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Proximate Drivers of Multi-Symbioses in Termitaria

Maria do Socorro Lacerda Rolim
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Proximate Drivers of Multi-Symbioses in Termitaria

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Og F. Fonseca de Souza

Co-adviser: Maria Avany Bezerra Gusmão

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ABSTRACT

ROLIM, Maria do Socorro Lacerda, D.Sc., Universidade Federal de Viçosa, August, 2026. **Proximate Drivers of Multi-Symbioses in Termitaria**. Adviser: Og Francisco Fonseca de Souza. Co-adviser: Maria Avany Bezerra Gusmão.

Why do some host organisms establish associations with multiple partners, whereas others do not? In termites, host-inquiline cohabitation raises questions about how such associations are established and maintained despite host defences. Here, we investigated whether host aggressiveness limits associations with multiple inquilines and whether opportunities for encounters with potential invaders are associated with defensive responses. We tested the aggressive responses of hosts and potential invaders during experimental encounters and compared hosts that differ in the number of inquilines they harbour. Contrary to our hypothesis, the host harbouring multiple inquilines exhibited higher aggressiveness, while potential invaders also responded aggressively. Furthermore, *Cornitermes cumulans* individuals were more aggressive in the region with higher inquiline richness per nest and more frequent host-inquiline co-occurrence. The comparison with the sympatric congeneric species *C. bequaerti* revealed levels of aggressiveness similar to those observed in *C. cumulans*. Our results show that host aggressiveness alone does not prevent associations with multiple inquilines and suggest that differences in opportunities for interactions with potential invaders may contribute to geographic variation in host defensive responses.

Keywords: termites; symbiosis; aggressiveness; inquilism; cohabitation

RESUMO

ROLIM, Maria do Socorro Lacerda, D.Sc., Universidade Federal de Viçosa, agosto de 2026. **Determinantes imediatos de multissimbioses em termiteiros.** Orientador: Og Francisco Fonseca de Souza. Coorientadora: Maria Avany Bezerra Gusmão.

Por que alguns hospedeiros estabelecem associações com múltiplos parceiros, enquanto outros não? Em cupins, a coabitação entre hospedeiros e inquilinos levanta questões sobre como essas associações são estabelecidas e mantidas diante das defesas do hospedeiro. Neste contexto, investigamos se a agressividade dos hospedeiros limita a associação com múltiplos inquilinos e se oportunidades de encontros com potenciais invasores estão associadas às respostas defensivas. Testamos as respostas agressivas de hospedeiros e potenciais invasores em encontros experimentais e comparamos hospedeiros que diferem no número de inquilinos que abrigam. Contrariamente à nossa hipótese, o hospedeiro que abrigava múltiplos inquilinos apresentou maior agressividade, enquanto os potenciais invasores também responderam agressivamente. Além disso, indivíduos de *Cornitermes cumulans* foram mais agressivos na região com maior riqueza de inquilinos nos ninhos e maior frequência de coocorrência entre hospedeiros e inquilinos. A comparação com a espécie congênica simpátrica *C. bequaerti* revelou níveis de agressividade semelhantes aos observados em *C. cumulans*. Nossos resultados mostram que a agressividade do hospedeiro, por si só, não impede associações com múltiplos inquilinos e sugerem que diferenças nas oportunidades de interação com potenciais invasores podem contribuir para a variação geográfica nas respostas defensivas dos hospedeiros.

Palavras-chave: cupins; simbiose; agressividade; inquilismo; coabitação

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General Introduction

Symbiotic associations are widespread and may involve one or several partners (Douglas 2016; Drew et al. 2021). Yet, organisms differ in the number and diversity of partners they maintain (Batstone et al. 2018). Understanding this variation requires examining the interactions that precede symbiotic establishment. A potential partner must first reach and interact with the host, and the outcome of this interaction may determine whether the association is rejected or established.

Host recognition is central to these interactions. In social insects, individuals discriminate colony members from unfamiliar organisms and adjust their responses accordingly (Shorter and Rueppell 2012; Grüter et al. 2018). Unfamiliar individuals may trigger aggressive responses, whereas familiarity with an individual can reduce aggressive responses towards that individual (Marins and DeSouza 2008; Ishikawa and Miura 2012; Van Wilgenburg et al. 2009). These responses act as behavioral mechanisms to mediate conflict and regulate potential partners (Estrela et al. 2016).

However, host defences alone cannot fully explain multi-symbiotic associations. Potential partners may avoid detection, evade attacks, resist removal, or otherwise overcome these defensive barriers. Inquiline termites, which live within nests constructed and maintained by other termite species, illustrate this possibility. Some are attacked when encountered by their hosts, yet establish persistent associations within host termitaria (Hugo et al. 2020). Others reduce conflict through niche segregation, either by avoiding dietary overlap or by remaining spatially separated within the nest (Florencio et al. 2013; Timmermans et al. 2025).

Multi-symbiosis may therefore arise from the outcomes of interactions with different potential partners (Montoya et al. 2023). The number of symbiotic partners should consequently reflect not only host defensive behaviour, but also opportunities for interaction and the ability of potential partners to overcome or avoid that defence. However, the strength of host defence need not be constant. Rather, defensive responses can be adjusted according to prior interactions with potential opponents, which may modify subsequent aggressive responses (Hsu et al. 2006; Van Wilgenburg et al. 2009). Thus, variation in encounter history may contribute to differences in defensive aggressiveness. For such effects to accumulate, however, potential opponents must be present and come into contact with the host.

This reasoning leads to two complementary expectations. If host defence constrains the establishment of symbiotic associations, hosts associated with more inquiline species should exhibit weaker aggressive responses towards potential partners. Conversely, if defensive behaviour is shaped by previous experience, hosts exposed to greater opportunities for host-inquiline encounters should exhibit stronger aggressive responses towards potential opponents. In termite systems, these expectations can be examined through host-inquiline interactions. Termite nests can harbour diverse communities of heterospecific organisms (Mathews 1977; Florencio et al. 2013), creating opportunities for hosts to encounter potential intruders.

Although host defence is expected to affect interactions with potential partners, defence alone does not fully explain the persistence of multi-symbiotic associations. Opportunities for encounters with heterospecific individuals may also shape how hosts respond to potential partners by influencing the experience accumulated through repeated interactions. Despite the importance of these processes, it remains unclear how host defence and opportunities for heterospecific interactions jointly shape multi-symbiotic associations. This question is particularly relevant in horizontally acquired systems, where hosts must interact with potential partners while limiting conflict and maintaining tolerable associations (Estrela et al. 2016). We therefore examine defensive interactions between termite hosts and inquilines, assessing how aggressive responses vary with familiarity between potential opponents and with opportunities for host-inquiline encounters.

Overview of chapters

This thesis is divided into three chapters. Chapter 1 provides a general introduction to multi-symbiotic associations in termite nests. It focuses on how host defence varies with opponent familiarity and opportunities

for host-inquiline encounters.

Chapter 2 tests whether host aggressiveness limits the establishment of multi-symbiotic associations. We compared the aggressiveness of termite hosts that differ in the number of inquiline species they harbour. Contrary to our expectation, the multi-symbiotic host was more aggressive than hosts associated with fewer or no inquilines. This result shows that strong host aggression does not necessarily prevent the establishment of multiple symbiotic associations.

Chapter 3 examines whether host aggressiveness varies across geographic contexts and whether this variation is associated with opportunities for encounters between hosts and inquilines. We compared *Corietermes cumulans* individuals from two regions differing in termite species richness. Individuals from the region with higher termite species richness showed greater aggressiveness and were associated with higher inquiline richness and more frequent co-occurrence of hosts and inquilines within the same nest. A congeneric host from the same region exhibited similar levels of aggressiveness, providing further evidence that aggressiveness may be associated with local context rather than host identity.

Together, the three chapters challenge the view that multi-symbiosis requires weak host defence. Our results show that hosts can remain highly aggressive while maintaining multiple symbiotic partners. Multi-symbiosis may instead emerge from interactions in which host defence, invader behaviour, and opportunities for repeated encounters jointly shape the outcome.

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Host aggressivity does not prevent multi-symbioses in termitaria

Maria Lacerda, Diogo Costa, Maria Avany Bezerra-Gusmão,
Luana Silveira, and Og DeSouza

Proximate drivers of multi-symbioses in termitaria

Abstract

Abstract

Why do some host organisms establish symbioses with multiple partners, while others do not? In heterospecific termite-termite cohabitation within a single termitarium, this is a common scenario that still needs to be unveiled. We tested the hypothesis that these multi-symbioses should be less likely in aggressive rather than tolerant termite hosts. To do so, we simulated – in experimental arenas – initial encounters between a multi-symbiotic termite host (*Cornitermes cumulans*) and a potential nest-invading termite, estimating the aggressiveness of both partners. For comparison, we ran equivalent assays using two other termite hosts that are known to house zero or a single inquiline species (*Nasutitermes ephratae* and *Constrictotermes cyphergaster*, respectively). To account for differences in aggressivity due to previous experience between partners, we arranged partners in a gradient of ecological and phylogenetic dissimilarities among each other within the arenas. This allowed a continuous estimate of a “degree of strangeness” between partners. Contrary to our hypothesis, the multisymbiotic termite host here studied presented far more aggressiveness than the other hosts. This suggests that host aggressiveness in itself is not enough to prevent termitaria invasion by multiple inquiline species. In fact, host aggressiveness elicited equivalent retaliation from invaders, suggesting that this may be one of the routes to multi-symbioses in these termitaria. Such routes are summarised in a schematic map, as a theoretical exercise to generate new hypotheses about the drivers of multi-symbioses in termitaria.

Keywords: termite behaviour, host-symbiont interaction; aggressiveness, nest invasion.

1.1 Introduction

Why are there organisms that engage in symbiotic associations with a multitude of species, whereas others connect with only a few partners, and still others live entirely on their own? (Batstone et al. 2018; Douglas 2016). Despite being a common scenario in termite-termite heterospecific cohabitation in a single termitarium, this is an issue still deserving clarification. To understand this issue, one could inspect the interspecific interindividual interactions between potential partners. After all, a symbiotic association begins when an individual encounters a potential partner and forms a successful bond. Such a binding will occur if the partner is not recognised as a stranger, triggering no confrontation and being thence welcomed, tolerated, or ignored (Grüter et al. 2018; Marins and DeSouza 2008; Parmentier et al. 2017). However, even when a potential partner is identified as an alien, and this recognition provokes conflict, a symbiotic relationship can still form, provided that the rejection is addressed. Thus, whether an organism will establish none, a few, or multiple symbiotic links depends on the number of successful outcomes of its interactions with a potential partner. As for multi-symbioses, it seems reasonable to suspect that they arise from cumulative runs of the above process. Each of these runs could follow a given route (as hypothesised in Fig. 1.1) that culminates in a successful symbiotic link.

Although quite relevant for understanding multisymbiotic systems in general, it is in termites that this issue remains arguably less solved. Aiming to shed some light on the problem, here we inspect a multisymbiotic system composed of termite species (so-called “inquilines”) that cohabit a termitarium along with their termite-building species (the “host”). The host of focus is *Cornitermes bequaerti*, a species widely known for housing a multitude of inquiline species in their nests.

Termites provide a good case study of multi-symbioses because their nests span the full range of cohabitant load, from zero to many inquiline species (Marins et al. 2016). Termites also provide the full spectrum of symbiotic links, from microorganisms (protists, archaea, and bacteria) inhabiting their gut (Brune and Dietrich 2015) to macroorganisms (fungi, arthropods including other termite species, and vertebrates) cohabiting their nests (Monteiro et al. 2017; Timmermans et al. 2025). Additionally, termites and their mounds can be regarded as an analogue of a typical host organism (for instance, a mature animal or plant). Whereas these latter hosts use their skin or bark as an initial barrier to invasion, termite mounds present a protective outer wall serving the same purpose. If this first line of defense is breached, a typical host engages its immune cells and defensive chemicals. A termite colony, in turn, mobilises its defensive forces in the form of soldiers and workers (DeSouza et al. 2016), some of which may also deploy chemical weapons (Prestwich 1984).

Indeed, termites provide a mirror for many of the routes underlying symbioses (Fig. 1.1) in other organisms. Just as mitochondria are completely imperceptible to their eukaryotic host, *Serritermes serrifer* termites also go unnoticed by living inside their host termitarium walls (Emerson and Krishna 1975). Similarly, many termite inquiline species do not overlap their diets with that of their hosts, presumably avoiding conflicts (Florencio et al. 2013). Other symbionts, such as the bacteria *Vibrio fischeri*, despite being recognised, are accepted by their squid hosts (Nyholm and McFall-Ngai 2004). Likewise, *Corotoca melantho* termitophile beetles, interact pacifically with their host, bypassing rejection through morpho-chemical mimicry (Pisno et al. 2019; Castiblanco et al. 2022b). As for the systems composed by plant-rhizobia or plant-pathogens, the bacteria are initially perceived as intruders, eliciting an unsuccessful defensive response by the host plant (Soto et al. 2009). *Inquilinitermes microcerus*, too, are attacked if met by their termite host (Hugo et al. 2020), but form otherwise stable colonies within their host termitaria.

However, not all symbiotic systems follow this conflictual logic. Some, such as lichens (Pichler et al. 2023), involve the coexistence of multiple partners, including fungi, algae or cyanobacteria, and other microorganisms. In these cases, the fungus employs broad chemical signals and relatively non-specific adhesion mechanisms, providing it with flexibility in recognising different partners. This flexibility allows the host fungus to be tolerant, accepting multiple symbionts and integrating them effectively. In such contexts, tolerance among partners appears to play a crucial role, facilitating the establishment of more complex and diverse symbiotic networks. After all, only hosts exhibiting flexible recognition traits will accept distinct partners.

