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MECANISMOS DETERMINANTES DA AGRESSIVIDADE
INTERCOLONIAL EM CUPIM

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2018

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**MECANISMOS DETERMINANTES DA AGRESSIVIDADE
INTERCOLONIAL EM CUPIM**

Dissertação apresentada ao Programa de Pós-Graduação em Ecologia e Conservação da Universidade Federal de Sergipe, como requisito parcial para obtenção do título de Mestre em Ecologia.

Orientadora: Dr.^a Ana Paula Albano Araújo

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por

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia e Conservação da Universidade Federal de Sergipe, como parte dos requisitos exigidos para a obtenção do título de Mestre em Ecologia e Conservação.

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RESUMO

O comportamento agressivo permite aos animais acesso aos recursos locais, podendo contribuir para o aumento do *fitness*. Uma estratégia para reduzir os custos envolvidos na defesa de territórios consiste na redução de conflitos entre vizinhos. Esta redução na agressividade pode ser determinada, por exemplo, pelo aumento de encontros e conseqüente habituação aos vizinhos. Insetos sociais apresentam um eficiente sistema de reconhecimento que garante a coesão do grupo e a proteção de intrusos em seus ninhos e territórios. Aqui, analisamos inicialmente o efeito da distância entre colônias e da disponibilidade de recursos na agressividade e respostas às pistas químicas intercoloniais em *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). Posteriormente, analisamos se a exposição prévia aos odores intercoloniais e o tipo de recurso consumido pelas colônias poderiam ser mecanismos envolvidos na modulação da agressividade intercolonial. A manipulação de recursos alimentares foi conduzida em campo, onde ninhos com diferentes distâncias entre si foram mantidos sem adição de iscas (controle) e com a adição de três ou 16 iscas de cana-de-açúcar/ninho, durante três meses. Bioensaios de agressividade, bioensaios de trilha linear e em Y foram conduzidos considerando-se todas as combinações de colônias dentro de cada tratamento. O efeito da exposição prévia aos odores intercoloniais e do tipo de recurso consumido sobre a agressividade intercolonial e escolha de pistas foi testado em laboratório. Nossos resultados mostraram que indivíduos mantidos sem adição ou com adição de três iscas/ninho exibiram maior número de lutas com seus vizinhos do que com indivíduos de colônias distantes. Indivíduos provenientes das colônias mantidas sem iscas (controle) seguiram distâncias mais curtas nas trilhas lineares quando comparado às colônias com adição de iscas. A agressividade intercolonial não foi influenciada pela exposição prévia aos odores intercoloniais e nem pelo consumo de recursos similares. No entanto, indivíduos previamente expostos aos odores intercoloniais foram mais atraídos para esses odores. Além disso, indivíduos provenientes de colônias que usaram recursos similares exibiram um maior número de vibrações entre si do que indivíduos provenientes de colônias que consumiram diferente tipo de recurso. Concluindo, nossos resultados mostram que a agressividade intercolonial em *N. aff. coxipoensis* parece ser dependente da oferta de recursos. Nossos resultados podem contribuir para uma melhor compreensão do uso do espaço por colônias de *N. aff. coxipoensis* e pode ser útil para explicações de padrões de coocorrência de espécies de cupins em condições naturais.

Palavras-chave: Agressividade, comunicação, Hipótese do Querido Inimigo, Isoptera, recursos, pista químicas

ABSTRACT

Aggressive behaviour can ensure animals access to local resources contributing to fitness increment. To reduce constant costs in the defence of territories, individuals could save energy with conflicts avoiding aggression with neighbour (Dear Enemy Hypothesis). This reduction in aggressiveness can be determined by the increase of encounters and consequently odor habituation among neighbours. Social insects have an efficient recognition system that guarantees social cohesion and protection against intruders in their colonies and territories. Here, we analysed the effect of distance among colonies and resource availability on the aggression level and responses to chemical cues of *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). We also tested if this pattern could be promoted by previous exposure to intercolonial odors and the type of resource consumed. Manipulation of resource offer was conducted in the field during three months, where nests with different distances were kept without addition of baits (control), with addition of three or 16 sugarcane baits/nest. Bioassays of aggressiveness, linear and Y-shaped trail following bioassays were carried out with all pairwise combinations of colonies in each treatment. The effect of previous exposure to intercolonial odor and the consumption of similar resources on aggressive behavior and choice of intercolonial cues was tested in laboratory conditions. Our results showed individuals from colonies with 0 and 3 baits/nest showed a higher number of fighting with neighbours than those from non-neighbours colonies. Termite workers from colonies without baits (control) followed shorter distance in the linear trails compared to those from colonies with addition of baits. Intercolonial aggressiveness was not affected by previous exposure to intercolonial odor and neither by the consumption of similar resources. However, individuals previously exposed to intercolonial odor were more attracted to these odors than individuals who had no prior exposure. In addition, individuals from colonies of *N. aff. coxipoensis* that use similar resources increased the number of vibration than individuals who consumed different food resources. We concluded that the response of intercolonial aggressiveness in *N. aff. coxipoensis* seems to be resource-dependent. These results may contribute to the comprehension of the use of space by *N. aff. coxipoensis* and could be useful to explain patterns of termite species co-occurrence at natural conditions.

Keywords: aggressiveness, communication, Dear Enemy Hypothesis, Isoptera, food resource, chemical signals.

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INTRODUÇÃO GERAL

A defesa de recursos consiste em um comportamento amplamente difundido entre os animais e pode garantir acesso a locais de alimentação, nidificação e reprodução, contribuindo assim para o incremento do *fitness*. A exibição de comportamentos agressivos pode interferir diretamente nos padrões de ocupação do habitat pelos animais e conseqüentemente nas dinâmicas de populações, comunidades e no funcionamento dos ecossistemas (Brown 1964; Both & Visser 2003). Apesar dos benefícios obtidos pela aquisição de recursos, comportamentos agressivos podem ser onerosos. Tais custos podem ser minimizados, por exemplo, quando há redução de conflitos constantes entre indivíduos de territórios vizinhos. Desta forma, poderia ser esperado menor agressividade entre vizinhos do que entre indivíduos de territórios distantes ('Hipótese do Querido Inimigo', Fisher 1954). Tal padrão tem sido observado em vários organismos (Heinze *et al.* 1996; Rosell & Bjørkøyli 2002; Moser-Purdy & Mennill 2016). Uma das formas de redução da agressividade entre vizinhos pode ser determinada pelo aumento da frequência de encontros e conseqüente habituação entre vizinhos (Ydenberg *et al.* 1988; Heinze *et al.* 1996; Dimarco *et al.* 2010). Tal comportamento pode ser esperado, principalmente, em organismos que possuem local fixo de nidificação, com forrageio no entorno do ninho.

Muitos insetos eussociais (ex. cupins, formigas, abelhas e vespas) são forrageadores de sítio-central e mantêm suas colônias em ninhos, apresentando castas especializadas em diferentes funções e com habilidade para discriminar entre indivíduos próprios e não-próprios às suas colônias. Esta discriminação é possível porque quando em contato interindividual, esses insetos reconhecem o odor dos hidrocarbonetos cuticulares, os quais podem ser determinados por fatores endógenos (ex. ligados ao gene) (Greenberg 1979; Fournier *et al.* 2016) e exógenos (ex. relacionados ao ambiente)

(Jutsum *et al.* 1979; Crosland 1989; Liang & Silverman 2000; Sorvari *et al.* 2008). Desta forma, insetos eussociais são capazes de defender seus ninhos contra possíveis invasores (Knaden & Wehner 2003); assim como suas áreas de forrageio (Levings & Adams 1984).

Cupins são insetos eussociais que apresentam importante papel ecológico na manutenção da diversidade local por alterar as propriedades físicas e químicas do solo e fornecer local de nidificação que permitem a coexistência de várias espécies (Lee & Wood 1971; Bignell & Eggleton 2000; Jouquet *et al.* 2011). Assim, o entendimento dos mecanismos que regulam o uso do habitat por esses organismos é fundamental para a compreensão dos padrões de coexistência de espécies. Apesar disso, poucos estudos têm abordado a defesa de territórios em cupins (Levings & Adams 1984; Adams & Levings 1987; Leponce *et al.* 1997) e como a utilização de pistas intercoloniais interfere nesse comportamento. Estudos recentes têm sugerido que a disponibilidade de recursos (ex. quantidade e qualidade) pode explicar os níveis de agressividade e modular a ocupação do habitat por diferentes espécies. Araújo *et al.* (2017) verificaram maior sobreposição de áreas de forrageio entre diferentes espécies de cupins nos extremos de baixa e alta disponibilidade de recurso, o que poderia ocorrer devido à falta de energia para defesa do território e devido à maior tolerância intercolonial, respectivamente. De fato, outros estudos também verificaram a importância da quantidade e variedade de recursos modulando a agressividade intercolonial. Cristaldo *et al.* (2016) mostraram que colônias vizinhas de *Nasutitermes* aff. *coxipoensis* foram menos agressivas entre si quando a disponibilidade de recursos foi reduzida e que os indivíduos optaram por seguir pistas de colônias que possuíam maior oferta de recursos. Já Florane *et al.* (2004) verificaram que a agressividade intercolonial é reduzida quando as colônias consomem recursos

similares, evidenciando que o ambiente pode interferir na composição de hidrocarbonetos cuticulares e conseqüentemente na discriminação intercolonial.

