but lacking agonism in others (Delaplane, 1991). Ishikawa & Miura (2012), for example, showed that when exposed to intruding ants, pseudergates of the termite *Hodotermopsis sjostedti* manage to change their defensive behaviour depending on social context, exhibiting low aggressiveness when paired with their soldiers, but very high levels of aggression in the presence of reproductives. Accordingly, Roisin et al. (1990) reported a similar response in *Nasutitermes princeps*, in which workers accompanied by soldiers, significantly decreased their defensive actions. Likewise, in fungus-culturing termites (*Macrotermes gilvus* and *Macrotermes carbonarius*), workers were seldom aggressive towards non-nestmates, a result that seemed to be not affected by geographic distance from where colonies were collected (Neoh et al., 2012). All these studies indicate that it is possible to have scenarios in which termite species adopt low aggressive behaviour, rather than the typical aggressiveness seen in the group.

Constrictotermes cyphergaster and *I. microcerus* represent a typical case of obligatory inquilinism, which means that, at least for inquilines, nest-sharing is mandatory (Shellman-Reeve, 1997). At the same time, one would expect this cohabitation to be not strictly in the interest of the host because, after all, nest construction is a costly, demanding process (Korb & Linsenmair, 1999). Given these facts, it is reasonable to think of a scenario in which hosts would endeavour to detect inquilines, whereas inquilines would try to go unnoticed by hosts. In such terms, it is quite possible that an evolutionary arms race would take place, leading hosts and inquilines to reach well-adjusted behavioral profiles. In doing so, both species would become highly specialized in each other (Kilner & Langmore, 2011).

Cohabitation is more than simply living in overlapping spaces. Rather, it is a result of multiple interactions among the parties involved. Our results support the hypothesis that upon encountering hosts, inquiline termites tend to adopt peaceful behaviour, thereby preventing confront escalation.

Conflict, although natural, may be a limiting factor for species that live in close relationship, especially if surpasses acceptable thresholds. In social insects, excessively aggression has been shown to cause even destruction of entire colonies. Here we have shown a set of behavioural adaptations which seems to allow *I. microcerus* to manage the amount of aggression received from *C. cyphergaster*. Once inevitably exposed to their hosts, inquilines behave peacefully and prevent confront escalation, even when direct contact with opponents is already established. Additionally, in our experimental conditions inquilines managed to perform evasive manoeuvres, such as reverse, bypassing and an intricate mechanism using defecation to repel hosts, suggesting that active avoidance is also in place. This unchallenging behaviour may play an important role in cohabitation since it seems to increase the chances for hosts and inquilines of sharing a single nest, without major detrimental consequences. While in accordance with previous reports on cohabitation among termites, our findings reinforce the growing view of conflict avoidance as an effective strategy for coexistence and adds new insights in terms of behavioural studies with cohabiting termite species.

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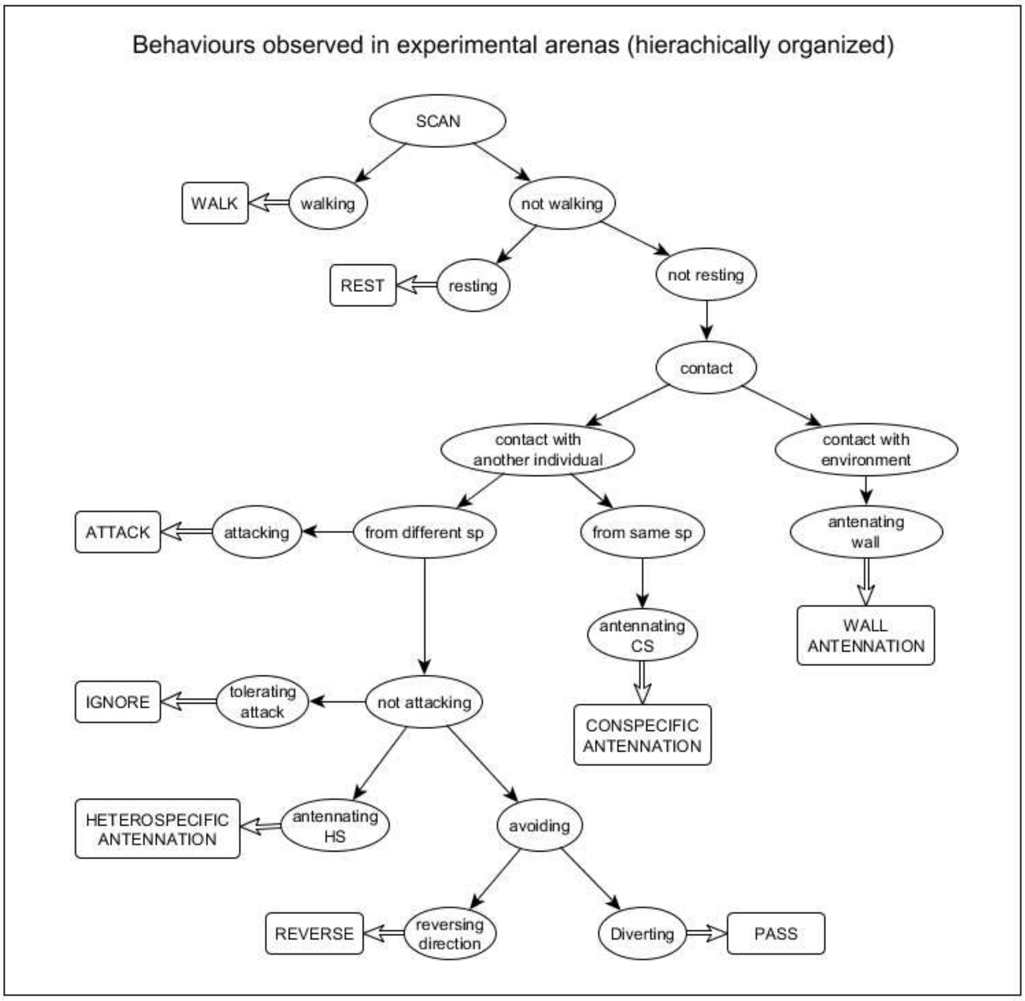
CHAPTER 2