If termites are to mirror also these systems, one should expect multisymbiotic termite hosts to exhibit lower levels of aggressivity. This sounds plausible: the network of cohabitants in termite mounds in Brazil and Cameroon share more properties with mutualistic plant-pollinator than to antagonistic host-parasitoid

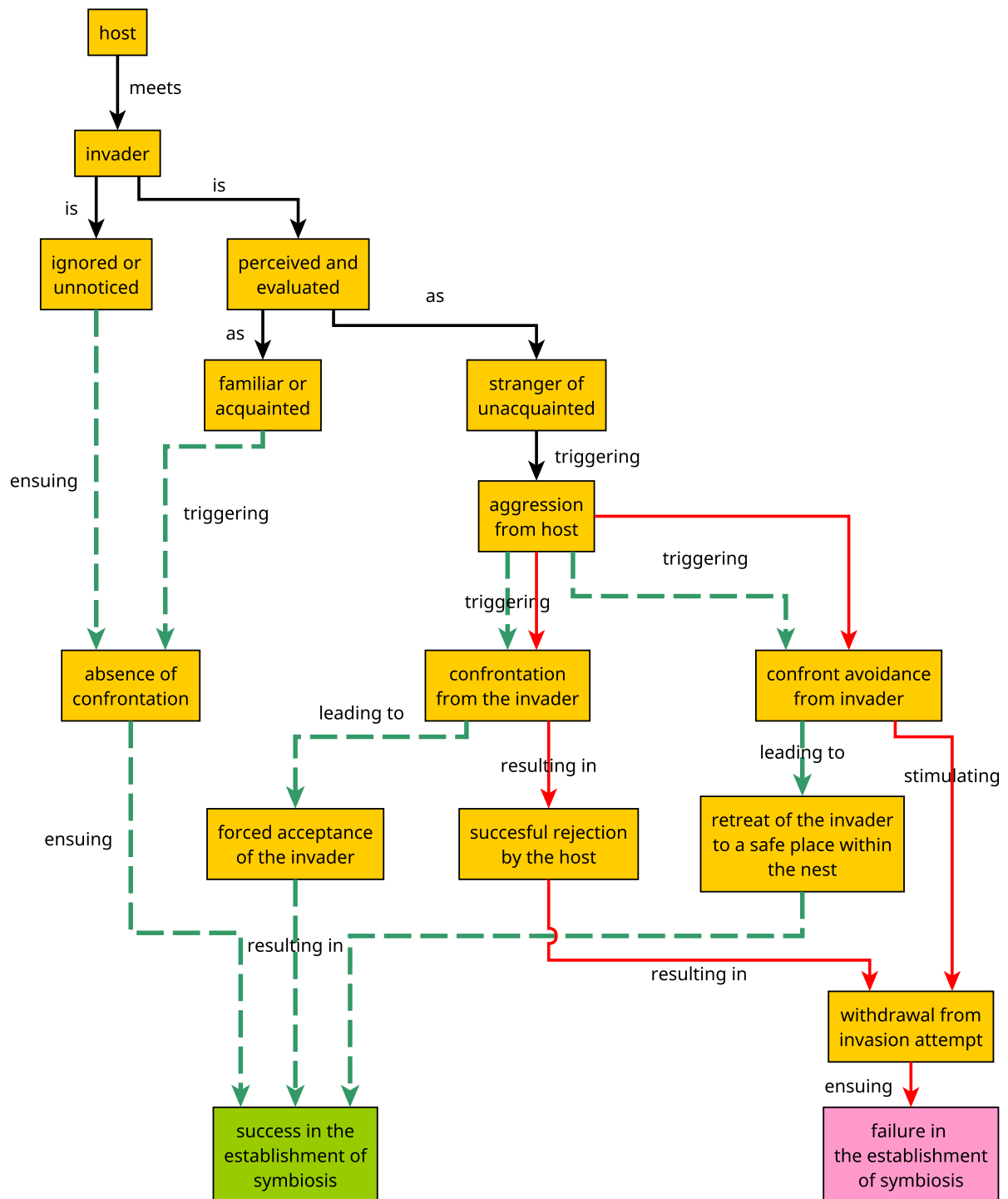


Figure 1.1: Hypothetical routes to symbiosis following the encounter between a potential host and its potential invader. Green dashed routes lead to the success of the symbiotic link while red solid routes lead to its failure.

networks (Campbell et al. 2016).

It seems reasonable to suspect that any specific case of multi-symbiosis would result from the iteration of independent successful invasion attempts, each of which profits from any of the three types of host failures referred to above (heedlessness, tolerance, inability to evict). It seems also reasonable to expect that, all other things being equal, a host minimally able to recognise and attack invaders would establish fewer symbiotic links than heedless or tolerant hosts.

That is to say, multi-symbioses should be less likely or, at least, less teeming in aggressive rather than tolerant hosts. Here we will focus on this hypothesis, inspecting how a termite host (*Cornitermes bequaerti*), which is known for cohabiting with many termite inquilines, responds to acquainted and stranger potential invaders when facing them in experimental arenas. We expect this host to be far less aggressive towards potential invaders than other termite hosts with a low symbiotic load. For comparison, we ran equivalent assays using two other termite hosts that are known to house zero or a single inquiline species (*Nasutitermes ephratae* and *Constrictotermes cyphergaster*, respectively).

1.2 Materials and Methods

Key terms used here are listed in Table 1.1.

Table 1.1: Terms definitions for this study.

Term	Definition
Colony:	A group of genetically related termites of the same species that share a nest and live together in an organised structure.
Nest:	The physical structure built by the host termite.
Host:	A termite that builds a nest and hosts other species.
Inquiline:	Termites that inhabit a nest built by another termite species.
Invader:	A termite individual introduced in the experimental arena to interact with the host, regardless of whether it is a natural inquiline of this host or not.

1.2.1 Rationale

To test our hypothesis, we experimentally simulated the first encounter between a nest-building termite and a potential intruder by allowing individuals to interact under controlled conditions. Termite individuals were confined to a single arena and video-recorded during their interactions. We subsequently analysed these recordings by quantifying aggressive and non-aggressive (i.e., tolerant) behaviours exhibited during encounters.

The assays involved a multisymbiotic host (*Cornitermes bequaerti*) as our species of primary interest and – for comparative purposes – two other host termites occurring within the same geographical region and biome. These other hosts (*Nasutitermes ephratae* and *Constrictotermes cyphergaster*) are known to house zero or one inquiline species, respectively (see Section 1.2.3 “Biological models”). This approach gives us a continuum in the number of inquiline species housed by these hosts (zero, one, many), avoiding a less informative categorical distinction between, say, a host with and a host without inquilines.

Host aggressiveness towards unfamiliar individuals is more nuanced than simple self/non-self recognition (Van Wilgenburg et al. 2010): hosts may evaluate the potential threat posed by interacting individuals and adjust their responses accordingly. In fact, consistent with the dear enemy hypothesis (Fisher 1954; Werba et al. 2022) and Emery’s rule (Emery 1909), defensive responses may depend on the degree of familiarity between interacting partners, with individuals showing greater tolerance towards familiar associates than towards unfamiliar individuals. Reduced aggression may therefore occur towards partners with whom

hosts share a history of non-conflicting interactions (Hamilton 1964; Grafen 1990; Aguilera-Olivares et al. 2016), whereas unfamiliar or previously harmful opponents may elicit stronger defensive responses.

Because previous interactions can modify subsequent responses towards potential opponents (Van Wilgenburg et al. 2009), differences in encounter history may contribute to variation in host aggressiveness. Therefore, we quantified the degree of strangeness between interacting individuals as a measure of how likely one individual was to be perceived as unfamiliar by another. This measure allowed us to evaluate how variation in partner similarity affected aggressive responses during encounters in the arena.

The degree of strangeness was based on dissimilarity between interacting individuals along two complementary axes: (i) ecological and (ii) phylogenetic (see Table 2.1). The ecological axis was defined according to nest origin, indicating whether individuals interacting in the arena originated from the same nest or from different nests. This distinction is particularly relevant in social insects, where discrimination between nestmates and non-nestmates underlies aggressive and rejection behaviours that prevent the incorporation of foreign individuals into colonies (Wilson 1979; van Zweden et al. 2009; Aguilera-Olivares et al. 2016). Individuals of the solitary bee *Manuelia postica*, for instance, generally show reduced aggression towards nestmates compared with non-nestmates (Flores-Prado et al. 2008), and may respond more strongly to chemical cues from neighbouring colonies than to cues from their own colony (Nehring et al. 2012). It seems plausible that, in termites too, individuals from the same nest show lower aggressive responses, whereas individuals from different nests are more likely to be recognised as potential intruders.

Beyond ecological similarity, interactions may also depend on the evolutionary relationship between individuals. Emery’s rule predicts that social parasites tend to associate with phylogenetically related hosts (Emery 1909), suggesting that evolutionary similarity may facilitate the establishment of interspecific associations. Experimental evidence also indicates that phylogenetic proximity can affect interactions between termite species by facilitating integration and acceptance of heterospecific individuals within host colonies (Bai et al. 2026). To capture this variation, we defined a phylogenetic axis of strangeness, distinguishing interactions involving individuals from the same species from those involving different species.

We therefore quantified strangeness according to the dissimilarity between interacting individuals across these ecological and phylogenetic dimensions (Table 1.2). Individuals sharing more attributes, such as nest origin or species identity, were considered less strange to each other, whereas individuals differing across these dimensions represented more unfamiliar interactions.

Table 1.2: The degree of strangeness between the focal individual and its interacting counterpart in a given arena, based on traits of this counterpart individual. = both individuals share the same trait in that given axis; ≠ individuals present distinct traits. See Section 1.2.1 “Rationale” for details.

Traits shared with the focal individual		strangeness
ecological axis:	phylogenetic axis:	
nest	species	
=	=	1
≠	=	2
=	≠	3
≠	≠	4

1.2.2 Study site and collection

We collected 18 termite nests in the Cerrado biome in Mário Viana Municipal Biological Reserve (RBMMV) (14°43’S, 52°21’W), located within the Universidade do Estado de Mato Grosso (UNEMAT) in Nova Xavantina, Mato Grosso, Brazil. It covers approximately 500 hectares, with an average altitude of 346 metres above sea level (Marimon et al. 2010). According to the Köppen classification, the regional climate is Aw. Annual precipitation ranges from 1300 to 1500 mm and a mean monthly temperature of 25°C, as recorded by the climatological station of Nova Xavantina.

All collected nests were transported to the laboratory, where they were kept under room conditions for up to 7 days. Food (paper fragments and grasses) and water were provided to prevent desiccation of the termites until the experiments were conducted.

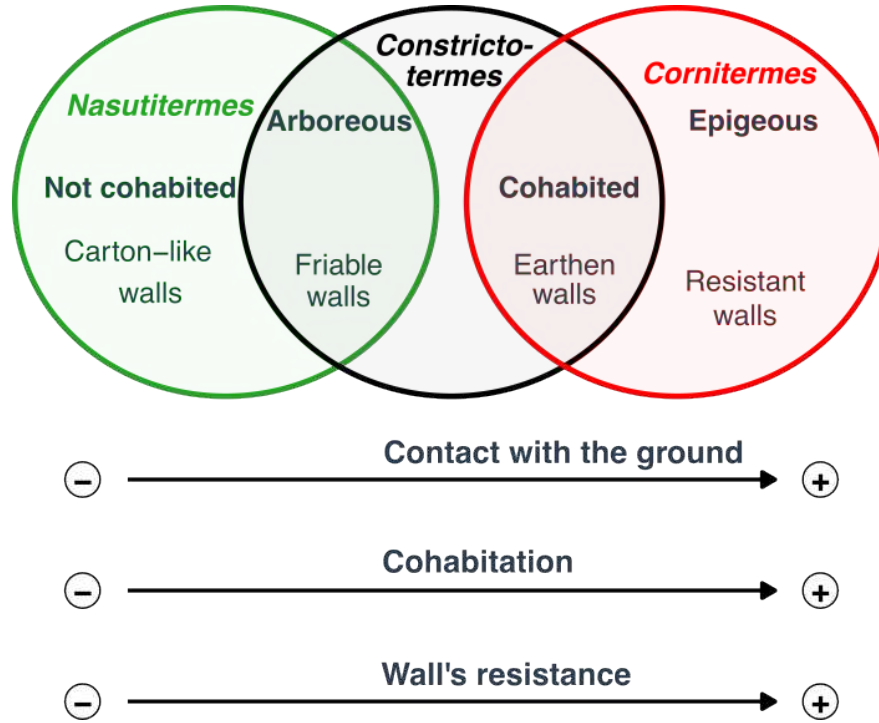


Figure 1.2: The set of nest traits shared by the three non-inquiline nest-building species used in our experiments, emphasizing the nest traits of *Constrictotermes* as a ‘transition’ between those of *Nasutitermes* and *Cornitermes*.

1.2.3 Biological models

At the above study site, we detected the coexistence of a set of termite species that were highly suitable for our test. Among these, three species (i) can build their own nest, (ii) vary in their natural propensity to hold inquilines, and (iii) are not known to invade other species’ nests. At this same location, we found seven other termite species which are known to invade other termite species’ nests, either facultatively or obligatorily. The facultative ones are those which can build their own nests (see [DeSouza et al. 2025](#), for details on termite inquilism). Being all sympatric, these species would have had opportunities to share some ecological history.