Neste trabalho, analisamos o efeito da distância entre colônias e da oferta de recurso na agressividade intercolonial em *Nasutitermes* aff. *coxipoensis* (Blattodea: Termitoidea: Termitidae). Adicionalmente, analisamos os mecanismos envolvidos na modulação do comportamento agressivo. A dissertação foi dividida em dois artigos. No primeiro artigo - “Does distance among colonies and resource availability explain the intercolonial aggressiveness in *Nasutitermes* aff. *coxipoensis*?” - analisamos se a agressividade intercolonial e a percepção de sinais químicos dependem da distância entre as colônias e da oferta local de recursos. Já no segundo artigo - “Attraction and vibration: effects of previous exposure and type of resource in the perception of allocolonial odors in termites” - testamos se a resposta à exposição prévia aos odores intercoloniais e o tipo de recurso consumido pelas colônias podem consistir nos mecanismos responsáveis pela modulação da agressividade intercolonial em *N.* aff. *coxipoensis*.

Os resultados deste estudo podem contribuir para a compreensão dos mecanismos que modulam a agressividade em cupins e dos padrões de coocorrência de diferentes espécies, tanto em escala local (ex. dentro do ninho) quanto em escalas maiores (ex. no entorno do ninho).

Comportamento agressivo e defesa de territórios em animais

O comportamento agressivo para garantia de recursos é comumente observado em diversos grupos animais e pode promover aumento do *fitness* dos indivíduos (Kontiainen *et al.* 2009; Tanner & Adler 2009; Ariyomo & Watt 2012; Injaian & Tibbetts 2015). As estratégias de defesa podem incluir comportamentos indiretos [ex. advertências (Moore 2007); displays (Viera *et al.* 2011); sinais visuais (Tibbetts & Lindsay 2008); pistas acústicas (Ladich & Myrberg 2006), sinalizações químicas (Malka *et al.* 2008)] ou confrontos físicos diretos [ex. luta (King 1973)]. Comportamentos agressivos atuam como uma forma de competição por interferência, uma vez que inibem a presença de outros organismos em uma área, dificultando o acesso aos recursos (Wilson 1975; Getty 1987). Desse modo, a territorialidade pode determinar os padrões de distribuição de indivíduos e espécies, alterando a dinâmica das populações e a estrutura das comunidades locais (Leponce *et al.* 1997; Lopez-Sepulcre & Kokko 2005; Ceccarelli 2007).

Apesar dos benefícios associados à defesa de recursos, a agressividade pode resultar em elevado custo energético, injúrias ou até mesmo risco de morte (King 1973). Estudos mostram que a agressividade pode ser modulada dependendo do contexto ecológico no qual o animal se encontra, como por exemplo, a disponibilidade local de recursos (Toobaie & Grant 2013), o valor potencial do recurso (Parker 1974), a distância do inimigo (ex. vizinho ou estranho) (Fisher 1954; Temeles 1994), e o período reprodutivo (Landys *et al.* 2010). A 'Hipótese do Querido Inimigo', por exemplo, prevê que a modulação na agressividade ocorre de forma que indivíduos de territórios vizinhos tendem a ser menos agressivos entre si do que entre indivíduos com territórios distantes (Fisher 1954). Esta hipótese já foi corroborada para vários *taxa* incluindo mamíferos (Rosell & Bjørkøyli 2002), aves (Stoddard *et al.* 1990, Briefer *et al.* 2008, Moser-Purdy

& Mennill 2016) e formigas (Thomas *et al.* 2007; Dimarco *et al.* 2010). Responder de forma menos agressiva aos vizinhos é um comportamento adaptativo, que permite a redução de gastos energéticos com conflitos constantes (Jaeger 1981). Um dos motivos que poderiam determinar menor agressividade entre vizinhos (Temeles 1994) está relacionado ao risco potencial que o vizinho ou o estranho possa representar. Vizinhos possuem territórios já estabelecidos, enquanto estranhos poderiam ser potenciais usurpadores dos territórios. Além disso, o aumento da frequência de encontros entre indivíduos vizinhos dentro dos territórios poderia promover habituação, reduzindo assim o comportamento agressivo entre os mesmos (Ydenberg *et al.* 1988, Langen *et al.* 2000, Dimarco *et al.* 2010).

Insetos eussociais (ex. cupins, formigas, abelhas e vespas) podem consistir em bons modelos para estudos de agressividade. Muitas destas espécies são forrageadores de sítio-central e mantêm suas colônias abrigadas em ninhos, apresentando castas especializadas para defesa, assim como habilidade para discriminar entre indivíduos próprios e não-próprios às suas colônias. A territorialidade tem sido observada em diversas espécies do grupo e é mediada através da comunicação e reconhecimento entre indivíduos (Traniello & Robson 1995; Adams 2016). Em formigas, por exemplo, a defesa do território pode ser baseada em sinais ambientais (ex. material de nidificação) que interferem no reconhecimento de forrageadores vizinhos e estranhos, modulando a agressividade (Heinze *et al.* 1996).

Comunicação em cupins (sinais químicos e vibração)

Os insetos exibem diferentes níveis de complexidade de organização social e desenvolveram eficientes sistemas para a troca de informações interindividuais (Leonhardt *et al.* 2016). A comunicação entre indivíduos da mesma colônia ocorre

principalmente através de sinais químicos (feromônios) e sinais vibroacústicos (Šobotník *et al.* 2010; Hunt & Richard 2013; Richard & Hunt 2013), que podem atuar de modo independente ou em conjunto (Holldobler 1999). A comunicação entre indivíduos ou membros do grupo é essencial para garantia da coesão social, uma vez que regula diversas atividades como a divisão do trabalho, exploração de recursos e as atividades defensivas (Billen 2011).

A informação química transmitida de um indivíduo para outro é essencial no reconhecimento entre indivíduos (próprios ou não-próprios à colônia), na identificação das castas e, ainda, constitui um componente importante na emissão de sinais de alarme e comportamento de territorialidade (Traniello & Robson 1995; Billen & Morgan 1998). O reconhecimento interindividual em insetos sociais é mediado principalmente por hidrocarbonetos presentes na cutícula dos insetos (Zweden & D’Ettorre 2010; Ozaki & Wada-Katsumata 2010). No caso dos insetos eussociais, cada colônia possui uma composição própria de hidrocarbonetos cuticulares (HCs) que pode variar de forma qualitativa e/ou quantitativa. A composição dos HCs é determinada por fatores genéticos (Dronnet *et al.* 2006) e ambientais, como por exemplo, o tipo de recurso consumido e o material de nidificação (Liang & Silverman 2000; Florane *et al.* 2004). Os cupins são capazes de reconhecer esse conjunto de sinais próprios às suas colônias. No entanto, quando encontram indivíduos que apresentam um sinal não-próprio, tendem à executar comportamentos agressivos (Van Zweden & D’Ettorre 2010).

Além da comunicação química, os cupins, assim como outros insetos, também podem se comunicar por meio de sons e vibração (Hunt & Richard 2013). A vibração é um típico comportamento de alarme, sendo observada em inúmeras espécies estudadas até o presente momento (Šobotník *et al.* 2010), com exceção do cupim inquilino *Inquilinitermes microcerus* (ver Cristaldo *et al.* 2016a). As vibrações podem ser

induzidas por diferentes estímulos químicos, seja intra (Cristaldo *et al.* 2015) ou interespecífico (Cristaldo *et al.* 2016a) e são caracterizadas por movimentos corporais verticais e longitudinais. Esses sinais de alarme vibracionais podem ser transmitidos rapidamente por longas distâncias no campo (+/- 1m) e dentro do ninho (+/- 0,3m) (Röhrig *et al.* 1999), podendo permitir uma rápida evacuação e/ou reações de defesa, através da atração de outros companheiros de ninho para a área. Em cupins, a vibração consiste não apenas em um sinal de alerta aos riscos, mas também tem papel central nas atividades de forrageio, como por exemplo, para informar aos companheiros de ninho a quantidade de recursos (Evans *et al.* 2005) e para detectar a presença de competidores (Evans *et al.* 2009).

Cupins: exploração de recursos, agressividade e territorialidade

O uso do habitat por cupins pode ter importantes consequências para a diversidade local. Durante suas atividades de forrageio e construção de ninhos, esses organismos promovem o revolvimento do solo, constroem túneis/ galerias e realizam a ciclagem de nutrientes, alterando as propriedades físicas e químicas do solo (Lee & Wood 1971; Bignell & Eggleton 2000). A alimentação desses organismos é constituída basicamente de matéria orgânica morta, que pode incluir um amplo gradiente de humificação (ex. madeira seca até materiais com mais alto grau de decomposição) (Donovan *et al.* 2001; Davies 2002), contribuindo assim para a ciclagem de nutrientes aumentando a fertilidade dos solos. Nos ambientes tropicais, esses insetos são considerados os principais consumidores de material vegetal (Jouquet *et al.* 2011). Os cupins podem digerir uma taxa particularmente alta de celulose (entre 74 e 99%) (Prins & Kreulen 1991). Adicionalmente, as estruturas físicas (ninhos) construídas pelos cupins para abrigar suas colônias servem de local de nidificação para vários outros invertebrados e também para vertebrados. Desta forma, os cupins permitem o

estabelecimento de várias outras espécies (Lee & Wood 1971; Bignell & Eggleton 2000; Jouquet *et al.* 2011). Assim, o entendimento dos mecanismos que regulam o uso do habitat por esses organismos é fundamental para a compreensão da diversidade local.

Os cupins podem ser classificados de acordo com o tipo de nidificação/ hábito de forrageio que apresentam: i) espécies de sitio único: se alimentam e nidificam no mesmo substrato; ii) espécies de hábito intermediário: nas quais não existe uma separação verdadeira entre o ninho e o alimento; e iii) forrageadores de sítio-central: para as quais há uma separação completa entre ninho e recurso, e os indivíduos necessitam sair do ninho para forragear (Abe 1987). Para alguns forrageadores (ex. de sítio central) as atividades de busca e exploração de recursos se iniciam e terminam no ninho (Traniello & Leuthold 2000). O tipo de forrageio pode variar entre espécies, incluindo tanto a exploração de recursos abaixo do solo (ex. através da construção de túneis) quanto acima do solo (por meio de galerias ou trilhas ao ar livre) (Sugio 1995; Almeida *et al.* 2016). A exploração de novas fontes de recursos é realizada coletivamente e mediada por sinais químicos (Traniello & Robson 1995; Traniello & Leuthold 2000) que são depositados nas trilhas (ex. feromônio de trilha) e podem acelerar o recrutamento e intensificar o forrageio (Arab *et al.* 2012). Além disso, as pistas químicas auxiliam na defesa do território de forrageio indicando aos companheiros de ninho a ocupação da área por possíveis competidores ou invasores (Traniello & Robson 1995).