Supplementary Material

Anexos

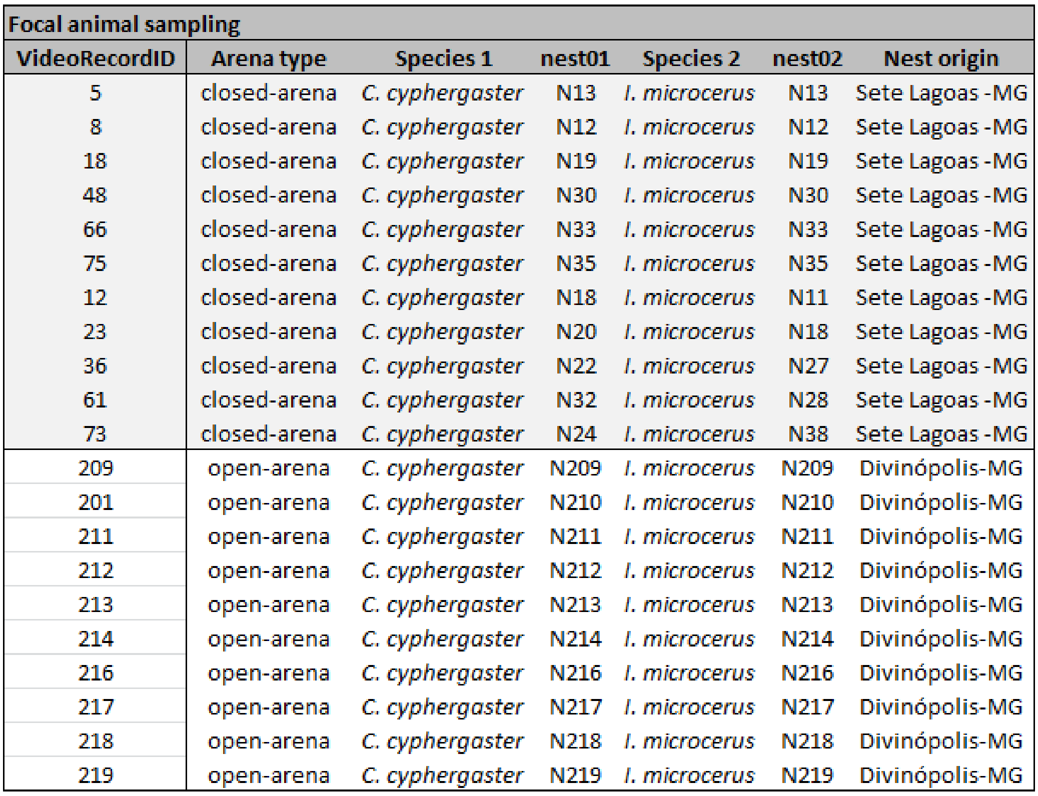


[Figure S1]

Figure 2.1: Behaviour observed in experimental closed arenas hierarchically organized. Boxes indicate the labels used in our analysis.