The non-inquiline nest-building termite species referred to above are: *Nasutitermes ephratae* (Holmgren, 1910) (Termitidae: Nasutitermitinae), *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae: Nasutitermitinae), and *Cornitermes bequaerti* Emerson, 1952 (Termitidae: Syntermitinae). On top of varying in their natural propensity to hold inquilines, these species present a set of nest traits forming a continuum in which *N. ephratae* and *Cor. bequaerti* are at the extremes, whereas *Con. cyphergaster* stands in the middle, sharing nest traits with both of them (Fig. 1.2).

N. ephratae builds arboreous nests, which have “cartonated” friable walls and houses no inquilines. *Cor. bequaerti* builds epigeous nests, which have resistant earthen walls and house a large number of inquiline species. *Con. cyphergaster* stands as a link between these two, building arboreous friable nests, which have earthen walls, nevertheless. This species is also known to start their nest in the soil, migrating it up in a tree as the nest matures. *Con. cyphergaster* normally houses a single obligatory inquiline species, even though multi-occupation of their nest by several inquilines has already been spotted at least once ([Costa et al. 2019](#)).

1.2.4 Experimental Setup

To perform the experimental combinations of individuals in the arenas, we used only termite species co-occurring in the study site described above (Section 2.2.4). These termites have been collected either from their own nest or from the nest of other termites under test here (Table S3). Since termite inquilines usually

present low host specificity (Campbell et al. 2016), these sympatric species could have *tried* to enter any of the nests of the hosts examined here. Whether or not they would be rejected by the host is precisely the matter under test here.

The assays involved observing interactions among termite individuals confined to a single arena. Experimental arenas were mounted by placing a PVC hosepipe ring (50 mm diameter, 3 cm long) on a flat, sand-blasted glass base. A flat transparent glass plate covered the arena. Before inserting the termites, the arenas were washed three times with mild detergent and running water to remove residues and unwanted substances. The arena structure enabled both conspecific and heterospecific encounters between individuals, simulating interactions between cohabitants in the nest. A plastic barrier, as high as the arena walls, halved its floor space, keeping heterospecific individuals apart before the beginning of the assay (Suppl. Mat. Fig. S1). This prevented conflicts before the assays started. The barrier was removed right before the assay began.

The number of individuals in each arena was adjusted to maximise their interactivity (Table S3). To do so, the groups were formed so that the ratio of the total area occupied by the individuals to the total area of the arena's floor was kept between 0.14 – 0.20. This implies that termites themselves occupied 14–20% of the walking floor area, within the range of densities maximising interactivity for confined termites (Miramontes and DeSouza 1996; DeSouza and Miramontes 2004). Furthermore, the proportions of workers and soldiers in arenas were adjusted to reflect natural field conditions, based on species descriptions in the available literature. When this information was not accessible, this proportion was determined in the field by collecting samples of individuals from field nests and counting workers and soldiers. This procedure was repeated approximately three times, one sample per nest.

Arenas comprised a gradient of the degree of “strangeness” between their composing individuals: nest builders and intruding termites (Tables 1.2 and S3; see also Section 1.2.1, “Rationale”). There were 38 arenas composed of two sets of individuals, as long as one of these sets consisted of individuals belonging to our pre-selected nest-building termite species (*N. ephratae*, *Con. cyphergaster*, and *Cor. bequaerti*). Each of these 38 arenas corresponded to one of 6 degrees of strangeness, each degree repeated at least 3 times (see Table S3).

1.2.5 Termite caste and behaviours to be observed

Pilot trials were conducted to select termite castes for the experimental arenas and define the behaviours to be recorded and observed in the experiments.

During this period, we tested different caste combinations (workers only, soldiers only, or a mixture of both) in six exploratory videos used exclusively to adjust the configuration of the experimental arena. These preliminary trials were later discarded and not included in the main behavioural analyses. The preliminary trials, in conjunction with previous literature (Hugo et al. 2020), guided the selection and definition of the behaviours to be observed in the experiments (see Table 1.3).

Not surprisingly, as already demonstrated by Castiblanco et al. (2022a), the presence of the soldiers was relevant for the activity of the individuals (soldiers + workers) in the arena. However, the workers themselves were the most interactive caste. As a result, we chose the workers as the focus of our observations while keeping soldiers in each testing group (see below).

Table 1.3: Behaviours exhibited by the focal individual during interactions with another individual in an experimental arena. Columns are *Behaviour*: the behaviours observed during interactions; *Definition*: the description of each behaviour; *Classification*: the categorisation of behaviours as aggressive or non-aggressive.

Behaviour	Definition	Classification
Antennate	Focal termite performs antennation upon contact with a conspecific or a heterospecific.	Nonaggressive
Attack	Focal termite performs aggressive actions, including biting, head-butting, or releasing chemicals to immobilize the other individual upon encountering a conspecific or a heterospecific.	Aggressive
Bypass	Focal termite continues moving without changing its own direction even after coming into contact with a conspecific or a heterospecific.	Nonaggressive
Kill	Focal termite causes the death of the other individual upon a fight.	Aggressive
Groom	Focal termite performs grooming upon encountering a conspecific or a heterospecific.	Nonaggressive
Reverse	Focal termite changes its own direction of movement upon encountering a conspecific or a heterospecific.	Nonaggressive
Ignore	Focal termite is stationary and does not react upon being encountered by a conspecific or a heterospecific.	Nonaggressive
Oral trophallaxy	Focal animal performs mutual oral contact with a conspecific or a heterospecific.	Nonaggressive
Receive proctodeal trophallaxis	Focal termite receives trophallaxis via anus contact with a conspecific or a heterospecific.	Nonaggressive
Provide proctodeal trophallaxis	Focal termite provides trophallaxis via anus contact with a conspecific or a heterospecific.	Nonaggressive
Vibration	Focal termite vibrates its body upon encountering a conspecific or a heterospecific.	Nonaggressive

1.2.6 Observational protocol

To investigate the interindividual interactions within each experimental arena, assays were video-recorded for 5 minutes. In total, 49 videos were recorded, a single video per arena. Before recording the videos, the termites were allowed to acclimatise in the arenas for approximately 30 minutes. After that, the plastic barrier was removed, allowing all termites from both groups to circulate and interact freely. These behaviours were registered using Boris software (Friard and Gamba 2016). To ensure consistency in the interpretation of behaviour, the videos were inspected by a single observer. We focused only on worker-worker interactions because previous observations showed this was the most interactive caste in our tests.

Observations consisted of recording the behaviour exhibited by a focal individual when it contacted another individual in the arena for 5 minutes from the beginning of the trial (that is, right after the removal of the barrier, see above). Interindividual contact was defined as occurring when the distance between workers' bodies did not exceed two antenna lengths. (Paiva et al. 2021).

1.2.7 Statistical analysis

To examine the effects of strangeness between interacting individuals on their tolerance or aggressiveness toward each other, we built two sets of candidate models. Each set consisted of models in which a single response variable, describing tolerance or aggressiveness exhibited by focal individuals, was regressed on the degree of strangeness exhibited by their interacting individuals. The identity of the focal individuals entered the model as a covariate.

Both sets of models departed from a global model given by the general equation:

$$y \sim s + h + s : h + d + nh + ni \quad (1.1)$$

where y is the aggressivity exhibited by the focal individual (see below) and s is the degree of strangeness between host and invader, as defined in Table 2.1. The host's identity is given by h . The interactions between $s:h$ allowed for distinct slopes in the regression lines representing distinct host identities. The absolute density experienced by the termites in each arena is given by d . The nest of origin of the host termites is given by na and the nest of origin of invading termites is given by ni .

The first set of models described the effects of the intruders' strangeness upon the proportion of *aggressive* behaviours exhibited by the host termites when confronted with these intruders. To do so, the proportion of aggressive behaviours exhibited by the hosts entered the global model (equation 1.1 above) as $yvar1$:

$$yvar1 = \frac{aggr}{nonAggr + aggr} \quad (1.2)$$

where $aggr$ is the number of aggressive behaviours and $nonAggr$ is the number of non-aggressive behaviours exhibited by the host.

The second set of models described the effects of the hosts' strangeness upon the proportion of *aggressive* behaviours exhibited by the invading termites when confronted with these hosts. To do so, the proportion of aggressive behaviours exhibited by these invaders entered the global model (equation 1.1 above) as a $yvar2$:

$$yvar2 = \frac{aggr}{nonAggr + aggr} \quad (1.3)$$

where $aggr$ is the number of aggressive behaviours and $nonAggr$ is the number of non-aggressive behaviours exhibited by the invaders.

These models were submitted to "model selection", a statistical approach differing from traditional hypothesis testing in that it can be used to identify a single best model, thus lending support to one particular hypothesis, or it can be used to make inferences based on weighted support from a complete set of competing models. This approach fits well with the advice of Chamberlin (1890) regarding best procedures for hypothesis testing.

Analyses followed the recommendations of Burnham and Anderson (2002) and were performed under R version 4.3.3 (R Core Team 2021), using package MuMIn (Bartoń 2022) under Generalised Linear Modelling (GLM). Initially, a global model (equation 1.1) was built to encompass all candidate models for a given $yvar$ (either equation 1.2 or 1.3). Analyses were carried out for each y -var independently. Models were built under Binomial errors. The fit and complexity of each candidate model were measured using the second-order Akaike Information Criterion (AIC_c). Models were ranked by AIC_c to identify the best, *i.e.*, the one with the lowest AIC_c . Inference on the existence of effects of explanatory variables on the hosts' aggressivity ($yvar1$) or invaders' aggressivity ($yvar2$) patterns was made based on the models whose AIC_c differed from the best model's values by two or fewer units. Full averaging was applied to these models, resulting in a final model for each y -var. This final model was used to plot the curves depicted in Figures 1.3 and 1.4.

After building the final model for each $yvar$, we proceeded to further model simplification, building alternative models by collapsing levels of the variable $hostId$ (equation 1.1) when their intercepts or slopes did not significantly differ, according to the AIC_c evaluation. Then we calculated the AIC_c for each of these alternative models and selected the model with the lowest AIC_c as the final result.

1.3 Results

Our results showed that host aggressiveness tends to increase as its interactor becomes more strange (Fig. 1.3; Table 1.4), indicating that hosts seek to repel potential invaders.

The degree of this response, however, varied among host species: *Cor. bequaerti* individuals were more aggressive than those of the other host species tested here. This is evidenced in Fig. 1.3, where the curve corresponding to *Cor. bequaerti* lies well above the curves representing the other host species here studied.

Additionally, while *Con. cyphergaster* and *N. ephratae* reacted significantly only to the highest levels of strangeness (level 4), *Cor. bequaerti* displayed generalised aggressiveness across all levels of strangeness (nestmate & non-nestmate conspecifics, nestmate & non-nestmate heterospecifics).

In other words, individuals belonging to host species whose nests harbour many inquilines (here, the multi-symbiotic *Cor. bequaerti*) were more aggressive than the individuals from the non-multi-symbiotic hosts here assayed (Fig. 1.3; Table 2.3). This result contradicts our initial hypothesis that multi-symbiotic hosts would be less aggressive than non-multi-symbiotic ones.

The invaders here studied (Table 1.5) also displayed aggressiveness towards unfamiliar individuals (Fig. 1.4; Table 1.5), indicating that invaders are not entirely passive.

The degree of invader responses, however, varied across hosts: the invaders of *Cor. bequaerti* were more aggressive than those of the other host species tested here. This is evidenced in Fig. 1.4, where the curve corresponding to invaders of *Cor. bequaerti* lies well above the curves representing the invaders of the other host species here studied.

Additionally, while invaders of *Con. cyphergaster* and *N. ephratae* reacted mainly to the highest levels of strangeness (level 4), the invaders of *Cor. bequaerti* displayed generalised aggressiveness towards all their interactors.

These results indicate that invaders – and not only hosts – do play a role in the processes underlying inquilinism in termitaria. This also indicates that the host's attitude somewhat modulates the invader's aggression. This is further evidenced by observing the reactions of the same invader when facing different hosts: *Embiratermes festivellus* was generally more aggressive when it encountered *Cor. bequaerti* host individuals than when it contacted other host individuals. As depicted in Fig. 1.4, the dots marked as emb – and corresponding to *E. festivellus* – occur at higher y-values when this invader interacted with *Cor. bequaerti* than when it interacted with the other hosts assayed here.

Among the non-aggressive behaviours, some could involve interspecific interactions (such as antennation, trophallaxy). In contrast, others could be considered "evasive" hosts and invaders ignored or bypassed each other without any reaction as they came near within the arenas (Tables S1 and S2). Such evasive behaviours were relatively frequent, accounting for $\approx 20\%$ of all behaviours even for aggressive hosts. These behaviours ultimately reduced direct contact between interactors, thereby preventing the triggering of aggression. This suggests that evasiveness would also be relevant in the processes underlying inquilinism in termitaria.