As interações agressivas podem resultar em diferentes comportamentos incluindo lutas, fuga e submissão (Haverty & Thorne 1989). A agressividade pode ser mais intensa em interações interespecíficas (Getty *et al.* 2000). Colônias que ocorrem na mesma área e que necessitam dos mesmos recursos podem ser mais agressivas entre

si, pois são potenciais competidores nos casos de escassez de alimento (Thorne & Haverty 1991).

A defesa do ninho e das áreas de forrageio contra possíveis invasores é realizada por soldados e por operários, que possuem mecanismos de defesa químicos e/ou mecânicos (Prestwich 1984). Os soldados constituem a principal casta de defesa e possuem uma variedade de adaptações defensivas, incluindo defesa mecânica (ex. mordida) e química (ex. secretam substâncias) que elícita o alarme (Noirot & Darlington 2000; Šobotník *et al.* 2010). No entanto, os operários além de realizarem as tarefas de manutenção da colônia (ex. limpeza do ninho e cuidado com a prole) e forrageio, também desempenham importante papel na defesa (Thorne 1982). As espécies mais dominantes conseguem proteger grandes áreas que apresentam recursos não explorados, excluindo competitivamente outras espécies que se alimentam do mesmo recurso (Leponce *et al.* 1997). Levings & Adams (1984), por exemplo, observaram agressividade intra e intercolonial em locais de sobreposição das trilhas e territórios de forrageio de *N. corniger* e *N. nigriceps*, com exclusão do número de ninhos.

Espécie estudada - Nasutitermes aff. coxipoensis

Colônias de *N. aff. coxipoensis* constroem ninhos epígeos formados por material cartonado escuro e bastante fino, que se desintegra facilmente. Indivíduos dessa espécie apresentam hábito de forrageio noturno (Almeida *et al.* 2016). A busca por recursos inicialmente é realizada em trilhas ao ar livre e essas trilhas são convertidas em túneis apenas quando são encontrados potenciais recursos (Almeida *et al.* 2016). Além disso, em locais com maiores densidades de recursos os cupins reduzem suas buscas, apresentando menores áreas de forrageio (Almeida *et al.* 2018).

Estudos prévios mostraram que *Nasutitermes* aff. *coxipoensis* modula seus níveis de agressividade e a aceitação e exploração de sinais químicos entre colônias vizinhas de acordo com a oferta local de recursos alimentares (Cristaldo *et al.* 2016). Quando as colônias foram mantidas com níveis baixos de recursos os indivíduos foram mais agressivos com vizinhos, por outro lado nos extremos de disponibilidade (ausência e alto nível de oferta de recursos) os indivíduos exibiram menor agressividade (Cristaldo *et al.* 2016). Adicionalmente, os operários são capazes de explorar e escolher trilhas com sinais químicos de colônias vizinhas com melhores disponibilidades de recurso (Cristaldo *et al.* 2016). Essa exploração de pistas intercoloniais pode interferir diretamente nas decisões de forrageio, seja auxiliando os indivíduos na obtenção de informações sobre novas fontes alimentares ou indicando a ocorrência de possíveis competidores.

REFERÊNCIAS

- ABE, T. 1987. Evolution life types in termites. *In* Evolution and coadaptation in biotic communities. pp. 125–148, University of Tokyo Press, Tokyo.
- ADAMS, E. S. 2016. Territoriality in ants (Hymenoptera : Formicidae): a review. *Myrmecological News* 101–118.
- ADAMS, E. S., and S. C. LEVINGS. 1987. Territory size and population limits in mangrove termites. *J. Anim. Ecol.* 56: 1069–1081.
- ALMEIDA, C. S., P. F. CRISTALDO, O. DESOUSA, L. BACCI, D. F. FLORENCIO, N. G. CRUZ, A. A. SANTOS, A. S. SANTANA, A. P. OLIVEIRA, A. P. S. LIMA, and A. P. A. ARAÚJO. 2018. Resource density regulates the foraging investment in higher termite species. *Ecol. Entomol.*
- ALMEIDA, C. S., P. F. CRISTALDO, D. F. FLORENCIO, N. G. CRUZ, A. A. SANTOS, A. P. OLIVEIRA, A. S. SANTANA, E. J. M. RIBEIRO, A. P. S. LIMA, L. BACCI, and A. P. A. ARAÚJO. 2016. Combined foraging strategies and soldier behaviour in *Nasutitermes* aff. *coxipoensis* (Blattodea: Termitoidea: Termitidae). *Behav. Processes* 126: 76–81.
- ARAB, A., Y. CAROLLO BLANCO, and A. M. COSTA-LEONARDO. 2012. Dynamics of foraging and recruitment behavior in the Asian subterranean termite *Coptotermes gestroi* (Rhinotermitidae). *Psyche* (New York) 2012.
- ARAÚJO, A. P. A., P. F. CRISTALDO, D. F. FLORENCIO, F. S. ARAÚJO, and O. DESOUSA. 2017. Resource suitability modulating spatial co-occurrence of soil-forager termites (Blattodea: Termitoidea). *Austral Entomol.* 56.
- ARIYOMO, T. O., and P. J. WATT. 2012. The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. *Anim. Behav.* 83: 41–46.
- BIGNELL, D. E., and P. EGGLETON. 2000. Termites in ecosystems. *In* A. T., D. E. Bignell, and M. Higashi (Eds.) *Termites: evolution, sociality, symbioses, ecology*. pp. 363–388, Kluwer Academic Publishers.
- BILLEN, J. 2011. Exocrine glands and their key function in the communication system of social insects. *Formos. Entomol.* 31: 75–84.
- BILLEN, J., and E. D. MORGAN. 1998. Pheromone communication in social insects: sources and secretions. *Pheromone Commun. Soc. Insects Ants Wasps Bees Termit.* 3–33.
- BOTH, C., and M. E. VISSER. 2000. Breeding territory size affects fitness: an

- experimental study on competition at the individual level. *J. Anim. Ecol.* 1021–1030.
- BOTH, C., and M. E. VISSER. 2003. Density dependence, territoriality, and divisibility of resources: from optimality models to population processes. *Am. Nat.* 161: 326–336.
- BRIEFER, E., T. AUBIN, K. LEHONGRE, and F. RYBAK. 2008. How to identify dear enemies: the group signature in the complex song of the skylark *Alauda arvensis*. *J. Exp. Biol.* 211: 317–326.
- BROWN, J. L. J. L. 1964. The evolution of diversity in avian territorial systems. *Wilson Bull.* 76: 160–169.
- CECCARELLI, D. M. 2007. Modification of benthic communities by territorial damselfish: A multi-species comparison. *Coral Reefs* 26: 853–866.
- CRISTALDO, P. F., A. P. A. ARAUJO, C. S. ALMEIDA, N. G. CRUZ, E. J. M. RIBEIRO, M. L. C. ROCHA, A. S. SANTANA, A. A. SANTOS, A. PASSOS, O. DE SOUZA, and D. F. FLORENCIO. 2016. Resource availability influences aggression and response to chemical cues in the Neotropical termite *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). *Behav. Ecol. Sociobiol.* 70: 1257–1265.
- CRISTALDO, P. F., V. JANDAK, K. KUTALOVA, V. B. RODRIGUES, M. BROTHANEK, O. JIRICEK, O. DESOUSA, J. SOBOTNIK, V. JANDÁK, K. KUTALOVÁ, V. B. RODRIGUES, M. BROTHÁNEK, O. JIŘÍČEK, O. DESOUSA, and J. ŠOBOTNÍK. 2015. The nature of alarm communication in *Constrictotermes cyphergaster* (Blattodea: Termitoidea: Termitidae): the integration of chemical and vibroacoustic signals. *Biol. Open* 4: 1649–1659.
- CRISTALDO, P.F., RODRIGUES, V.B., ELLIOT, S., ARAÚJO, A.P.A AND O. DESOUSA. 2016a. Heterospecific detection of host alarm cues by an inquiline termite species (Blattodea: Isoptera: Termitidae). *Anim. Behav.* 120: 43-49.
- CROSLAND, M. W. J. 1989. Kin recognition in the ant *Rhytidoponera confusa* I. Environmental odour. *Anim. Behav.* 37: 912–919.
- DAVIES, R. G. 2002. Feeding group responses of a Neotropical termite assemblage to rain forest fragmentation. *Oecologia* 133: 233–242.
- DIMARCO, R. D., A. G. FARJI-BRENER, and A. C. PREMOLI. 2010. Dear enemy phenomenon in the leaf-cutting ant *Acromyrmex lobicornis* : Behavioral and genetic evidence. *Behav. Ecol.* 21: 304–310.