[Table S1]

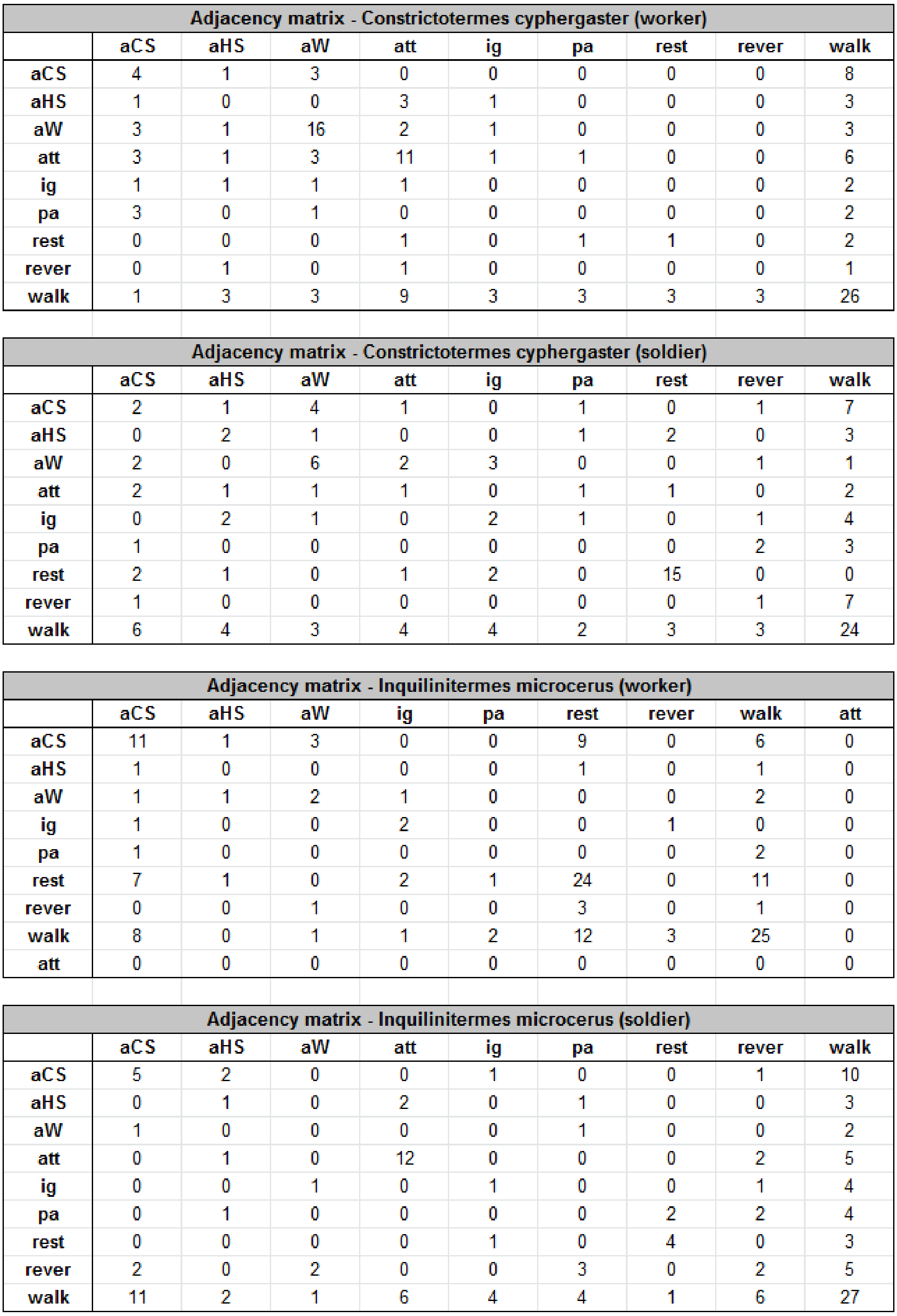
Table 2.1: List of five-minutes videos recorded and used for behavioural observation. *VideoRecordID* refers to the name attributed to each movie; *nest01* refers to the nest from which Species 1 was collected, *nest02* refers to the nest from which Species 2 was collected, *Nest origin* refers to the sampling location.