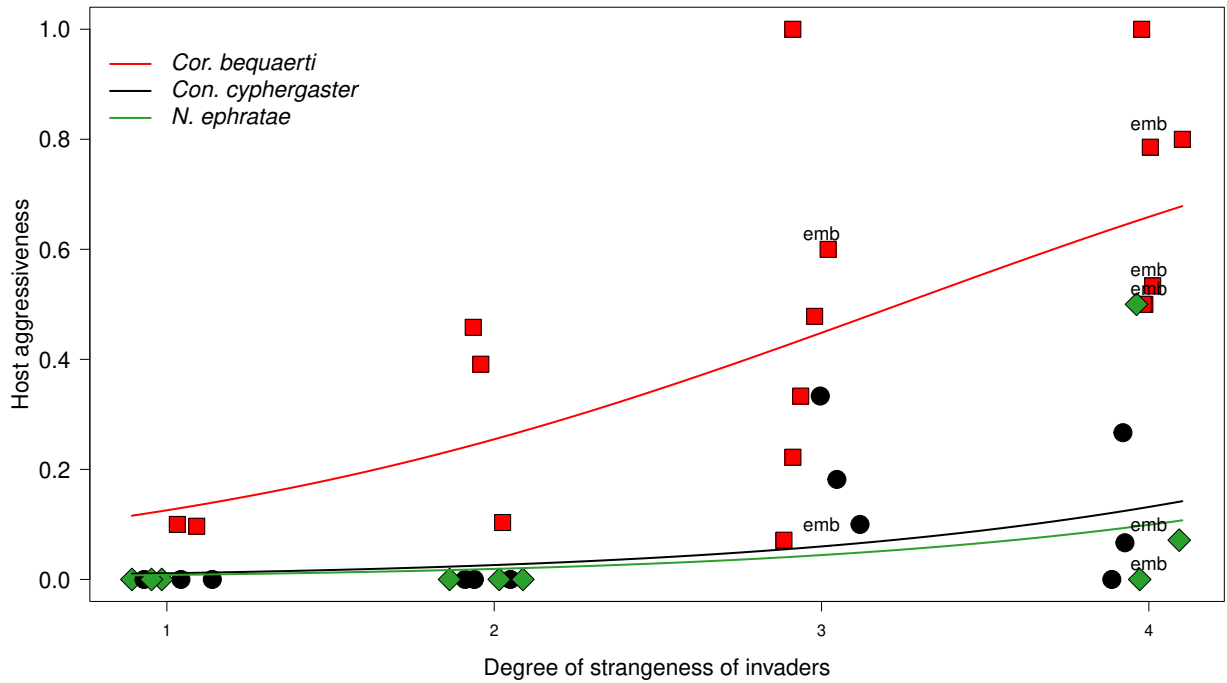


Figure 1.3: Host aggressiveness (y-var) increases as the invader becomes stranger (x-var), with multi-symbiotic hosts (*Cornitermes bequaerti*) being generally more aggressive than the other two hosts (*Constrictotermes cyphergaster* and *Nasutitermes ephratae*). Each dot represents an experimental arena containing two groups of termites: (i) hosts and (ii) potential invaders. Host aggressiveness is defined as the proportion of aggressive behaviours relative to the total number of behaviours exhibited by hosts when meeting invaders in the arena. The dots marked with emb correspond to the invader *Embiratermes festivellus*. The degree of strangeness increases as hosts and invaders become less acquainted, as defined in Table 1.2. See statistical details in Tables 1.4 and 1.5.

Table 1.4: Models with substantial empirical evidence ($\Delta \text{AIC} \leq 2$) predicting host or invader aggressivity (y-vars) when facing each other in experimental arenas. The response variable is the proportion of aggressive behaviours exhibited by the focal partner upon contact with the other partner (*i.e.*, host $\times$ invader or vice-versa), relative to the total of behaviours exhibited by this focal partner. i = intercept; s = degree of strangeness between interactors, as in Table 1.2; h = host species identification; nh = nest origin of the host; ni = nest origin of the invader; d = absolute density experienced by termites in the experimental arena, as in Table S3; df = degrees of freedom; LogLik = log-likelihood; AICc = second-order Akaike Information Criterion (AICc); Δ = AICc difference between the best model and the focal model; Weight = Akaike weight. A colon (:) indicates an interaction between variables. Full parameterisation for final global models is given in Table 1.5.

model	df	logLik	AICc	Δ	weight
<i>yvar = host aggressiveness towards invaders</i>					
<i>global model: $y \sim s + h + s : h + nh + ni + d$</i>					
$y \sim i + h + s + h : s$	6	-66.363	147.4	0.00	0.555
$y \sim i + h + s$	4	-69.331	147.9	0.44	0.445
<i>yvar = invader aggressiveness towards hosts</i>					
<i>global model: $y \sim s + h + s : h + nh + ni + d$</i>					
$y \sim i + h + s + h : s$	6	-51.096	116.9	0.00	1.000

Table 1.5: Parameter estimates for the final models predicting host or invader aggressivity (y-vars) when facing each other in experimental arenas. Final models are derived from the models with substantial empirical evidence, as detailed in Table 1.4. Terms marked with an asterisk (*) present confidence interval ranges that do not span zero. Terms: i = intercept, here representing the host *Constrictotermes cyphergaster*; h = host id, with levels $h[\text{Cor}]$ for *Cornitermes bequaerti* and $h[\text{Nas}]$ for *Nasutitermes ephratae*; s = strangeness degree, as in Table 1.2; d = absolute density experienced by termites in the experimental arena, as in Table S3.

	Estimate	Adj_SE	CI_Lower	CI_Upper	
<i>yvar</i> = host aggressivity towards invaders					
$y \sim i + h + s + h : s$					
i	-5.843	1.125	-8.048	-3.638	*
h[Cor]	3.404	1.217	1.019	5.789	*
h[Nas]	-31.785	2817.226	-5553.549	5489.979	
s	1.014	0.325	0.378	1.650	*
h[Cor] : s	-0.284	0.370	-1.010	0.442	
h[Nas] : s	7.925	704.307	-1372.517	1388.366	
<i>yvar</i> = invader aggressivity towards hosts					
$y \sim i + h + s + h : s$					
i	-8.077	1.906	-11.813	-4.342	*
h[Cor]	5.826	1.943	2.017	9.634	*
h[Nas]	-32.534	4424.901	-8705.339	8640.271	
s	1.707	0.531	0.666	2.749	*
h[Cor] : s	-1.004	0.547	-2.077	0.069	
h[Nas] : s	8.272	1106.225	-2159.929	2176.474	

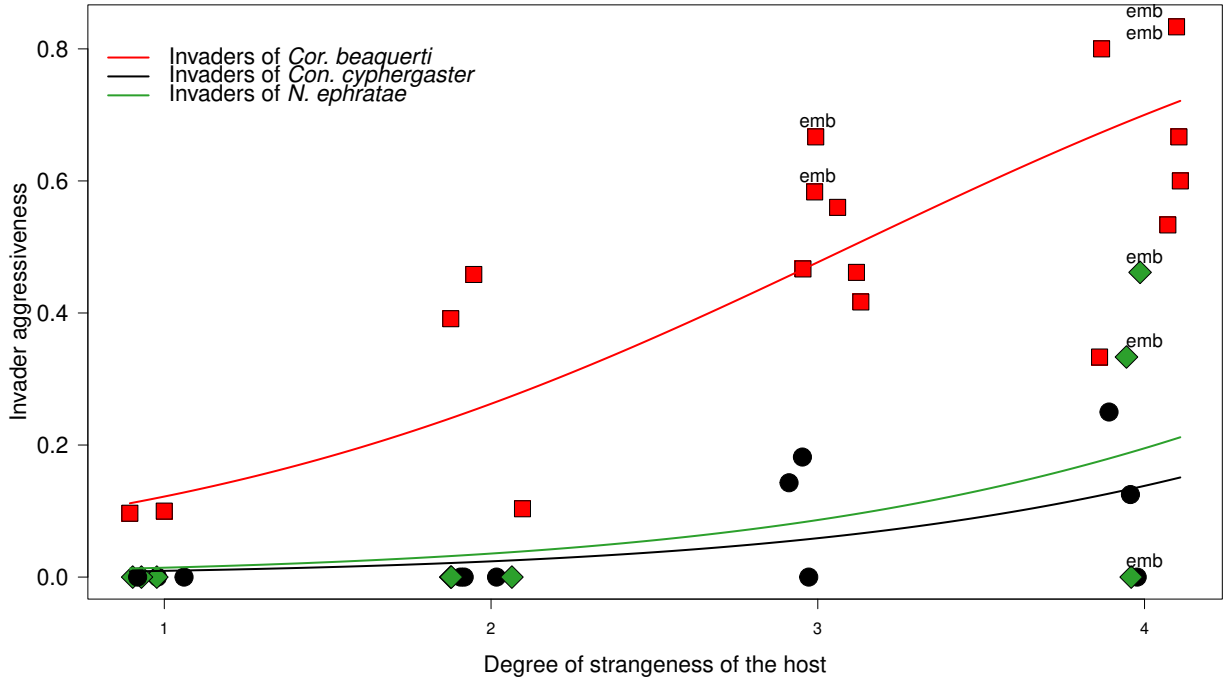


Figure 1.4: Invader aggressiveness increases as the host becomes stranger, with invaders of multi-symbiotic hosts (*Cornitermes bequaerti*) being generally more aggressive than the invaders of the other two hosts (*Constrictotermes cyphergaster* and *Nasutitermes ephratae*). Each dot represents an experimental arena containing two groups of termites: (i) hosts and (ii) potential invaders. Invader aggressiveness is defined as the proportion of aggressive behaviours relative to the total number of behaviours exhibited by invaders when meeting hosts in the arena. The degree of strangeness increases as hosts and invaders become less acquainted, as defined in Table 1.2. The dots marked with emb correspond to the invader *Embiratermes festivellus*. See statistical details in Tables 1.4 and 1.5.

1.4 Discussion

Our initial expectation was that termite hosts associated with a greater diversity of inquilines (*i.e.*, multi-symbiotic hosts) would be less aggressive towards invaders than those hosts that harbour fewer inquilines. Our results, however, contradicted this expectation. Individuals of the multi-symbiotic host *Cor. bequaerti* proved themselves consistently more aggressive than individuals of *Con. cyphergaster* and *N. ephratae*, which harbour fewer or no inquiline species at all (Fig. 1.3; Table 1.4).

This seems to indicate that the high aggressiveness of *Cor. bequaerti* is not sufficient to prevent invasions. Likewise, the lower aggressiveness of *Con. cyphergaster* and *N. ephratae* is not enough to ease multi-symbioses. In other words, the success or failure of invasions do not depend exclusively on the host's attitude towards the invader. It is plausible that other factors could help the invader establish itself.

In fact, as depicted in the hypothetical exercise of Fig. 1.1, there are more routes (4 green routes) favouring the invader in establishing the symbiotic link than routes (2 red routes) favouring the host in preventing it. Interestingly, even when the host exhibits overt aggressivity, there are two routes leading to the success of the symbiotic link. This occurs, for instance, in plant-rhizobium and plant-pathogen interactions (Soto et al. 2009), in which the invader mounts a counterattack against the host plant. In termites, too, a similar scenario occurs. Encounters between termite hosts and some staphylinid termitophiles elicit aggression by termite workers towards the beetles. These latter, in turn, stand still while termites quickly retreat, as if scared off by a substance exuded by the beetles (Kistner 1982).

Our results seem to point towards the same phenomena, closely mirroring the routes depicted in Fig. 1.1. As soon as the host detected the invader in the experimental arenas, it initiated aggressive reactions (Fig. 1.3, Table S1). Such aggressiveness was stronger when the invader was less familiar to the host (that is, it presented a higher “index of strangeness”, see M&M).

In their turn, the invaders reacted aggressively instead of fleeing the host’s attacks (Fig. 1.4, Table S2; see also Fig. 1.1). This aggressiveness on the part of the invaders can be characterised as a counterattack rather than a natural predisposition. After all, among all hosts here assayed, *Cor. bequaerti* presented the highest levels of aggressiveness (the highest curve in Fig. 1.3). Accordingly, invaders of this host also exhibited higher levels of aggressiveness than the invaders of the other hosts (Fig. 1.4). More specifically, *E. festivellus* invaders reacted more aggressively when encountering *Cor. bequaerti* hosts than when encountering *N. ephratae*. When faced with *Cor. bequaerti*, the invader’s aggressiveness rose up to approximately 0.80 (at strangeness degree 4) (Fig. 1.4, red dots marked with “emb”). Conversely, when faced with *N. ephratae* hosts, the aggressiveness of *E. festivellus* was significantly lower, reaching a maximum of 0.50 (at strangeness degree 6) (Fig. 1.4, green dots marked with “emb”).

Therefore, it is plausible to consider that invaders may retaliate against the hosts’ aggression, adjusting their own aggressiveness in response to the intensity of the host attack they encounter (Van Wilgenburg et al. 2010). Retaliation on the part of invaders has already been observed by Hugo et al. (2020) for the case of *Inquilinitermes microcerus* inquilines encountering its host *Con. cyphergaster* in experimental arenas. It seems, then, reasonable to state that the host’s attitude can shape the invader’s behaviour. In itself, this result adds a nuance to previous reports on termite inquilines acting pacifically (Florencio et al. 2013). One can now say that termite inquilines are pacific as long as they are not challenged.

Retaliation, however, seems not the only option available for the invader. If the host’s aggressiveness is triggered by the detection of the invader, then an alternative would be to act evasively. That is, the invader could act stealthily, entering the nest undetected by the host and thereby avoiding the costs of an attack. Indeed, we observed in our assays that some invaders showed no reaction when coming into contact with the host (ignore or bypass behaviours; Table S2) and, in these cases, the hosts also showed no reaction (Table S1). Thus, invaders that do not react seem to avoid being perceived by the host, ensuring a silent entry into the nest. If several invaders adopt this same strategy, multi-symbiosis could be established precisely through this evasive strategy adopted by the invader.

All in all, our results support the notion that a very aggressive host is not a guarantee against multiple inquilines in a termite nest. Host defence against invaders is often mediated by a suite of behavioural, chemical, morphological and architectural traits, rather than by aggression alone (Grüter et al. 2018). These results confirm, at the behavioural level, previous environmental-level analysis (Marins et al. 2016) on the determinants of within-termitaria biodiversity. According to this report, the presence of a builder colony has an important negative effect on within-mound termite species richness. Such an effect, however, is much smaller than the effect of the physical attributes of the mound itself.