- DONOVAN, S. E., P. EGGLETON, and D. E. BIGNELL. 2001. Gut content analysis and a new feeding group classification of termites. *Ecol. Entomol.* 26: 356–366.
- DRONNET, S., C. LOHOU, J. P. CHRISTIDES, and A. G. BAGNÈRES. 2006. Cuticular hydrocarbon composition reflects genetic relationship among colonies of the introduced termite *Reticulitermes santonensis* feytaud. *J. Chem. Ecol.* 32: 1027–1042.
- EVANS, T. A., R. INTA, J. C. S. LAI, S. PRUEGER, N. W. FOO, E. W. FU, and M. LENZ. 2009. Termites eavesdrop to avoid competitors. *Proc. R. Soc. B Biol. Sci.* 276: 4035–4041.
- EVANS, T. A., J. C. S. LAI, E. TOLEDANO, L. MCDOWALL, S. RAKOTONARIVO, and M. LENZ. 2005. Termites assess wood size by using vibration signals. *Proc. Natl. Acad. Sci.* 102: 3732–3737.
- FISHER, J. 1954. Evolution and bird sociality. *In* J. Huxley, A. Hardy, and E. Ford (Eds.) *Evolution as a Process*. pp. 71–83, Allen & Unwin, London.
- FLORANE, C., J. BLAND, C. HUSSENER, and A. RAINA. 2004. Diet-mediated inter-colonial aggression in the *Formosan subterranean* termite *Coptotermes formosanus*. *J. Chem. Ecol.* 30: 2559–2574.
- FOURNIER, D., J. C. DE BISEAU, S. DE LAET, A. LENOIR, L. PASSERA, and S. ARON. 2016. Social structure and genetic distance mediate nestmate recognition and aggressiveness in the facultative polygynous ant *Pheidole pallidula*. *PLoS One* 11: 1–20.
- FRABERGER, R. J., and M. AYASSE. 2007. Mating behavior, male territoriality and chemical communication in the european spiral-horned bees, *Systropha planidens* and *S. curvicornis* (Hymenoptera: Halictidae). *J. Kansas Entomol. Soc.* 80: 348–360.
- GETTY, G. M., M. I. HAVERTY, and V. R. LEWIS. 2000. Agonistic behavior between recently collected and laboratory cultured *Reticulitermes* spp. (Isoptera: Rhinotermitidae) from northern California. *Pan-Pac. Entomol.* 76: 243–250.
- GETTY, T. 1987. Dear enemies and the prisoners-dilemma - Why should territorial neighbors form defensive coalitions. *Am. Zool.* 27: 327–336.
- GREENBERG, L. 1979. Genetic component of bee odor in kin recognition. *Science.* 206: 1095–1097.
- HAVERTY, M. I., and B. L. THORNE. 1989. Agonistic behavior correlated with

- hydrocarbon phenotypes in dampwood termites, *Zootermopsis* (Isoptera: Termitidae). *J. Insect Behav.* 2: 523–543.
- HEINZE, J., S. FOITZIK, A. HIPPERT, and B. HÖLLDOBLER. 1996. Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. *Ethology* 102: 510–522.
- HÖLLDOBLER, B. 1999. Multimodal signals in ant. *J Comp Physiol* 129–141.
- HUNT, J. H., and F. J. RICHARD. 2013. Intracolony vibroacoustic communication in social insects. *Insectes Soc.* 60: 403–417.
- INJAIAN, A., and E. A. TIBBETTS. 2015. Advertised quality and resource value affect aggression and social vigilance in paper wasp contests. *Anim. Behav.* 102: 259–266.
- JAEGER, R. G. 1981. Dear enemy recognition and the costs of aggression between salamanders. *Science Am. Nat.* 117: 962–974.
- JOUQUET, P., S. TRAORÉ, C. CHOOSAI, C. HARTMANN, and D. BIGNELL. 2011. Influence of termites on ecosystem functioning. *Ecosystem services provided by termites. Eur. J. Soil Biol.* 47: 215–222.
- JUTSUM, A. R., T. S. SAUNDERS, and J. M. CHERRETT. 1979. Intraspecific aggression In the leaf-cutting ant *Acromyrmex octospinosus*. *Anim. Behav.* 27: 839–844.
- KING, J. A. 1973. The ecology of aggressive behavior. *Annu. Rev. Ecol. Syst.* 64: 117–138.
- KNADEN, M., and R. WEHNER. 2003. Nest defense and conspecific enemy recognition in the desert ant *Cataglyphis fortis*. *J. Insect Behav.* 16: 717–730.
- KONTIAINEN, P., H. PIETIÄINEN, K. HUTTUNEN, P. KARELL, H. KOLONEN, and J. E. BROMMER. 2009. Aggressive ural owl mothers recruit more offspring. *Behav. Ecol.* 20: 789–796.
- LADICH, F., and A. A. MYRBERG. 2006. Agonistic behavior and acoustic communication. *In Communication in Fishes.* pp. 121–148.
- LANDYS, M. M., W. GOYMANN, I. SCHWABL, M. TRAPSCHUH, and T. SLAGSVOLD. 2010. Impact of season and social challenge on testosterone and corticosterone levels in a year-round territorial bird. *Horm. Behav.* 58: 317–325.
- LANGEN, T. A., F. TRIPET, and P. NONACS. 2000. The red and the black: habituation and the dear-enemy phenomenon in two desert *Pheidole* ants. *Behav. Ecol. Sociobiol.* 48: 285–292.

- LEE, K. E., and T. G. WOOD. 1971. Termites and soil. Academic Press London, New York.
- LEONHARDT, S. D., F. MENZEL, V. NEHRING, and T. SCHMITT. 2016. Ecology and evolution of communication in social insects. *Cell* 164: 1277–1287.
- LEPONCE, M., Y. ROISIN, and J. M. PASTEELS. 1997. Structure and dynamics of the arboreal termite community in New Guinean coconut plantations. *Biotropica* 29: 193–203.
- LEVINGS, S. C., and E. S. ADAMS. 1984. Intra- and interspecific territoriality in *Nasutitermes* (Isoptera: Termitidae) in a Panamanian mangrove forest. *J. Anim. Ecol.* 53: 705–714.
- LIANG, D., and J. SILVERMAN. 2000. “You are what you eat”: Diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* 87: 412–416.
- LOPEZ-SEPULCRE, A., and H. KOKKO. 2005. Territorial defense, territory size, and population regulation. *Am. Nat.* 166: 317–329.
- MALKA, O., S. SHNIEOR, T. KATZAV-GOZANSKY, and A. HEFETZ. 2008. Aggressive reproductive competition among hopelessly queenless honeybee workers triggered by pheromone signaling. *Naturwissenschaften* 95: 553–559.
- MATHEWS, A. G. A. 1977. Studies on termites from the Mato Grosso state, Brazil. Academia Brasileira de Ciências, Rio de Janeiro, Brasil.
- MOORE, P. A. 2007. Agonistic behavior in freshwater crayfish. *In* Evolutionary ecology of social and sexual systems: crustaceans as model organisms. pp. 90–114.
- MOSER-PURDY, C., and D. J. MENNILL. 2016. Large vocal repertoires do not constrain the dear enemy effect: a playback experiment and comparative study of songbirds. *Anim. Behav.* 118: 55–64.
- NOIROT, C., and J. P. E. C. DARLINGTON. 2000. Termite nests: architecture, regulation and defence. *In* T. ABE, D. E. Bignell, and M. Higashi (Eds.) Termites: evolution, sociality, symbioses, ecology. pp. 121–139, Kluwer Academic, Netherlands.
- OZAKI, M., and A. WADA-KATSUMATA. 2010. Perception and olfaction of cuticular compounds. *Insect Hydrocarb. Biol. Biochem. Chem. Ecol.* 207–221.
- PARKER, G. . 1974. Assessment strategy and the evolution of animal conflicts. *J. Theor. Biol.* 47: 223–243.
- PRESTWICH, G. D. 1984. Defense mechanisms of termites. *Annu. Rev. Entomol.* 29:

201–232.

- PRINS, R. A., and D. A. KREULEN. 1991. Comparative aspects of plant cell wall digestion in insects. *Anim. Feed Sci. Technol.* 32: 101–118.
- RICHARD, F. J., and J. H. HUNT. 2013. Intracolony chemical communication in social insects. *Insectes Soc.* 60: 275–291.
- RÖHRIG, A., W. H. KIRCHNER, and R. H. LEUTHOLD. 1999. Vibrational alarm communication in the African fungus-growing termite genus *Macrotermes* (Isoptera, Termitidae). *Insectes Soc.* 46: 71–77.
- ROSELL, F., and T. BJØRKØYLI. 2002. A test of the dear enemy phenomenon in the *Eurasian beaver*. *Anim. Behav.* 63: 1073–1078.
- SHELTON, T. G., and J. K. GRACE. 1996. Review of agonistic behaviors in the Isoptera. *Sociobiology* 28: 155–176.
- ŠOBOTNÍK, J., A. JIROŠOVÁ, and R. HANUS. 2010. Chemical warfare in termites. *J. Insect Physiol.* 56: 1012–1021.
- SORVARI, J., P. THEODORA, S. TURILLAZZI, H. HAKKARAINEN, and L. SUNDSTRÖM. 2008. Food resources, chemical signaling, and nest mate recognition in the ant *Formica aquilonia*. *Behav. Ecol.* 19: 441–447.
- STODDARD, P. K., M. D. BEECHER, C. L. HORNING, and M. S. WILLIS. 1990. Strong neighbor-stranger discrimination in song sparrows. *Cooper Ornithol. Soc.* 92: 1051–1056.
- SUGIO, K. 1995. Trunk trail foraging of the fungus-growing termite *Macrotermes carbonarius* (Hagen) in southeastern Thailand. *Tropics* 4: 211–222.
- TANNER, C. J., and F. R. ADLER. 2009. To fight or not to fight: context-dependent interspecific aggression in competing ants. *Anim. Behav.* 77: 297–305.
- TEMELES, E. J. 1994. The role of neighbours in territorial systems: When are they “dear enemies”? *Anim. Behav.* 47: 339–350.
- THOMAS, M. L., C. M. PAYNE-MAKRISÂ, A. V. SUAREZ, N. D. TSUTSUI, and D. A. HOLWAY. 2007. Contact between supercolonies elevates aggression in Argentine ants. *Insectes Soc.* 54: 225–233.
- THORNE, B. L. 1982. Termite-termite interactions: workers as an agonistic caste. *Psyche* (New York) 89: 133–150.
- THORNE, B. L., and M. I. HAVERTY. 1991. A review of intracolony, intraspecific, and interspecific agonism in termites. *Sociobiology* 19: 115–145.