Focal animal sampling						
VideoRecordID	**Arena type**	**Species 1**	**nest01**	**Species 2**	**nest02**	**Nest origin**
5	closed-arena	*C. cyphergaster*	N13	*I. microcerus*	N13	Sete Lagoas -MG
8	closed-arena	*C. cyphergaster*	N12	*I. microcerus*	N12	Sete Lagoas -MG
18	closed-arena	*C. cyphergaster*	N19	*I. microcerus*	N19	Sete Lagoas -MG
48	closed-arena	*C. cyphergaster*	N30	*I. microcerus*	N30	Sete Lagoas -MG
66	closed-arena	*C. cyphergaster*	N33	*I. microcerus*	N33	Sete Lagoas -MG
75	closed-arena	*C. cyphergaster*	N35	*I. microcerus*	N35	Sete Lagoas -MG
12	closed-arena	*C. cyphergaster*	N18	*I. microcerus*	N11	Sete Lagoas -MG
23	closed-arena	*C. cyphergaster*	N20	*I. microcerus*	N18	Sete Lagoas -MG
36	closed-arena	*C. cyphergaster*	N22	*I. microcerus*	N27	Sete Lagoas -MG
61	closed-arena	*C. cyphergaster*	N32	*I. microcerus*	N28	Sete Lagoas -MG
73	closed-arena	*C. cyphergaster*	N24	*I. microcerus*	N38	Sete Lagoas -MG
209	open-arena	*C. cyphergaster*	N209	*I. microcerus*	N209	Divinópolis-MG
201	open-arena	*C. cyphergaster*	N210	*I. microcerus*	N210	Divinópolis-MG
211	open-arena	*C. cyphergaster*	N211	*I. microcerus*	N211	Divinópolis-MG
212	open-arena	*C. cyphergaster*	N212	*I. microcerus*	N212	Divinópolis-MG
213	open-arena	*C. cyphergaster*	N213	*I. microcerus*	N213	Divinópolis-MG
214	open-arena	*C. cyphergaster*	N214	*I. microcerus*	N214	Divinópolis-MG
216	open-arena	*C. cyphergaster*	N216	*I. microcerus*	N216	Divinópolis-MG
217	open-arena	*C. cyphergaster*	N217	*I. microcerus*	N217	Divinópolis-MG
218	open-arena	*C. cyphergaster*	N218	*I. microcerus*	N218	Divinópolis-MG
219	open-arena	*C. cyphergaster*	N219	*I. microcerus*	N219	Divinópolis-MG

[Table S2]

Table 2.2: Adjacency matrices containing the behavioural change for host's and inquiline's caste, used to draw graphs in yEd (Abbreviations: aCS=conspecific antennation, aHS=heterospecific antennation, aW=wall antennation, ig=ignore, pa=pass, rever=reverse, att=attack).



Adjacency matrix - Constrictotermes cyphergaster (worker)

	aCS	aHS	aW	att	ig	pa	rest	rever	walk
aCS	4	1	3	0	0	0	0	0	8
aHS	1	0	0	3	1	0	0	0	3
aW	3	1	16	2	1	0	0	0	3
att	3	1	3	11	1	1	0	0	6
ig	1	1	1	1	0	0	0	0	2
pa	3	0	1	0	0	0	0	0	2
rest	0	0	0	1	0	1	1	0	2
rever	0	1	0	1	0	0	0	0	1
walk	1	3	3	9	3	3	3	3	26

Adjacency matrix - Constrictotermes cyphergaster (soldier)

	aCS	aHS	aW	att	ig	pa	rest	rever	walk
aCS	2	1	4	1	0	1	0	1	7
aHS	0	2	1	0	0	1	2	0	3
aW	2	0	6	2	3	0	0	1	1
att	2	1	1	1	0	1	1	0	2
ig	0	2	1	0	2	1	0	1	4
pa	1	0	0	0	0	0	0	2	3
rest	2	1	0	1	2	0	15	0	0
rever	1	0	0	0	0	0	0	1	7
walk	6	4	3	4	4	2	3	3	24

Adjacency matrix - Inquilinitermes microcerus (worker)

	aCS	aHS	aW	ig	pa	rest	rever	walk	att
aCS	11	1	3	0	0	9	0	6	0
aHS	1	0	0	0	0	1	0	1	0
aW	1	1	2	1	0	0	0	2	0
ig	1	0	0	2	0	0	1	0	0
pa	1	0	0	0	0	0	0	2	0
rest	7	1	0	2	1	24	0	11	0
rever	0	0	1	0	0	3	0	1	0
walk	8	0	1	1	2	12	3	25	0
att	0	0	0	0	0	0	0	0	0

Adjacency matrix - Inquilinitermes microcerus (soldier)

	aCS	aHS	aW	att	ig	pa	rest	rever	walk
aCS	5	2	0	0	1	0	0	1	10
aHS	0	1	0	2	0	1	0	0	3
aW	1	0	0	0	0	1	0	0	2
att	0	1	0	12	0	0	0	2	5
ig	0	0	1	0	1	0	0	1	4
pa	0	1	0	0	0	0	2	2	4
rest	0	0	0	0	1	0	4	0	3
rever	2	0	2	0	0	3	0	2	5
walk	11	2	1	6	4	4	1	6	27