This brings to light an alternative hypothesis for the multioccupation of *Cor. bequaerti* nests by many inquilines, as opposed to the nests of *Con. cyphergaster* and *N. ephratae*. Epigeous (that is, in full contact with the soil) nests of *Cornitermes* spp. may harbour up to twenty-six species simultaneously (Costa et al. 2009). In contrast, arboreal nests of *Con. cyphergaster* and *N. ephratae* typically contain, respectively, one inquiline species or none at all. If the physical attributes of the termitarium are more effective than the presence of its builder, invasions should succeed better in accessible as opposed to inaccessible nests. This seems to be quite in line with the notion that an arboreal nest habit should limit the contact with predators, pathogens and parasites in social insects in general (Boomsma et al. 2005) and termites in particular (Starr and Karsai 2017). The arboreal nests of *N. ephratae* and *Con. cyphergaster*, being less accessible than the epigeous nests of *Cor. bequaerti*, would be less invaded simply because they face a lower number of invasion attempts. This will greatly reduce the number of routes to symbioses (Fig. 1.1) being experienced by invaders, thereby decreasing the likelihood of successful establishment. Multi-symbioses, then, should be more likely in accessible nests as opposed to inaccessible ones.

In summary, our results indicate that it is not enough for the host to be aggressive to prevent invasion of its nest. After all, this aggressiveness can be overcome by the invader’s ability to retaliate or to evade detection. In the end, the success of multiple invasions seems to depend on a cascade of events that begins the moment the invader locates and reaches the nest. Only after this first step would the interactions between host and invader begin to determine the outcome of the invasion. Therefore, the success or failure of

an invasion depends first on the invader's ability to find and access the nest and then on the result of the host-invader interactions.

In conclusion, multi-symbioses in termitaria seem to result from a cascade of events involving nest accessibility, host aggressiveness, and invaders' retaliation or evasive behaviour. The combined interaction of these multiple factors would determine the establishment and maintenance of multi-symbiosis. Evidently, these are hypotheses that still require specific testing. We present them here as a roadmap for further efforts toward the full disclosure of the reasons behind multi-symbioses in termites and, by extension, other organisms.

1.5 Supplementary Material

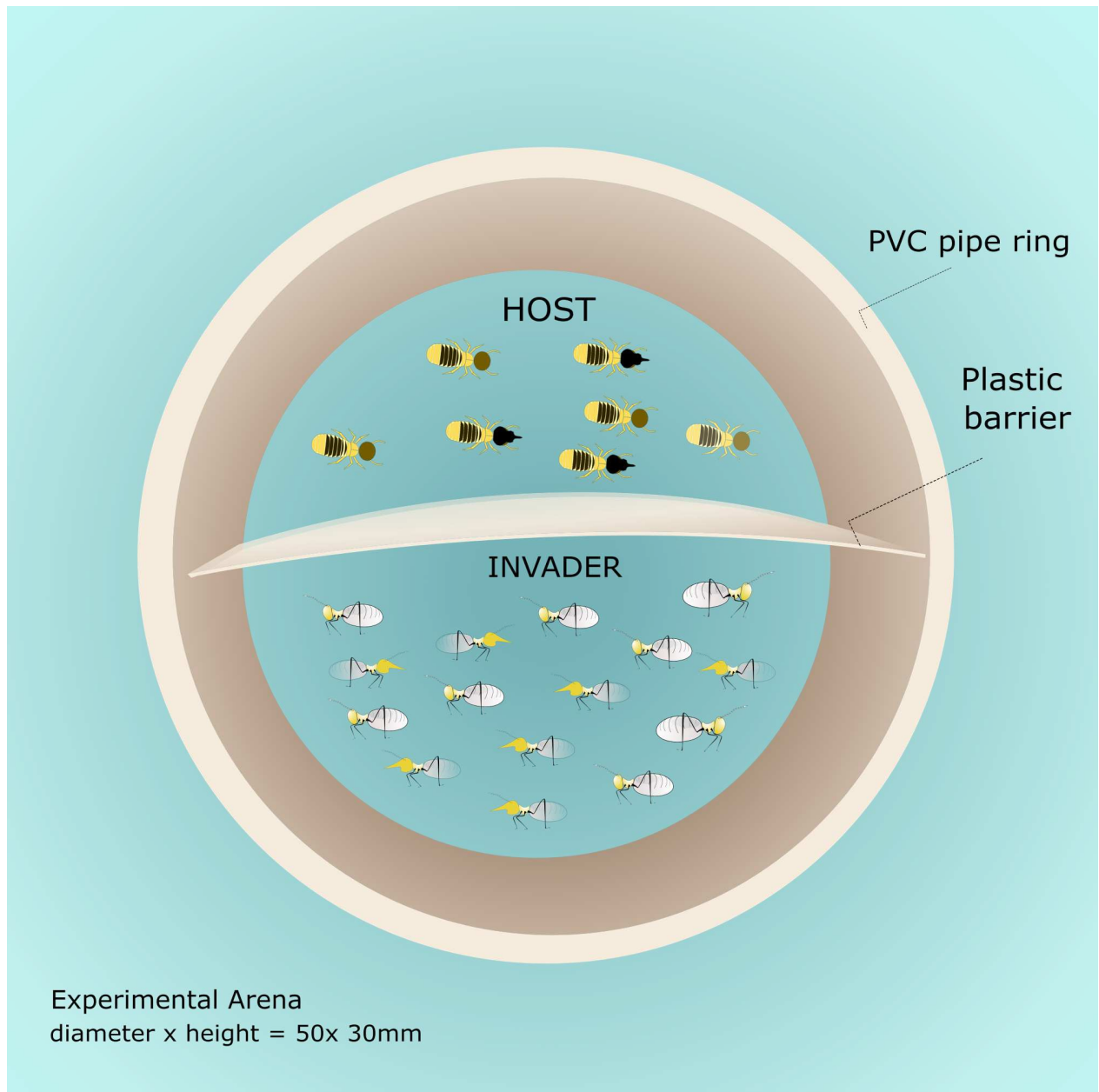


Figure S1: Schematic representation of the top view of the experimental arena containing Host and Invader.

Table S1: The number of behaviours exhibited by a host when confronted with their potential invaders in experimental arenas. For detailed descriptions of each behaviour, see 1.3. ant = antennation, byp = bypass, ign = ignore, atk = attack, grm = groom, kill = kill, oralt = oral trophallaxis, provp = provide proctodeal trophallaxis, rcvp = receive proctodeal trophallaxis, rev = reverse, vib = vibrate, tot = total.

Host	ant	byp	ign	atk	grm	kill	oralt	provp	rcvp	rev	vib	tot
<i>Con. cyphergaster</i>	116	85	24	33	0	0	1	1	0	55	4	319
<i>Cor. bequaerti</i>	93	53	9	121	0	8	3	0	2	38	6	333
<i>N. ephratae</i>	101	20	35	18	1	0	20	2	1	20	29	247

Table S2: The number of behaviours exhibited by potential invaders when confronting a host in experimental arenas. For detailed descriptions of each behaviour, see 1.3. ant = antennation, byp = bypass, ign = ignore, atk = attack, grm = groom, kill = kill, oralt = oral trophallaxis, provpproct = provide proctodeal trophallaxis, rcvp = receive proctodeal trophallaxis, rev = reverse, vib = vibrate, tot = total.

Invader	ant	byp	ign	atk	grm	oralt	prvp	rcvp	rev	vib	tot
<i>Con. cyphergaster</i>	33	23	4	0	0	0	1	0	13	0	74
<i>Cor. bequaerti</i>	15	14	0	15	0	0	0	0	2	10	56
<i>E. festivellus</i>	2	2	3	11	0	0	0	0	1	0	19
<i>Inquilinitermes</i> spp.	4	3	3	0	0	0	0	0	1	0	11
<i>Microcerotermes exiguus</i>	4	2	3	5	0	0	0	4	0	0	18
<i>Nasutitermes ephratae</i>	21	8	3	15	1	13	2	0	11	1	75
<i>Silvestritermes euamignathus</i>	4	0	1	3	0	0	0	0	0	0	9
<i>Spinitermes trispinosus</i>	4	5	2	9	0	0	0	0	7	0	27

Table S3: Number of worker and soldier termites, non-aggressive and aggressive interactions, spatial density, nest characteristics, and species identity when confined in experimental arenas. *strg*: a simpler degree of dissimilarity between individuals A x individuals B; *nestA*: identification of nest A; *nestB*: identification of nest B; *indAGen*: genus of species A; *indAEp*: specific epithet of species A; *indBGen*: genus of species B; *indBEp*: specific epithet of species B; *NonAggr*: number of non-aggressive encounters; *Aggr*: number of aggressive encounters; *density*: spatial density (*d*); *nType*: nest type (*arb* = arboreal, *epi* = epigeic); *nMat*: nest construction material (*clay* = clay/earth, *cart* = carton); *status*: status of the group in the arena (*host* = host species, *invader* = inquiline/invader species). Genus abbreviations: *corni* = *Cornitermes*; *const* = *Constrictotermes*; *embir* = *Embiratermes*; *inqui* = *Inquilinitermes*; *micro* = *Microcerotermes*; *nasuti* = *Nasutitermes*; *silve* = *Silvestritermes*; *spini* = *Spinitermes*. Specific epithet abbreviations: *bequa* = *bequaerti*; *cumul* = *cumulans*; *cyphe* = *cyphergaster*; *ephra* = *ephratae*; *euami* = *euamignathus*; *exigu* = *exiguus*; *festi* = *festivellus*; *fur* = *fur*; *micro* = *microcerus*; *trisp* = *trispinosus*.

strg	nestA	nestB	indAGen	indAEp	indBGen	indBEp	NonAggr	Aggr	density	nType	nMat	status
2	N01	N01	const	cyphe	const	cyphe	39	0	0.12	arb	clay	host
2	N01	N01	const	cyphe	const	cyphe	35	0	0.12	arb	clay	invader
2	N01	N06	const	cyphe	const	cyphe	14	0	0.12	arb	clay	host
2	N01	N06	const	cyphe	const	cyphe	17	0	0.12	arb	clay	invader
1	N01	N01	const	cyphe	const	cyphe	39	0	0.14	arb	clay	host
2	N01	N02	const	cyphe	const	cyphe	35	0	0.14	arb	clay	host
2	N01	N06	const	cyphe	const	cyphe	14	0	0.14	arb	clay	host
2	N01	N07	const	cyphe	const	cyphe	17	0	0.14	arb	clay	host
1	N02	N02	const	cyphe	const	cyphe	17	0	0.12	arb	clay	host
1	N02	N02	const	cyphe	const	cyphe	17	0	0.14	arb	clay	invader
1	N03	N03	corni	cumul	corni	cumul	28	4	0.16	epi	clay	host
1	N03	N03	corni	cumul	corni	cumul	28	4	0.16	epi	clay	invader
3	N03	N03	corni	cumul	silve	euami	5	7	0.20	epi	clay	host
3	N03	N03	corni	cumul	silve	euami	5	3	0.20	epi	clay	invader
1	N04	N04	corni	bequa	corni	bequa	28	3	0.16	epi	clay	host
1	N04	N04	corni	bequa	corni	bequa	28	3	0.20	epi	clay	invader
5	N04	N04	corni	bequa	spini	trisp	7	5	0.18	epi	clay	host
5	N04	N04	corni	bequa	spini	trisp	12	11	0.14	epi	clay	host
6	N05	N18	nasuti	ephra	embir	festi	7	6	0.19	arb	cart	invader
6	N05	N18	nasuti	ephra	embir	festi	5	5	0.19	arb	cart	host
2	N05	N21	nasuti	ephra	nasuti	ephra	23	0	0.14	arb	cart	host
2	N05	N22	nasuti	ephra	nasuti	ephra	21	0	0.14	arb	cart	host
1	N05	N05	nasuti	ephra	nasuti	ephra	27	0	0.14	arb	cart	host
1	N05	N05	nasuti	ephra	nasuti	ephra	27	0	0.14	arb	cart	invader
2	N05	N21	nasuti	ephra	nasuti	ephra	23	0	0.14	arb	cart	invader
2	N05	N22	nasuti	ephra	nasuti	ephra	21	0	0.14	arb	cart	invader
1	N06	N06	const	cyphe	const	cyphe	28	0	0.12	arb	clay	host
1	N06	N06	const	cyphe	const	cyphe	28	0	0.14	arb	clay	invader
5	N06	N06	const	cyphe	inqui	micro	27	3	0.20	arb	clay	host
5	N06	N11	const	cyphe	inqui	micro	11	4	0.20	arb	clay	host
6	N06	N12	const	cyphe	inqui	micro	16	0	0.20	arb	clay	host
5	N06	N06	const	cyphe	inqui	micro	9	0	0.19	arb	clay	invader
6	N06	N11	const	cyphe	inqui	micro	9	3	0.19	arb	clay	invader
6	N06	N12	const	cyphe	inqui	micro	7	1	0.19	arb	clay	invader
5	N11	N11	const	cyphe	inqui	fur	18	4	0.20	arb	clay	host
6	N11	N12	const	cyphe	inqui	micro	28	2	0.20	arb	clay	host
5	N11	N11	const	cyphe	inqui	fur	9	2	0.19	arb	clay	invader
6	N11	N12	const	cyphe	inqui	micro	2	0	0.19	arb	clay	invader
5	N12	N12	const	cyphe	inqui	micro	2	1	0.20	arb	clay	host
5	N12	N12	const	cyphe	inqui	micro	6	1	0.19	arb	clay	invader
1	N16	N16	corni	bequa	corni	bequa	9	1	0.16	epi	clay	host
2	N16	N25	corni	bequa	corni	bequa	13	11	0.16	epi	clay	host
2	N16	N28	corni	bequa	corni	bequa	26	3	0.16	epi	clay	host
1	N16	N16	corni	bequa	corni	bequa	9	1	0.20	epi	clay	invader
2	N16	N25	corni	bequa	corni	bequa	13	11	0.20	epi	clay	invader
2	N16	N28	corni	bequa	corni	bequa	26	3	0.20	epi	clay	invader
3	N16	N16	corni	bequa	embir	festi	5	7	0.19	epi	clay	invader
3	N16	N16	corni	bequa	embir	festi	4	6	0.20	epi	clay	host
6	N16	N25	corni	bequa	spini	trisp	10	5	0.15	epi	clay	invader
6	N16	N25	corni	bequa	spini	trisp	4	4	0.16	epi	clay	host
6	N21	N18	nasuti	ephra	embir	festi	6	3	0.19	arb	cart	invader
6	N21	N18	nasuti	ephra	embir	festi	39	3	0.19	arb	cart	host
2	N21	N22	nasuti	ephra	nasuti	ephra	26	0	0.14	arb	cart	host
1	N21	N21	nasuti	ephra	nasuti	ephra	24	0	0.14	arb	cart	host
1	N21	N21	nasuti	ephra	nasuti	ephra	24	0	0.19	arb	cart	invader
2	N21	N22	nasuti	ephra	nasuti	ephra	26	0	0.19	arb	cart	invader
6	N22	N18	nasuti	ephra	embir	festi	5	0	0.19	arb	cart	invader
6	N22	N18	nasuti	ephra	embir	festi	8	0	0.19	arb	cart	host
1	N22	N22	nasuti	ephra	nasuti	ephra	36	0	0.14	arb	cart	host
1	N22	N22	nasuti	ephra	nasuti	ephra	36	0	0.18	arb	cart	invader
3	N25	N25	corni	bequa	embir	festi	3	6	0.15	epi	clay	invader
3	N25	N25	corni	bequa	embir	festi	13	1	0.20	epi	clay	host
3	N25	N25	corni	bequa	silve	euami	7	2	0.20	epi	clay	host
3	N25	N25	corni	bequa	silve	euami	8	7	0.18	epi	clay	invader
5	N25	N25	corni	bequa	spini	trisp	14	12	0.18	epi	clay	invader
5	N25	N25	corni	bequa	spini	trisp	8	4	0.14	epi	clay	host
2	N28	N30	corni	bequa	corni	bequa	28	18	0.16	epi	clay	host