- TIBBETTS, E. A., and R. LINDSAY. 2008. Visual signals of status and rival assessment in *Polistes dominulus* paper wasps. *Biol. Lett.* 4: 237–239.
- TOOBAIE, A., and J. W. A. GRANT. 2013. Effect of food abundance on aggressiveness and territory size of juvenile rainbow trout, *Oncorhynchus mykiss*. *Anim. Behav.* 85: 241–246.
- TRANIELLO, J. F. A., and R. H. LEUTHOLD. 2000. Behavior and ecology of foraging in termites. *In* T. Abe, D. E. Bignell, and M. Higashi (Eds.) *Termites: evolution, sociality, symbioses, ecology*. pp. 141–168, Kluwer Academic, London, UK.
- TRANIELLO, J. F. A., and S. K. ROBSON. 1995. Trail and territorial communication in social insects. *In* *Chemical ecology of insects 2*. pp. 241–286, Springer.
- VIERA, V. M., V. A. VIBLANC, O. FILIPPI-CODACCIONI, S. D. CÔTÉ, and R. GROSCOLAS. 2011. Active territory defence at a low energy cost in a colonial seabird. *Anim. Behav.* 82: 69–76.
- WILSON, E. O. 1975. *Sociobiology*. United States.
- YDENBERG, R. C., L. A. GIRALDEAU, and J. B. FALLS. 1988. Neighbors, strangers, and the asymmetric war of attrition. *Anim. Behav.* 36: 343–347.
- ZWEDEN, J. S., and P. D'ETTORRE. 2010. Nestmate recognition in social insects and the role of hydrocarbons. 222–243.
- VAN ZWEDEN, J. S., and P. D'ETTORRE. 2010. Nestmate recognition in social insects and the role of hydrocarbons. *Chem. Commun.* 222–243.

CAPÍTULO 1

**Does distance among colonies and resource availability
explain the intercolonial aggressiveness in *Nasutitermes* aff.
coxipoensis?**

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ABSTRACT

Aggressive behaviour can ensure animals access to local resources. To reduce constant costs in the defence of territories, species could save energy with conflicts avoiding aggression with neighbour or in situations with abundance of resources. In the present study we analysed the effect of distance among colonies and resource availability on the aggression level and responses to chemical cues of *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). Manipulation of resource offer was conducted in the field, where nests with different distances were kept without addition of baits (control), with addition of three or 16 sugarcane baits/nest. After three months, aggressiveness, linear and Y-shaped trail following bioassays were carried out with all pairwise combinations of colonies in each treatment. Our results showed that aggressive index of *N. aff. coxipoensis* was affected by the resource availability. However, individuals from colonies with 0 and 3 baits/nest showed a higher number of fighting with neighbours than those from non-neighbours colonies. Termite workers from colonies without baits (control) followed shorter distance in the linear trails compared to those from colonies with addition of baits. In all treatments, there was no preference of workers in relation to the choice of chemical cues from own or other colonies. The response of intercolonial aggressiveness in *N. aff. coxipoensis* seems to be resource-dependent. These results may contribute to the comprehension of the use of space by *N. aff. coxipoensis* and could be useful to explain patterns of termite co-occurrence at different spatial scales, from local (inside the nest – e.g. cohabitation of nests by inquilines) to regional (e.g. around the nest).

Key words: chemical cues, defense, nasty neighbour, territoriality

INTRODUCTION

The protection of resources (*e.g.* food, mates and territories) is ubiquitous behaviour among animals and a fundamental predictor of fitness. This protection occurs via aggressive behaviours (specialized aggressive displays or physical attacks), which determine the access to resources. Intra- and interspecific aggressive behaviours may interfere in the dynamics of populations and the structure of communities, determining patterns of spatial distribution of individuals and species (Brown 1964, Both & Visser 2003). The defence of territories through aggressive behaviours is displayed in a range of taxa, such as mammals (Broadbooks 1970, Schulte-Hostedde & Millar 2002), fishes (Triefenbach & Zakon 2008, Lehtonen *et al* 2010), birds (Stoddard *et al* 1990, Temeles 1994) and insects (Heinze *et al* 1996, Nagamitsu & Inoue 1996, Tanner & Adler 2009). Although aggressiveness confers the protection of resources (Alcock 2011) allowing greater fitness, this behaviour involves energy expenditure (Viera *et al* 2011). Thus, animals tend to adopt strategies to balance the costs and benefits involved in display this behaviour (Both & Visser 2003). Among these strategies, the reduction of aggression towards individuals from neighbouring compared to those from distant territories (*'Dear Enemy Hypothesis'*, Fisher 1954) has been observed in several organisms (Heinze *et al* 1996, Rosell & Bjørkøyli 2002). This reduction in aggressiveness can be determined by the increase in the frequency of encounters and consequent habituation among neighbours, which allows them to save energy with constant conflicts (Ydenberg *et al* 1988, Heinze *et al* 1996, Dimarco *et al* 2010). Such behaviour often occurs in animals that forage around the nests, as observed in some social organisms.

In eusocial insects (bees, ants, termites and wasps) the ability to recognize nestmates allows the maintenance of cohesion and integrity of the group (Holldobler & Michener 1980) Thus, such colonies can defend their nests against possible invaders, as

well as their foraging territories (Levings & Adams 1984). However, studies about territoriality in termites have been shown incongruent results. Termite species may both be more aggressive with neighbouring (Dunn & Messier 1999 - "*Nasty Neighbours Hypothesis*") than with distant colonies (Kaib *et al* 2002 - "*Dear Enemy Hypothesis*"). Several factors may determine such distinct responses, for example: the variation in aggressiveness among species or individuals from social groups, the type of stranger, social circumstances and ecological factors (see Christensen & Radford 2018, for review). Recent studies have shown, for example, that resource offer may explain the use of habitat by different termite species (Araújo *et al* 2017), as well as the modulation of foraging efforts, acceptance to chemical cues and levels of intercolonial aggressiveness for the termite *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae) (Almeida *et al* 2018; Cristaldo *et al* 2016). Although some studies have shown that termites can exhibit territorial behaviour (Levings & Adams 1984, Adams & Levings 1987, Leponce *et al* 1997), the effect of distance among colonies and resource availability is still poorly studied.

Termite species present different nesting and foraging habits, including distinct levels of separation between nest and food (Abe 1987). The different species can also forage in tunnels ("belowground"), galleries ("aboveground") and/or through exposed columns on soil surface ("open air") (Almeida *et al* 2016). Aggressive behaviours to protect the territories could be more evident in central-place species that forage in the open air. *Nasutitermes* aff. *coxipoensis* is a species widely distributed in the Neotropical region. Colonies of this species forage through trails which are converted into galleries when resource is found, which minimizes their foraging costs (Almeida *et al* 2018).

As in other social insects, foraging and territory defence in termites are mediated by chemical signals (Traniello & Robson 1995). Although these signals emit

information to nestmates, they are often exploited by neighbouring colonies (Cristaldo 2018a). The detection and exploitation of neighbouring cues can provide several benefits, including the perception of intrusive individuals who could potentially be a threat. The response to chemical cues by neighbouring colonies according to resource availability has been previously studied in *N. aff. coxipoensis* (Cristaldo *et al* 2016), however, the effect of distance among colonies on the perception and acceptance of chemical signals in termites is still an open question. In the present study, we evaluate whether the intercolonial aggressiveness and the response to chemical cues in *N. aff. coxipoensis* depends on the distance among colonies and the local resource availability. Specifically, we test the following hypotheses: (1) the termite *N. aff. coxipoensis* exhibits greater intercolonial aggressiveness: (i) among individuals of colonies spatially more distant and (ii) in situations of low resource offer; and (2) individuals show greater acceptance and choice of chemical signals: (i) in colonies spatially closer and (ii) in situations of high resource availability.

MATERIAL AND METHODS

Ethics statements

The permit for termite sampling was provided by ICMBio/IBAMA (n°. 47652-1). No specific permits were required for the described laboratory studies, which have been carried out using a species that is neither endangered nor protected.

Study site

The manipulation of resource availability was conducted using nests of *N. aff. coxipoensis* at Santa Isabel Biological Reserve (10°43'29.2" S, 36°50'24.9"W), in municipality of Pirambu, state of Sergipe, Brazil (Fig. 1), during August to November

2017. The regional vegetation consists of grasslands (grasses and sedges) and post-beach, sandbank, palm trees, wetlands and marshes. The site of sample consists of grasslands, with homogeneous coverage in all its extension.

The climate is characterized as humid megathermal and sub-humid, with average annual temperature around 26 °C and average annual rainfall of 1400 mm (Nimer 1972), presenting dry (October-March) and rainy seasons (April-September) well defined (Pereira *et al* 2011).

Food resource manipulation in field

The food resource availability was manipulated in three sites (equidistant for at least 500 m), in which eight active nest of *N. aff. coxipoensis* were selected, totalizing 24 nests. All selected nests were similar in volume. The distance among nests varied from 3 to 190 meters. At each site, one of the treatments was established: (i) no addition of sugarcane baits around the nests (control), (ii) addition of three sugarcane baits around the nests or (iii) addition of 16 sugarcane baits around the nests. The sugarcane baits (15 cm x 2 cm radius) were arranged surrounding the selected nests. The treatments were kept in the field for three months, replacing the baits weekly. The sugarcanes used were fresh (cut recently) and we used always the same variety of sugarcane.

After three months, only five nests were alive in the treatment with 16 baits. The nests were removed from the field and taken to the Laboratory of Ecological Interactions of the Federal University of Sergipe, São Cristóvão, Sergipe, where the behavioural bioassays were performed. Specimens were identified by comparison with samples from the Laboratory of Ecological Interactions, where voucher specimens are deposited. Specimens present at Laboratory of Ecological Interactions (UFS) were previously

identified by the Termitology Laboratory at the University of Brasilia (#UnB-10616, 10617, 10619, 10620 and 10621).

Aggression and survival bioassays

Aggression bioassays were performed for all pairwise combinations of colonies (with different distances) within of each treatment established in the field (resource availability). For this, 10 individuals (four workers and one soldier/colony) were placed at the same time on opposite side of Petri dish (7 x 1.5 cm) covered with filter paper. The number and caste ratio (soldiers: workers) of termite groups used in the bioassays were chosen according to natural caste proportions. For each intercolonial pairwise combination, three repetitions were conducted, totalizing 261 Petri dishes. The individuals of each colony were previously marked in the pronotum region with a mixture of gouache and glue (white or black) (Marins *et al* 2017), in order to discriminate intercolonial behaviours.

The Petri dishes with pairwise individuals were video-recorded during 2 min/dishes. After the aggression bioassays, Petri dishes were maintained in B.O.D. incubator (26 °C and darkness) to record the termite survival. The number of dead individuals was quantified at 2-hours intervals until all individuals were dead.

Videos from aggressive bioassays were analysed for quantification of intercolonial interactions among all individuals in the Petri dishes, considering positive behaviours (trophallaxis, antennation and allogrooming), negative (biting and fighting) and vibration (alert). Intracolony behaviours were not evaluated. The counting of the numbers of behaviours were performed at intervals of 15s, by two observers simultaneously. Then, the intercolonial aggressive index (AI) was calculated considering the number of negative behaviours/total number of behaviours.

Response to chemical cues: acceptance and choice of intercolonial cues

Linear and Y-shaped trail-following bioassays were conducted to test the acceptance and choice of intercolonial cues for all pairwise combinations of colonies (with different distances) within of each treatment established in the field (resource availability). For this, whole body extracts were prepared for each colony by immersion of 50 freeze-killed workers in hexane (10 μ L/worker) for 24 h. After 24 h, the final volume of concentrated extract was quantified, and more hexane was added until the volume of 500 μ l.

The acceptance of intercolonial cues was tested through linear bioassays on filter paper (see details in Cristaldo *et al.* 2014, 2016). Hamilton[®] micro-syringe (10 μ l) was used to form trails on the filter paper. Each trail had 10 cm, the first 6 cm of the trail consisting of the odour from the colony of the tested individual (“*own cues*”), and from 5 cm with the odour of another colony (“*neighbouring cues*”). In this way, there was an overlap of the two odours (1 cm) in the central part of the trail. The control consists of individual following trails (10 cm) with cues of their own colony. Ten repetitions were performed for each combination of colonies, considering the nests within each treatment established in the field, totalizing 1530 trails.

The choice of intercolonial cues was tested through Y-shaped trail-following bioassays (see details in Cristaldo *et al.* 2014, 2016), which consisted of a 3 cm path with two arms of 7 cm each, forming an angle of 120° between them. Each arm was formed by the odour of the tested colony or odour of another colony. The choice of tested individual between these signals was noted. Ten repetitions were performed for each combination of colonies, totalizing 1320 trails.

Statistical analyses

Data were analysed in R software using Linear Generalized Models (GLM) (R Development Core Team 2016), followed by residual analyses to check the suitability of the model and the error distribution. Model simplification, when necessary, was conducted by extracting explanatory terms from the initial model and evaluating the subsequent change in deviance. Contrast analyses were performed to check the differences in the variations among different treatment levels (Crawley 2012).

To check whether the intercolonial behaviours (*y-axis*) is affected by the “distance of colonies” (*x-axis*₁) and “resource offer” (*x-axis*₂), data were submitted to linear regression under Normal error distribution. Similar analyses were performed to check the effect of these variables (“distance of colonies” and “resource offer”) in the mean time to death after aggressive encounters (*y-axis*). The mean time to death was previously calculated by survival analyses under Weibull distribution using *survival* package.

Data from linear bioassays were submitted to linear regression under Normal error distribution to test whether distance followed by individuals in the trails (*y-axis*) was affected by the effect of the distance of colonies (*x-axis*₁), resource offer (*x-axis*₂) and origin of cues (*own cues x neighbouring cues*) (*x-axis*₃). Similar model was performed to test the choice of intercolonial cues, however, under Binomial error distribution. The response variable was considered the proportion of choice of cues from another colony/total of choices.

RESULTS

Aggression and survival x distance among colonies and resource availability

The intercolonial Aggressive Index (AI) was not significantly affected by the distance among colonies ($F_{1,63} = 0.25$, $P = 0.61$), but was significantly influenced by

resource offer ($F_{2,61} = 6.52$, $P = 0.002$) (Fig. 2A). Colonies from control treatment (0 baits) and those maintained with 3 baits/nest did not differ among them ($P = 0.27$) and presented higher AI compared to colonies with 16 baits/nest ($P < 0.001$, Fig. 2A).

Regarding to positive behaviours (trophallaxis, antennation and allogrooming), the number of antennation was significantly affected only by resource offer (Tab. 1). Individuals did not perform trophallaxis and allogrooming behaviours. Similarly, the number of biting was significantly affected only by resource offer (Tab. 1). However, the number of fighting was significantly affected by resource availability and distance among colonies (Tab. 1); individuals from colonies with 0 and 3 baits/nest showed a higher number of fighting with neighbours than those from non-neighbours colonies. Vibration was not significantly affected neither by distance among colonies nor resource offer (Tab. 1).

The mean time to death after aggressive encounters was not affected by distance among colonies (*Deviance* = 1.39, *d.f.*=66, $P = 0.23$), however, was significantly affected by resource offer (*Deviance* = 9.32, *d.f.*= 64, $P = 0.009$). Colonies without baits (control) presented shorter time to death compared with colonies with 3 and 16 baits/nest (*Deviance* = 8.67, *d.f.*= 66, $P = 0.003$ (Fig. 2B).

Response to chemical cues x distance among colonies and resource offer

Trail distance followed was not significantly affected by distance among colonies ($F_{1,151} = 0.03$, $P < 0.85$) and by the origin of cues (“own cues” vs. “neighbour cues”) ($F_{1,148} = 0.64$, $P = 0.42$), however, was significantly affected by resource offer ($F_{2,149} = 30.17$, $P < 0.001$) (Fig. 4). Control colonies followed less distance in the trails compared with colonies with 3 and 16 baits/nests (Fig. 3).

For Y-shaped bioassays, the proportion of choices was not significantly

affected by distance among colonies ($X^2 = 0.01$, $d.f.= 131$, $P= 0.88$) and by resource offer ($X^2 = 1.48$, $d.f.= 131$, $P= 0.47$).

Table 1. Effect of distance among colonies and treatment (“resource offer”; [number of baits/nest]) in the intercolonial behaviours in *Nasutitermes* aff. *coxiensis*.

Source	d.f. resid.	F	P	
Antennation				
Distance among colonies	63	1.24	0.26	<i>n.s</i>
Resource availability	61	5.36	0.007	**
Biting				
Distance among colonies	63	0.03	0.85	<i>n.s</i>
Resource availability	61	4.61	0.01	*
Fighting				
Distance among colonies	63	4.61	0.03	*
Resource availability	61	5.52	0.006	**
Vibration				
Distance among colonies	63	0.01	0.91	<i>n.s</i>
Resource availability	61	1.93	0.15	<i>n.s</i>

n.s. = not significant; * $P < 0.05$; ** $P < 0.01$.

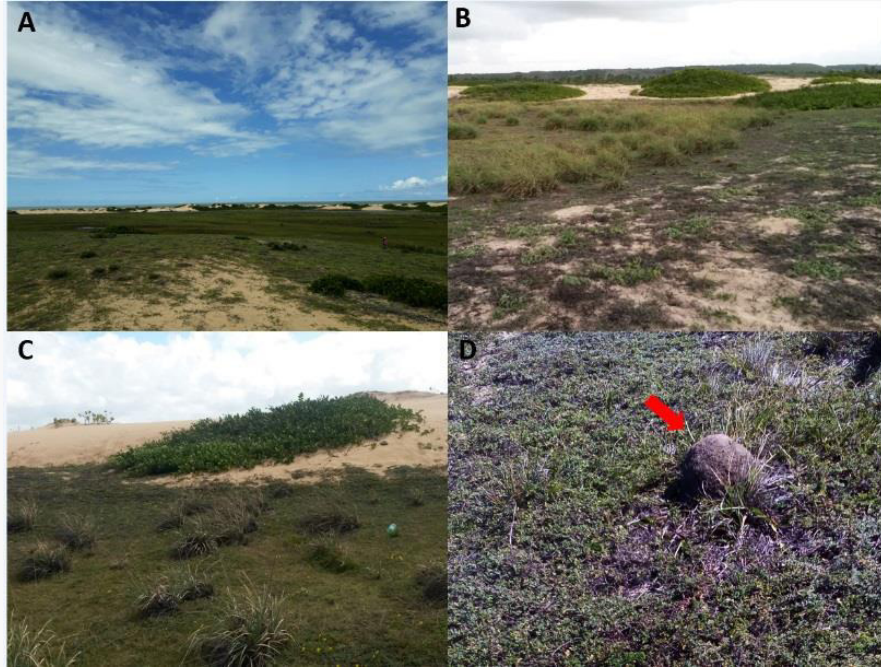


Fig. 1. Photographs of the field in which food resource manipulation was conducted (A-C) and view of one *Nasutitermes* aff. *coxipoensis* nest (D). Santa Isabel Biological Reserve, Pirambu, state of Sergipe, Brazil. 2018.