Continued on next page...

Table S3 – continued from previous page

strg	nestA	nestB	indAGen	indAEp	indBGen	indBEp	NonAggr	Aggr	density	nType	nMat	status
2	N28	N30	corni	bequa	corni	bequa	28	18	0.15	epi	clay	invader
4	N28	N30	corni	bequa	embir	festi	2	8	0.20	epi	clay	invader
4	N28	N30	corni	bequa	embir	festi	1	4	0.20	epi	clay	host
4	N28	N25	corni	bequa	silve	euami	0	2	0.20	epi	clay	host
4	N28	N25	corni	bequa	silve	euami	1	2	0.20	epi	clay	invader
5	N28	N28	corni	bequa	spini	trisp	11	14	0.18	epi	clay	invader
6	N28	N25	corni	bequa	spini	trisp	7	8	0.18	epi	clay	invader
5	N28	N28	corni	bequa	spini	trisp	0	5	0.14	epi	clay	host
6	N28	N25	corni	bequa	spini	trisp	6	6	0.16	epi	clay	host
4	N30	N28	corni	bequa	embir	festi	1	5	0.20	epi	clay	invader
4	N30	N28	corni	bequa	embir	festi	7	8	0.20	epi	clay	host
6	N30	N28	corni	bequa	spini	trisp	10	15	0.18	epi	clay	invader
6	N30	N28	corni	bequa	spini	trisp	3	11	0.16	epi	clay	host

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Geographic variation in host-guest aggressiveness among *Cornitermes* populations

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Proximate drivers of multi-symbioses

Abstract

Why do individuals of the same host species differ in their defensive responses towards potential invaders? Although aggression can protect termite nests against heterospecific intruders, defensive responses may vary according to the ecological context in which hosts interact with potential nest invaders. We tested the hypothesis that geographic variation in host aggressiveness is associated with differences in opportunities for interactions with potential inquilines. To do so, we compared the aggressive responses of *Cornitermes cumulans* individuals from two geographically distinct regions: Nova Xavantina (Mato Grosso) and Viçosa (Minas Gerais). We then evaluated whether these regions differed in inquiline richness and in the occurrence of host–inquiline co-occurrence within nests, two ecological patterns consistent with variation in opportunities for host–inquiline interactions. Finally, we compared the aggression of *Cornitermes bequaerti* individuals from Nova Xavantina to determine whether behavioral differences reflected the geographic context rather than the host species identity. Contrary to the expectation that aggressiveness would be mainly determined by host identity, *C. cumulans* individuals from the two regions differed in their aggressive responses. Nova Xavantina also showed greater inquiline richness and a higher frequency of simultaneous host–inquiline occurrence, suggesting that hosts from this region experience a different ecological context, with potentially more opportunities for interactions with heterospecific organisms. Moreover, the similar responses of *C. cumulans* and *C. bequaerti* individuals from Nova Xavantina indicate that behavioural differences are more consistent with regional context than with host species identity. Together, these results suggest that host defensive responses are flexible and may vary according to the ecological context in which hosts interact with heterospecific organisms in termite nests.

Keywords: termite behaviour; defensive response; cohabitation; symbiosis.

2.1 Introduction

Animals often exhibit some form of aggression when encountering each other (Tanner and Adler 2009; Van Wilgenburg et al. 2009). Such aggression can manifest in different forms, including physical confrontations, vocalizations, and threatening displays, and can serve multiple adaptive functions (Frommen 2020). An important function of aggression is the acquisition and defense of resources (Boiten et al. 2023; Peiman and Robinson 2010). Because access to resources can attract other individuals, aggression may also be directed towards individuals attempting to gain access to them.

Social insects are no exception in this regard: they also defend their nests against invaders (Shorter and Rueppell 2012; Kleineidam et al. 2017; Grüter et al. 2018). After all, the nests of social insects in general, and termites in particular, provide plenty of nutrients and a stable microclimate, being highly attractive to usurping organisms (Redford 1984; Korb and Linsenmair 2000; de Oliveira et al. 2016; Parmentier 2021; Tuma et al. 2025). Once such invaders are detected entering a termitarium, they should be perceived as a threat by the resident termites who built that given nest.

An individual's propensity to react to threats is often affected by a suite of factors, varying from environmental context to genetic propensity (Boissy 1995; Ishikawa and Miura 2012; Parmentier et al. 2015). Among these factors, prior experience in an aggressive context affects an animal's propensity to fight (Hsu et al. 2006). Such experience can modify subsequent aggressive responses, so that previous encounters may affect how an individual responds to later challenges. In the Argentine ant (*Linepithema humile*) this propensity to fight can be triggered by the mere exposure to an opponent, with no need for this encounter to escalate to a fight (Van Wilgenburg et al. 2009).

As is the case with ants, termite nests are also invaded by a wide variety of invertebrates (Dejean et al. 1997; Costa et al. 2009; Monteiro et al. 2017; Pires-Silva et al. 2021). Termites also engage in conflicts with such organisms (Marins and DeSouza 2008; Ishikawa and Miura 2012; Yang et al. 2025). However, defensive responses may depend not only on the immediate characteristics of opponents but also on prior interactions with potential threats. Therefore, if prior experience with interindividual conflicts affect termites similarly, differences in opportunities for encounters with potential invaders could lead to variation in defensive responses among host termites.

Here, we test this hypothesis by comparing the aggressive responses of *Cornitermes cumulans* individuals when facing potential nest invaders in experimental arenas. Assays involved contestants collected in two distinct regions differing by 6 degrees of latitude and markedly differing in termite species richness. As an independent test of whether behavioural differences were associated with regional context rather than host species identity, we further compared the aggressiveness of *C. bequaerti*, a congeneric host occurring in the same region as the more aggressive *C. cumulans* individuals.

2.2 Materials and Methods

2.2.1 Rationale

We investigated whether the termite host *Cornitermes cumulans* (see Section 2.2.5), exposed to different local contexts, differed in its aggressiveness towards potential invaders. To test this, we compared *C. cumulans* individuals collected from nests across two geographically distinct regions located 1,585 km apart. These regions differed in termite species richness, which may reflect differences in the diversity of potential heterospecific interactions available to hosts. Regions with higher termite species richness may harbour a greater diversity of potential invaders, increasing opportunities for hosts to encounter heterospecific organisms. Such differences in encounter opportunities may expose hosts to a broader range of stimuli and potentially contribute to variation in defensive responses.

Our hypothesis would find support if hosts from the termite species-rich region were consistently more aggressive than hosts from the species-poor region. Of course, factors other than species richness could lead to differences in host aggressivity between regions. To address this, we devised three further tests. First, we quantified, in each region, its inquiline species richness and the co-occurrence of hosts and inquilines in the same nest. Inquiline species richness represents the diversity of potential heterospecific interactors and thence potential threatening pressure to the host. Host-inquiline co-occurrence in a single nest estimates

the actual opportunity for host-guest interaction, providing hints about the pressure a host suffers from invaders.

Finally, we measured the aggressiveness of the congeneric host species *Cornitermes bequaerti*, which occurs in the inquiline species-rich region. This comparison allowed us to evaluate whether the behavioural pattern observed in *C. cumulans* was associated with regional context rather than host species identity. Under our hypothesis, *C. bequaerti* from the inquiline species-rich region should show aggressive responses more similar to those of *C. cumulans* from the same region than to those of *C. cumulans* from the inquiline species-poor region. Conversely, a similarity between *C. bequaerti* and *C. cumulans* from the inquiline species-poor region would weaken the hypothesis that regional context contributes to variation in host aggressiveness.

2.2.2 Ethics statement

All necessary permits were obtained for the described study, which complied with all relevant regulations of Brazil. This includes collecting and transportation permits (#SISBIO 94569) from IBAMA (The Brazilian Institute for the Environment and Renewable Natural Resources) as well as tacit approval from the Brazilian Federal Government implied by granting the authors the post of Scientific Researcher.

2.2.3 Terms definition

We adopt De Bary's definition of symbiosis as "the living together of unlike organisms" (De Bary 1879). Termites are monophyletic social cockroaches (Blattodea) belonging to the Infra-order Isoptera (Krishna et al. 2013). The terms "termitarium" (plural: termitaria), "mound", or "nest" denote the physical structure built by termites. A "colony" denotes a group of genetically related termites of the same species that share a nest and live together in an organised structure. In this study, a "host" refers to a termite species that constructs and occupies a nest, whereas an "inquiline" refers to a termite species that inhabits a nest built by another termite species.

2.2.4 Study site and collection

We collected termite nests from two distinct study areas in Brazil, belonging to the Atlantic Forest and Cerrado biomes, respectively. The first sampling site was the Mário Viana Municipal Biological Reserve (RB-MMV), located within the Universidade do Estado de Mato Grosso (UNEMAT), in Nova Xavantina, Mato Grosso, Brazil (14°43'S, 52°21'W). The reserve is situated within the Cerrado biome, covers approximately 500 hectares, and has an average altitude of 346 metres above sea level (Marimon et al. 2010). According to the Köppen classification, the regional climate is Aw, with annual precipitation ranging from 1300 to 1500 mm and a mean annual temperature of approximately 25°C, as recorded by the local climatological station in Nova Xavantina. A total of 6 termite nests were collected in this site.

The second study site was an urban fragment of the Atlantic Forest biome, known as "Mata da Biologia", located at the Universidade Federal de Viçosa (UFV), in the municipality of Viçosa, Minas Gerais, southeastern Brazil (20°45'S, 42°51'W). The site lies within the Atlantic Forest biome and comprises a mosaic of forest formations, with the sampled area characterised as Semideciduous Seasonal Forest. The total area of the fragment is approximately 75 hectares, with elevation ranging from 725 to 745 m above sea level. The regional climate is classified as Cwb (Köppen), characterised by mild temperatures, hot and rainy summers, and cool, dry winters (Miranda Neto et al. 2012; Camargos et al. 2013). Mean annual precipitation and temperature are 1,314 mm and 21.8°C (Almeida et al. 2016). The Viçosa region lies at 670–730 m a.s.l. and is characterised by a mountainous topography (Rocha et al. 2020). A total of 7 termite nests were collected in this site.

The latitude difference between Nova Xavantina, MT (approx. 14°40'24"S) and Viçosa, MG (approx. 20°45'14"S) is about 6 degrees and 5 minutes (6.08°), which corresponds to a north-south distance of roughly 675 km. This difference in latitude results in marked differences in mean annual temperature, with Nova Xavantina averaging approximately 24°C and Viçosa approaching 19°C. Cancellato et al. (2014) observed that north-to-south increments in latitude and decrements in temperature resulted in decreasing termite richness in Atlantic Forest biome in Brazil.

The nests collected for the behavioural assays were transported to the laboratory, where they were maintained under ambient conditions for up to seven days. Food (paper fragments and grasses) and water were provided to prevent termite desiccation until the experiments were conducted. Soldier specimens were collected for taxonomic identification and deposited as voucher specimens in the Termitology Section of the Entomological Museum of the Universidade Federal de Viçosa.

To investigate whether geographical differences in aggressiveness were associated with variation in the potential for interspecific interactions, an additional dataset comprising 42 *C. cumulans* nests from Nova Xavantina and 36 from Viçosa was examined. Nests from Nova Xavantina were opened at the Universidade do Estado de Mato Grosso (UNEMAT), whereas those from Viçosa were opened at the Universidade Federal de Viçosa (UFV). In each nest, the presence or absence of host and inquiline species was recorded to quantify inquiline species richness per host nest and host-inquiline co-occurrence frequency.