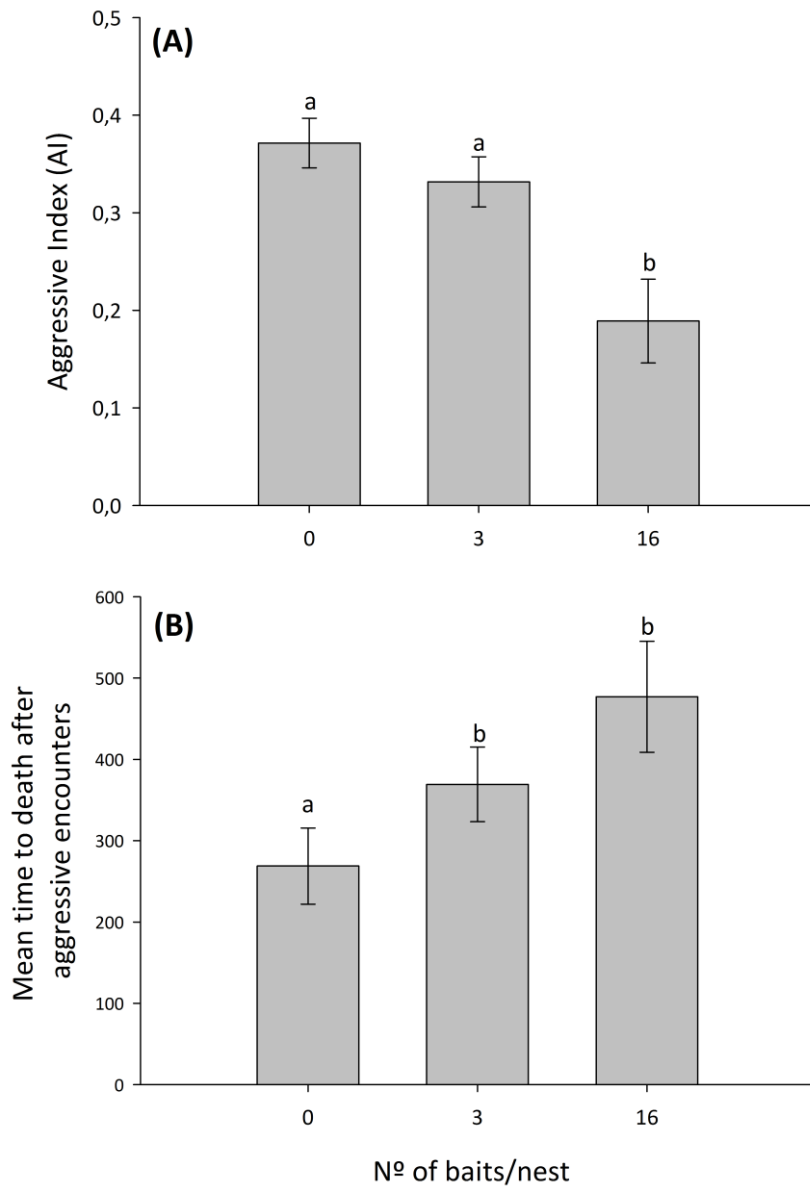


Fig. 2. Effects of resource availability (baits/nest) on *Nasutitermes* aff. *coxipoensis* aggressive index (A) and mean time to death after aggressive encounter (B). Different letters indicate significant differences among treatment ($P < 0.05$).

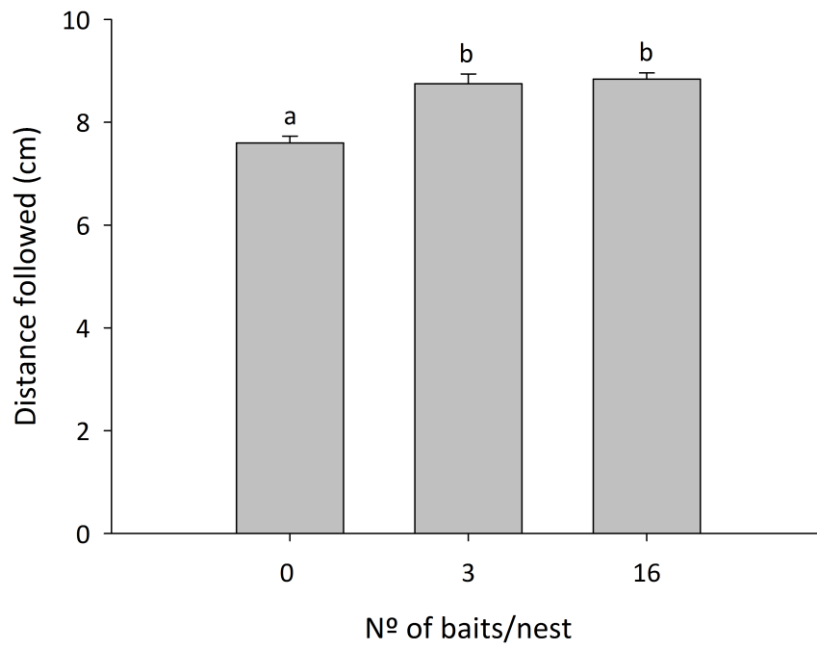


Fig. 3. Effect of resource availability (baits/nest) in mean trail distance followed by *Nasutitermes* aff. *coxipoensis* individuals in linear bioassays with whole worker body extracts. Different letters indicate significant differences among treatment ($P < 0.05$).

DISCUSSION

Our results demonstrated that intercolonial aggressiveness and the response to chemical cues in *N. aff. coxipoensis* colonies are modulated, mainly, by resource offer (Fig. 2 and 3). However, the most expensive level of aggression (*i.e.* number of fighting) was higher between individuals of neighbouring colonies compared to those from distant ones, being such pattern intensified in colonies with 0 and 3 baits/nest. In general, our results indicate that resource offer is a key factor in the response of intercolonial aggressiveness and the acceptance of chemical cues in termites; and that in situations of scarcity of resources, termites seem to defend their territories more intensely compared with situations of abundance of resources.

Individuals from *N. aff. coxipoensis* showed a higher aggressive with neighbours than those from non-neighbour colonies (“*Nasty Neighbours Hypothesis*”). Temeles (1994) suggests that nasty neighbour may be more common in organisms that territory defence is focused in a single purpose, such as defence of food or nest. In fact, termite seems to follow this rule, since they forage around the nests and have a limited living area (Abe 1987). For these organisms, protection against neighbours may be greater due the risk imposed by them (*i.e.* neighbours would represent potential competitors for resources and consequently a greater threat) (Gordon 1989). Such pattern seems to be prevalent in social insects (Muller & Manser 2007, Newey *et al* 2010), including ants (*Oecophylla smaragdina* - Newey *et al* 2010; *Pristomyrmex pungens* - Sanada-Morimura *et al* 2003) and termites (*Nasutitermes corniger* - Dunn & Messier 1999). In the present study, the higher aggressiveness observed in colonies with 0 and 3 baits/nest (“low resource offer”) reinforces this idea (Fig. 2A). On the other hand, the opposite behaviour (reducing aggression with neighbours - “*Dear Enemy Hypothesis*”) may be more evident in organisms that the defence of territory has multiple

purposes (*e.g.* sexual pattern, mating sites, among others), since for these organisms the individuals from distant territories would represent a higher risk (Temeles 1994).

Our results strongly indicate that resource offer is a key factor in the response of aggressive behaviour in termite species. Therefore, we hypothesized that resource offer *per se* could explain the divergent patterns already observed in the aggressive response among termite colonies with different spatial distances (*Nasty Neighbours x Dear Enemy*). That is, the occurrence of nasty neighbours or dear enemy effect depends of resource offer in the environment: in situations of low resource offer, neighbours may represent a higher threat, however, in extreme abundance of resources the opposite is expected.

Other studies with termites support our results and have shown evidence of the effect of resource offer in the response of intercolonial aggression (*e.g.* Adams & Levings 1987) and also in the exposure to foraging risks (Korb and Linsenmair 2002). The aggressive response in function of the resource offer can have effects at the population and community levels, influencing the distribution of colonies (see Lepage & Darlington 2000) as well as the overlapping of foraging areas by different species (Araújo *et al* 2017). In natural situations of resource scarcity, but that still providing energy for defence, termite colonies could guarantee access to the minimum amount of resources for their maintenance; and therefore, more hostile behaviours (*e.g.* fighting) could be more intense between individuals of neighbouring colonies than those from distant colonies. Araújo *et al* (2017) demonstrated that in situations with intermediate resource offer, colonies from different termite species could non-overlapping their foraging areas compared to sites with low resource offer, either to avoid direct conflicts or simply due the reduction of their foraging area. In fact, Almeida *et al* (2018) showed that in low resource offer, colonies of *N. aff. coxipoensis* intensify the searching for

resources, expanding their foraging efforts. This behaviour could result in higher rates of intercolonial encounters and conflicts, altering the distribution of colonies in the field. Here we observe that the mortality rate of individuals after aggressive encounters was affected not only by the greater aggressiveness *per se*, but also by the starvation of individuals. Although colonies with 0 and 3 baits showed the same level of aggression (Fig. 2A), colonies with 0 bait died faster (Fig. 2B). In natural situations with scarcity of resource, such mortality seems to be compensated by a higher allocation of energy from the colonies to the production of new individuals, as already observed for *N. aff. coxipensis* (Cristaldo *et al* 2018b).

The resource offer also affected the distance followed by workers in the trails (Fig. 3). The shorter distances followed by workers from colonies with 0 bait/nest may reflect the low quality of chemical cues from these colonies compared with colonies reared with 3 and 16 baits/nest. Social insects may use chemical cues from other individuals or species to access habitat quality. This ability may be particularly important for colonies under low resource offer since they could make a faster and more accurate evaluation during foraging (Cristaldo 2018a). *Nasutitermes aff. coxipoensis*, for example, can recognize and follow the chemical cues from neighbouring colonies, however, they usually choose chemical cues from individuals of colonies reared under greater resources offer (Cristaldo *et al* 2016).