2.2.5 Biological models

The biological models used in this study were the mound-building termites *Cornitermes cumulans* and *Cornitermes bequaerti*, both of which are known to harbour a diverse assemblage of inquiline termites (DeSouza et al. 2025). Behavioural assays focused on *C. cumulans* from Viçosa (MG) and Nova Xavantina (MT), with *C. bequaerti* from Nova Xavantina included as a congeneric comparison. In addition, ecological analyses of inquiline species richness and host-inquiline co-occurrence were conducted using *C. cumulans* nests from both localities.

2.2.6 Nest processing and inspection

To quantify inquiline species richness and host-inquiline co-occurrence, a total of 78 *C. cumulans* nests were examined, comprising 42 nests from Nova Xavantina (MT) and 36 nests from Viçosa (MG). Each nest was carefully opened and systematically divided into multiple fragments, which were individually inspected on white trays to maximise the detection of host and inquiline species. For each nest, the presence or absence of the host species and of each inquiline species was recorded.

2.2.7 Experimental Setup

The first stage of this study consisted of behavioural assays designed to compare aggressiveness among host populations. The focal host species included *C. cumulans* populations from Viçosa (MG) and Nova Xavantina (MT), together with *C. bequaerti* from Nova Xavantina. These hosts were paired with heterospecific inquiline termite species recorded in the study areas. Individuals were collected either from their colonies of origin or from nests of other species included in the assays (Table 2.1).

Behavioural assays were conducted in standardised circular arenas (PVC rings; 50 mm diameter, 3 cm height) placed on sand-blasted glass bases and covered with transparent glass plates (Fig. 2.2). All arenas were cleaned before use with detergent and running water to remove chemical residues. A removable partition separated the groups before each trial and was removed immediately prior to the beginning of the interaction.

Group size was standardised to maximise encounter probability while maintaining 14–20% arena coverage, a range known to enhance interaction rates under confined conditions (Miramontes and DeSouza 1996; DeSouza and Miramontes 2004). Worker and soldier proportions followed published descriptions or field subsamples when necessary. Each treatment was replicated three times per nest. Experimental arenas represented a gradient of interspecific "strangeness" between hosts and inquilines (Table 2.1), with each arena comprising two interacting groups.

2.2.8 Observational protocol

Behavioural assays were video-recorded to quantify interindividual interactions within each experimental arena during the first stage of the study. A single 5-minute recording was obtained per arena, resulting in 34 videos. Prior to recording, termites were allowed to acclimatise in the arenas for approximately 30 minutes.

Table 2.1: Degrees of interaction strangeness used in the behavioural assays. Increasing values represent increasing ecological and phylogenetic dissimilarity between interacting termites.

Degree	Description
1	Same nest, same species
2	Different nest, same species
3	Same nest, different species and same subfamily
4	Different nest, different species and same subfamily
5	Same nest, different species and different subfamily
6	Different nest, different species and different subfamily
7*	Outgroup

* Degree 7 represents an outgroup consisting of a termite species not recorded as an inquiline within the *Cornitermes*-associated system. By selecting a species with no documented history of co-occurrence with the focal hosts, we established a reference representing maximum ecological and phylogenetic dissimilarity.

After acclimatisation, the plastic partition was removed, allowing individuals from both experimental groups to interact freely. All recordings were analysed using BORIS software (Friard and Gamba 2016). To ensure consistency in behavioural scoring, all videos were analysed by a single observer.

Observations focused on worker-worker interactions, as this caste consistently showed the highest frequency of contacts across treatments involving *C. cumulans* (Viçosa and Nova Xavantina) and *C. bequaerti* (Nova Xavantina). An interindividual contact was defined as an encounter in which the distance between workers did not exceed two antenna lengths (Paiva et al. 2021). The behavioural events extracted using BORIS were quantified for each video and subsequently used to assess host aggressiveness (see subsection 2.2.9).

2.2.9 Data analysis

The behavioural analyses revealed that *Cornitermes cumulans* from Nova Xavantina (ccuMT) was more aggressive than *C. cumulans* from Viçosa (ccuMG). This result motivated the hypothesis that differences in aggressiveness are associated with variation in the potential for interspecific interactions. To investigate whether this difference in aggressiveness could be associated with variation in the potential for interspecific interactions, we quantified two indirect ecological proxies: (i) inquiline termite species richness per host nest and (ii) host-inquiline co-occurrence frequency.

Behavioural analysis

We investigated whether aggressiveness varied according to the degree of familiarity between termites from two different regions (see Section 2.2.5) using Generalised Linear Models (GLMs) with binomial error distribution and logit link function.

For each experimental arena, the response variable was defined as the proportion of aggressive behaviours recorded during the observation period:

$$p = \frac{compAggr}{compTot} \quad (2.1)$$

where compAggr is the number of aggressive behaviours and compTot is the total number of behavioural interactions observed. Models were fitted using compTot as binomial weights.

The explanatory variables were familiarity between interacting individuals (gradenr) and host population (hostLocal). An interaction between both explanatory variables was included:

$$p \sim gradenr * hostLocal \quad (2.2)$$

Model simplification was performed by collapsing levels within `hostLocal`. Competing models were compared using Akaike's Information Criterion (AIC) and analyses of deviance (Chi-square tests), and the most parsimonious model was retained.

Inquiline species richness

To estimate the potential for interspecific interactions, we quantified the richness of inquiline termite species per host nest. Mean inquiline richness was calculated as

$$\bar{s} = \frac{\sum s_i}{N} \quad (2.3)$$

where s_i is the number of inquiline species recorded in the i th host nest and N is the total number of host nests sampled, including nests without inquilines (i.e. $s = 0$).

To test whether inquiline richness differed between localities, we fitted a Generalised Linear Model (GLM) with Poisson error distribution and log link function:

$$s \sim \text{HostLocal} \quad (2.4)$$

Only host nests were included in this analysis; therefore, nests occupied exclusively by inquilines were excluded before model fitting. Model estimates were back-transformed using the exponential function to obtain the estimated mean richness per host nest. Model adequacy was assessed by comparing residual deviance with residual degrees of freedom.

Host-inquiline co-occurrence

As a second test for the potential for interspecific interactions, each host nest was classified into one of three mutually exclusive occupancy categories: (i) host only, (ii) inquiline only, or (iii) host and inquiline co-occurring.

Differences in occupancy frequencies between localities were evaluated using Pearson's chi-squared test based on contingency tables. Percentages were calculated relative to the total number of host nests sampled in each locality.

All analyses were conducted in R version 4.6.0 (R Core Team 2026).

2.3 Results

Our results (Table 2.2) revealed that hosts from the same geographical region did not differ in aggressiveness, even if they belonged to distinct species (*C. cumulans* × *C. bequaerti* from Nova Xavantina). However, hosts did differ in aggressiveness if they belonged to the same species but were located in a distinct geographical region ((*C. cumulans* from Viçosa (MG) × *C. cumulans* from Nova Xavantina (MT)) (Table 2.3)

Consistent with this pattern, inquiline termite species richness per host nest differed between the two *C. cumulans* localities (Table 2.4). The estimated richness was 2.032 inquiline species per nest for *C. cumulans* from Nova Xavantina (ccuMT), whereas *C. cumulans* from Viçosa (ccuMG) had an estimated richness of 0.304 species per nest (Figure 2.1). Thus, inquiline richness was approximately seven times higher in ccuMT than in ccuMG nests (GLM with Poisson error distribution: $\beta = 1.899 \pm 0.398$ SE, $z = 4.766$, $P < 0.001$).

Host-inquiline co-occurrence also differed significantly between the two *C. cumulans* localities (Pearson's $\chi^2 = 17.10$, $df = 2$, $P < 0.001$). Co-occurrence was more frequent in Nova Xavantina (ccuMT) than in Viçosa (ccuMG) (Table 2.5).

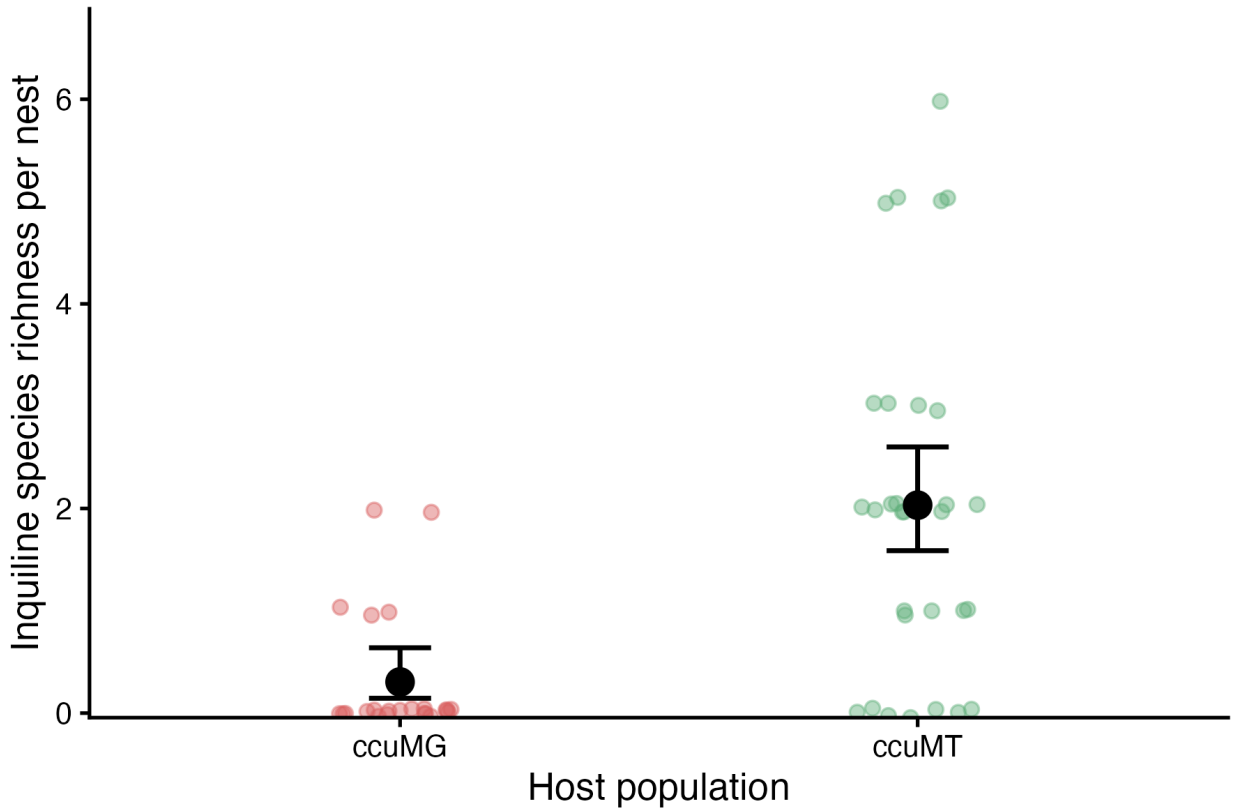


Figure 2.1: Inquiline termite species richness per nest in *C. cumulans* populations from Viçosa (Minas Gerais) and Nova Xavantina (Mato Grosso). Points represent observed values for individual nests. Black points and error bars represent model-estimated mean richness and 95% confidence intervals, respectively, from a Poisson generalized linear model.

Table 2.2: Parameter estimates from the binomial GLM testing the effect of host origin ($xvar = \text{hostLocal}$) on aggressiveness. Aggressiveness was measured as the proportion of aggressive behaviours ($yvar = \text{compAggr} / \text{compTot}$). HostLocal comprised three levels: *cbeqMT* (*C. bequaerti*, Mato Grosso), *ccuMT* (*C. cumulans*, Mato Grosso), and *ccuMG* (*C. cumulans*, Viçosa, Minas Gerais).

Term	Estimate	Std. Error	z value	$Pr(> z)$
(Intercept)	-0.4353	0.1224	-3.558	0.000374
hostLocalccuMG	-1.4224	0.2031	-7.004	2.49e-12
hostLocalccuMT	-0.3150	0.3187	-0.988	0.322992

Table 2.3: Alternative models used to compare host aggressiveness among three host groups. The explanatory variable `hostLocal` comprised three levels: *Cornitermes bequaerti* from Nova Xavantina (cbeqMT), *Cornitermes cumulans* from Viçosa (ccuMG), and *C. cumulans* from Nova Xavantina (ccuMT). In each model, host groups enclosed in square brackets "[...]" were combined into a single category for statistical comparison.

Model	df	AIC
Response = proportion of aggressive behaviours by the host		
m1: $y \sim \text{hostLocal}$ (cbeqMT, ccuMG, ccuMT)	3	272.57
m1a: $y \sim \text{hostLocal}$ ([ccuMG, ccuMT], cbeqMT)	2	280.51
m1b: $y \sim \text{hostLocal}$ ([ccuMT, cbeqMT], ccuMG)	2	271.57

Table 2.4: Estimated mean richness of inquiline termite species per *Cornitermes cumulans* host nest across the two studied localities. Estimated richness values represent back-transformed predictions from the model (log link). Abbreviations: *ccuMT* = *C. cumulans* in Nova Xavantina (MT); *ccuMG* = *C. cumulans* in Viçosa (MG).

HostLocal	Estimate	SE	z	P	Estimated richness
ccuMG	-1.1896	0.3780	-3.147	0.00165	0.304
ccuMT	1.8987	0.3984	4.766	1.88×10^{-6}	2.032

Table 2.5: Proportion of *Cornitermes cumulans* nests occupied solely by the host, solely by inquilines, or co-occupied by both across the two studied localities (%). Percentages are calculated relative to the total number of nests recorded (n) within each locality. Abbreviations: *ccuMT* = *C. cumulans* in Nova Xavantina (MT); *ccuMG* = *C. cumulans* in Viçosa (MG).

HostLocal (n)	Host only	Inquiline only	Host + Inquiline
ccuMT ($n = 42$)	16.7%	26.2%	57.1%
ccuMG ($n = 36$)	50.0%	36.1%	13.9%

2.4 Discussion

Individuals of *Cornitermes cumulans* from Viçosa and Nova Xavantina differed in aggressiveness, whereas *C. cumulans* and *C. bequaerti* individuals from Nova Xavantina showed similar levels of aggressiveness (See. Tables 2.2 and 2.4). If aggressiveness were mainly determined by host identity, individuals belonging to the same host species should respond more similarly across regions than individuals from different host species within the same region. The observed pattern does not support host identity as the main determinant of aggressiveness. Instead, it suggests that local context, as represented by distinct geographic regions, may contribute to differences in host aggressive responses.

One possible explanation for such context-dependent differences is that defensive responses are not fixed traits, but flexible behaviours that can be adjusted according to the perceived threat posed by unfamiliar individuals (Ishikawa and Miura 2012; Parmentier et al. 2015; de Bruijn et al. 2016). In insects, aggression may vary depending on the context of encounters and the characteristics of the opponent, allowing individuals to modulate their responses according to the circumstances in which interactions occur (Parmentier et al. 2015). Therefore, individuals exposed to opportunities for encounters with different heterospecific species may differ in how they assess unfamiliar individuals and in the intensity of their defensive responses.

One mechanism that may contribute to such variation is previous experience with potential opponents. Previous encounters or information about impending threats can alter subsequent defensive responses in

social insects (Hsu et al. 2006; Van Wilgenburg et al. 2009; Kleeberg et al. 2014). In particular, colonies may increase aggression after previous exposure to antagonistic interactions, suggesting that defensive responses can be adjusted according to the opportunity of encounters with potential opponents. Thus, individuals or colonies experiencing different encounter may differ in their subsequent aggressive responses. If previous experience contributes to shaping defensive responses, differences among hosts should depend on the opportunities available for such experiences to accumulate, as observed in social insects (Hsu et al. 2006; Van Wilgenburg et al. 2009). However, such experience can only accumulate through encounters with potential opponents. Consequently, regional differences in encounter opportunities may lead to differences in previous experience among hosts, contributing to the differences in aggressiveness observed between regions.

In the termite system investigated here, such encounter opportunities may arise from interactions with different heterospecific individuals occupying host nests. Termite nests are occupied or invaded by heterospecific individuals, including inquilines (Costa et al. 2009; Pires-Silva et al. 2021), and termites respond defensively when heterospecific individuals enter or occupy their nests (Marins et al. 2016; Parmentier 2021; Yang et al. 2025). Therefore, host-inquiline associations may represent recurrent contexts in which hosts encounter heterospecific individuals and evaluate them as potential threats, providing opportunities for previous experience to accumulate.

Ecological data provide indirect evidence that the potential for host-inquiline encounters differed between regions. Inquiline richness was higher in Nova Xavantina than in Viçosa, and host-inquiline co-occurrence was also more frequent in this region (Figure 2.1; Table 2.5). These ecological patterns occurred in the same direction as the behavioural differences observed in *C. cumulans*, with individuals from Nova Xavantina showing greater aggressiveness than those from Viçosa (Table 2.2). Together, these results are consistent with the hypothesis that host-inquiline associations represent recurrent contexts in which hosts encounter heterospecific individuals and evaluate them as potential threats, thereby providing opportunities for interspecific interactions. Consequently, geographic variation in these ecological pressures helps explain the divergence in behavioural responses across populations.

This interpretation is further supported because individuals of *C. bequaerti* from Nova Xavantina showed aggressiveness similar to that of *C. cumulans* individuals from the same region, whereas *C. cumulans* individuals from Nova Xavantina were more aggressive than those from Viçosa (Table 2.2). By providing an independent test of whether the behavioural pattern is restricted to *C. cumulans*, this comparison demonstrates that the similarity between different host species within the same region contrasts with differences between individuals of the same species from different regions. Consequently, these patterns strengthen the interpretation that the behavioural response is associated with local context rather than being a species-specific restriction to *C. cumulans*.

Although these ecological patterns support a role for encounter opportunities, inquiline richness and host-inquiline co-occurrence provide only indirect measures of such interactions. They do not quantify the frequency of individual encounters or the experience accumulated by hosts. Nevertheless, their concordance with behavioural differences provides convergent evidence consistent with a role of encounter opportunities in shaping host aggressiveness. Beyond the occurrence of potential inquilines, encounter opportunities may also depend on the conditions in which hosts and heterospecific organisms interact. Physical characteristics of termite nests may affect the establishment and distribution of associated organisms within termitaria (Costa et al. 2009, 2019). In addition, environmental conditions may affect the occurrence and composition of heterospecific organisms associated with termite nests (Monteiro et al. 2017). These factors were not evaluated here, but may contribute to explaining regional differences in encounter opportunities and host aggressiveness.

2.5 Supplementary material

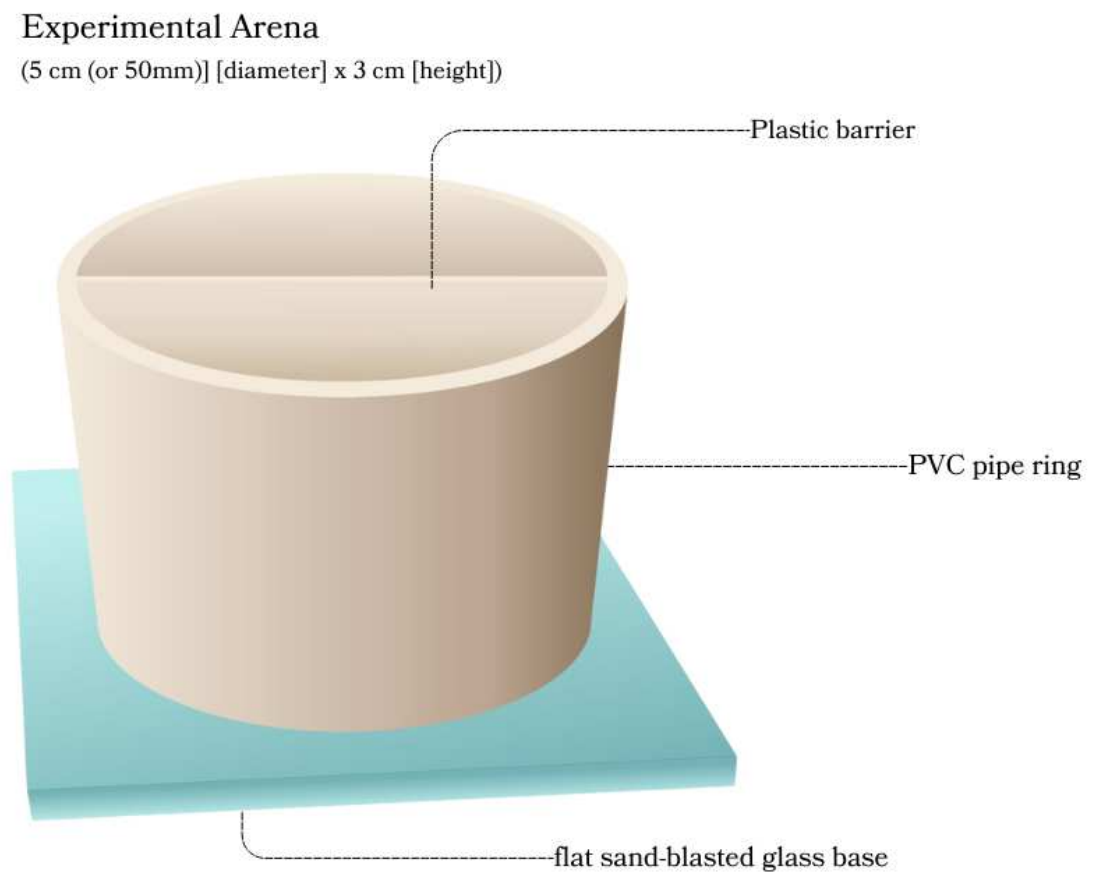


Figure 2.2: Experimental arena used for termite behavioural assays. Components of the arena, including a PVC ring (5 cm external diameter $\times$ 3 cm height), a transparent plastic barrier used during acclimation, and a sand-blasted glass base.

Table 2.6: Number of worker and soldier termites and the area they occupied when confined in experimental arenas, and the resulting spatial density. *arena*: arena identification; *nestH*: host nest identification; *nestI*: inquiline nest identification; *sphost*: host species; *spinq*: inquiline species; *grade*: The grade (1–7) indicates how much sphost and spinq differ, from 1 (very similar) to 7 (very different); *nHostW*: number of host workers; *nHostS*: number of host soldiers; *nInqW*: number of inquiline workers; *nInqS*: number of inquiline soldiers; *aHost*: area occupied by the hosts; *aInq*: area occupied by the inquilines; *aFloor*: available floor area of the arena; *density*: spatial density (d) defined as $d = aHost$ or $aInq/aFloor$. Areas in mm². Species codes: mex= *Microcerotermes exiguus*; cbeMT = *Cornitermes bequaerti* Mato Grosso; ccuMT = *Cornitermes cumulans* Mato Grosso; ccuMG = *Cornitermes cumulans* Minas Gerais; efe = *Embiratermes festivellus*; nkm = *Nasutitermes kemneri*; nep = *Nasutitermes ephratae*; str = *Spinitermes trispinosus*; seu = *Silvestritermes euamignathus*.

arena	nestH	nestI	sphost	spinq	grade	nHostW	nHostS	nInqW	nInqS	aHost	aInq	aFloor	density
a01	n01	nxx	ccuMG	ccuMG	2	13	1	13	1	204.9	204.9	1962.5	0.19
a02	n01	n01	ccuMG	ccuMG	1	15	1	14	1	47.6	343.9	1962.5	0.19
a03	n01	n10	ccuMG	seu	4	15	1	15	1	290.5	137.7	1962.5	0.18
a04	n01	n07	ccuMG	nep	7	13	1	8	2	204.9	120.6	1962.5	0.20
a05	n03	n10	ccuMG	seu	4	15	1	15	1	204.9	197.6	1962.5	0.19
a06	n04	n04	ccuMG	ccuMG	1	13	1	13	1	204.9	204.9	1962.5	0.20
a07	n04	n07	ccuMG	nep	7	13	1	8	4	204.9	120.6	1962.5	0.19
a08	n05	nxx	ccuMG	ccuMG	2	13	1	13	1	204.9	204.9	1962.5	0.20
a09	n05	n05	ccuMG	ccuMG	1	13	1	13	1	204.9	204.9	1962.5	0.20
a10	n05	n07	ccuMG	nep	7	13	1	8	8	204.9	120.6	1962.5	0.19
a11	n06	nxx	ccuMG	ccuMG	2	13	1	13	1	204.9	204.9	1962.5	0.20
a12	n06	n10	ccuMG	seu	4	15	1	15	1	290.5	137.7	1962.5	0.18
a13	n13	n21	cbeMT	nkm	7	13	1	8	2	204.9	120.6	1962.5	0.19
a14	n16	n16	cbeMT	cbeMT	1	13	1	13	1	204.9	204.9	1962.5	0.20
a15	n16	n16	cbeMT	emb	3	15	1	15	1	219	167	1962.5	0.19
a16	n16	n22	cbeMT	nkm	7	13	1	8	2	204.9	120.6	1962.5	0.20
a17	n16	n25	cbeMT	cbeMT	1	8	2	8	2	120.6	120.6	1962.5	0.19
a18	n16	n25	cbeMT	str	3	14	1	12	1	204.9	204.9	1962.5	0.15
a19	n16	n28	cbeMT	cbeMT	2	13	3	13	1	204.9	204.9	1962.5	0.20
a20	n25	n25	cbeMT	emb	3	15	1	15	1	219	167	1962.5	0.15
a21	n25	n25	cbeMT	seu	3	15	1	15	1	290.5	137.7	1962.5	0.18
a22	n25	n25	cbeMT	seu	5	15	1	15	1	290.5	137.7	1962.5	0.18
a23	n28	n28	cbeMT	str	5	13	1	13	1	219.2	89.4	1962.5	0.18
a24	n28	n25	cbeMT	seu	4	15	1	15	1	290.5	137.7	1962.5	0.20
a25	n28	n25	cbeMT	str	6	13	1	13	1	204.9	204.9	1962.5	0.15
a26	n28	n30	cbeMT	emb	4	15	1	15	1	219	167	1962.5	0.15
a27	n28	n30	cbeMT	cbeMT	4	13	1	13	1	204.9	197.6	1962.5	0.20
a28	n30	n28	cbeMT	emb	4	15	1	15	1	219	167	1962.5	0.19
a29	n30	n28	cbeMT	str	6	13	1	13	1	204.9	204.9	1962.5	0.15
a30	n03	n03	ccuMT	ccuMT	1	13	1	13	1	204.9	204.9	1962.5	0.20
a31	n03	n03	ccuMT	seu	3	15	1	15	1	290.5	137.7	1962.5	0.20
a32	n03	n05	ccuMT	nkm	7	13	1	8	2	204.9	120.6	1962.5	0.19
a33	n04	n04	cbeMT	cbeMT	1	13	1	13	1	204.9	204.9	1962.5	0.18
a34	n05	n05	cbeMT	str	5	4	1	28	1	67.8	299.6	1962.5	0.19
totals						453	37	444	49				

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