In summary, our results showed the importance of the resource offer in the response of intercolonial aggression in termites. We hypothesize that extreme levels of resource offer (low and high) can generate different patterns of intercolonial aggression among termite colonies with different spatial distances. This mechanism could be useful to explain patterns of termite co-occurrence at different spatial scales, from local (inside the nest – *e.g.* cohabitation of nests by inquilines) to regional (*e.g.* around the nest).

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REFERENCES

- Abe T (1987) Evolution life types in termites. In: Evolution and coadaptation in biotic communities. University of Tokyo Press, Tokyo, pp 125–148
- Adams ES, Levings SC (1987) Territory size and population limits in mangrove termites. *J Anim Ecol* 56:1069–1081.
- Alcock J (2011) *Comportamento Animal: Uma abordagem evolutiva.*, 9a. edição. Artmed, Porto Alegre
- Almeida CS, Cristaldo PF, DeSouza O, et al (2018) Resource density regulates the foraging investment in higher termite species. *Ecol Entomol online first*.
- Almeida CS, Cristaldo PF, Florencio DF, et al (2016) Combined foraging strategies and soldier behaviour in *Nasutitermes* aff. *coxipoensis* (Blattodea: Termitoidea: Termitidae). *Behav Processes* 126:76–81.
- Araújo APA, Cristaldo PF, Florencio DF, et al (2017) Resource suitability modulating spatial co-occurrence of soil-forager termites (Blattodea: Termitoidea). *Austral Entomol* 56: 235-243.
- Both C, Visser ME (2003) Density dependence, territoriality, and divisibility of resources: from optimality models to population processes. *Am Nat* 161:326–336.
- Broadbooks HE (1970) Home ranges and territorial behavior of the yellow-pine Chipmunk, *Eutamias amoenus*. *J Mammal* 51:310–326.
- Brown JL (1964) The evolution of diversity in avian territorial systems. *Wilson Bull* 76:160–169.
- Christensen C, Radford AN (2018) Dear enemies or nasty neighbors? Causes and consequences of variation in the responses of group-living species to territorial intrusions. *Behav Ecol* 0:1–10.
- Crawley MJ (2012) *The R Book*. John Wiley & Sons, Ltd, Chichester, UK
- Cristaldo PF (2018a) Trail pheromones in termites. In: *Termites and sustainable management*. Springer, pp 145–158
- Cristaldo PF, Almeida CS, Cruz NG, et al (2018b) The role of resource density on energy allocation in the neotropical termite *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). *Neotrop Entomol* 47: 329-335
- Cristaldo PF, Araújo APA, Almeida CS, et al (2016) Resource availability influences aggression and response to chemical cues in the Neotropical termite *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). *Behav Ecol Sociobiol* 70:1257–1265.
- Cristaldo PF, DeSouza O, Krasulová J, et al (2014) Mutual use of trail-following chemical cues by a termite host and its inquiline. *PLoS One* 9:1–9.
- Dimarco RD, Farji-Brener AG, Premoli AC (2010) Dear enemy phenomenon in the leaf-cutting ant *Acromyrmex lobicornis*: Behavioral and genetic evidence. *Behav Ecol* 21:304–310.
- Dunn R, Messier S (1999) Evidence for the opposite of the Dear Enemy Phenomenon in termites. *J Insect Behav* 12:461–464.
- Fisher J (1954) Evolution and bird sociality. In: Huxley J, Hardy A, Ford E (eds) *Evolution as a Process*. Allen & Unwin, London, pp 71–83
- Gordon DM (1989) Ants distinguish neighbours from strangers. *Oecologia* 81:198–200.
- Heinze J, Foitzik S, Hippert A, Hölldobler B (1996) Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderii*. *Ethology* 102:510–522.
- Hölldobler B, Michener CD (1980) Mechanisms of identification and discrimination in social Hymenoptera. In: *Evolution of social behavior: hypotheses and empirical*

- tests. Verlag Chemie GmbH., pp 35–58
- Kaib M, Franke S, Francke W, Brandl R (2002) Cuticular hydrocarbons in a termite : phenotypes and a neighbour \pm stranger effect. *Physiol Entomol* 27:189–198.
- Korb J, Linsenmair KE (2002) Evaluation of predation risk in the collectively foraging termite *Macrotermes bellicosus*. *Insectes Soc* 49:264–269.
- Lehtonen TK, McCrary JK, Meyer A (2010) Territorial aggression can be sensitive to the status of heterospecific intruders. *Behav Processes* 84:598–601.
- Lepage M, Darlington JPEC (2000) Population dynamics of termites. In: *Termites: evolution, sociality, symbioses, ecology*. Springer, pp 333–361
- Leponce M, Roisin Y, Pasteels JM (1997) Structure and dynamics of the arboreal termite community in New Guinean coconut plantations. *Biotropica* 29:193–203.
- Levings SC, Adams ES (1984) Intra- and interspecific territoriality in *Nasutitermes* (Isoptera: Termitidae) in a Panamanian mangrove forest. *J Anim Ecol* 53:705–714.
- Marins A, Cristaldo PF, L. R. Paiva, et al (2017) A new approach to mark termites (*Cornitermes cumulans* (Kollar) Blattodea: Isoptera) for laboratory bioassays. *Braz J Biol impress*.
- Muller CA, Manser MB (2007) “Nasty neighbours” rather than “dear enemies” in a social carnivore. *Proc R Soc B Biol Sci* 274:959–965.
- Nagamitsu T, Inoue T (1996) Aggressive foraging of social of floral resource part. *Oecologia* 110:432–439.
- Newey PS, Robson SKA, Crozier RH (2010) Weaver ants *Oecophylla smaragdina* encounter nasty neighbors rather than dear enemies. *Ecology* 91:2366–2372.
- Nimer E (1972) Climatologia da região Nordeste do Brasil. *Introdução à climatologia dinâmica*. *Rev Bras Geogr* 34:3–51.
- Pereira ERR, Freitas JC, Andrade ARS, Sousa VG (2011) Variability in number of rainy days in state of Sergipe-SE. *Appl. Res. & Agrotec.* 4:45-64.
- R Development Core Team (2016) R: A language and environment for statistical computing. Vienna, Austria
- Rosell F, Bjørkøyli T (2002) A test of the dear enemy phenomenon in the *Eurasian beaver*. *Anim Behav* 63:1073–1078.
- Sanada-Morimura S, Minai M, Yokoyama M, et al (2003) Encounter-induced hostility to neighbors in the ant *Pristomyrmex pungens*. *Behav Ecol* 14:713–718.
- Schulte-Hostedde AI, Millar JS (2002) “Little chipmunk” syndrome? Male body size and dominance in captive yellow-pine chipmunks (*Tamias amoenus*). *Ethology* 108:127–137.
- Stoddard PK, Beecher MD, Horning CL, Willis MS (1990) Strong neighbor-stranger discrimination in song sparrows. *Cooper Ornithol Soc* 92:1051–1056.
- Tanner CJ, Adler FR (2009) To fight or not to fight: context-dependent interspecific aggression in competing ants. *Anim Behav* 77:297–305.
- Temeles EJ (1994) The role of neighbours in territorial systems: When are they “dear enemies”? *Anim Behav* 47:339–350.
- Traniello JFA, Robson SK (1995) Trail and territorial communication in social insects. In: *Chemical ecology of insects 2*. Springer, pp 241–286
- Triefenbach FA, Zakon HH (2008) Changes in signalling during agonistic interactions between male weakly electric knifefish, *Apteronotus leptorhynchus*. *Anim Behav* 75:1263–1272.
- Viera VM, Viblanc VA, Filippi-Codaccioni O, et al (2011) Active territory defence at a low energy cost in a colonial seabird. *Anim Behav* 82:69–76.
- Ydenberg RC, Giraldeau LA, Falls JB (1988) Neighbors, strangers, and the asymmetric war of attrition. *Anim Behav* 36:343–347.

CAPÍTULO 2

Attraction and vibration: effects of previous exposure and type of resource in the perception of allocolonial odors in termites

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ABSTRACT

Social insects have an efficient recognition system that guarantees social cohesion and protection against intruders in their colonies and territories. However, the energy costs in constant conflicts with neighboring colonies could promote a reduction in the fitness of colonies. Here, we evaluated the effect of previous exposure to allocolonial odor and the consumption of similar food resources on aggressive behavior and choice of allocolonial cues in *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). Our results showed that intercolonial aggressiveness was not affected by previous exposure to allocolonial odor and by the consumption of similar food resources. However, individuals previously exposed to allocolonial odor were more attracted to these odors than individuals who had no prior exposure to allocolonial odor. In addition, individuals from colonies of *N. aff. coxipoensis* that use similar food resources increased alertness via a greater number of vibration than individuals who consumed different food resources. In general, our results indicate that colonies of *N. aff. coxipoensis* perceive allocolonial cues that have been previously exposed, and that the consumption of similar resources triggers an alert signal between individuals. Additional studies are necessary to assess how widespread this capacity of perception is present among the different Isoptera groups and the consequences of colony recognition odor cues on termite space use.

Key words: communication, habitat use, Isoptera, vibration, social behavior

INTRODUCTION

Social insects (termites, ants, wasps and bees) are one of the most successful groups of organisms in terrestrial environments. The success of these insects is mainly due to the efficient recognition and communication systems, which guarantees social cohesion and protection against intruders in their colonies and territories (Leonhardt *et al.* 2016). The ability to discriminate between nestmates and non-nestmates allows altruistic behaviors to be directed to related individuals, one of the conditions favoring the evolution and maintenance of sociality (Van Zweden & D’Ettorre 2010).

The recognition system in social insects is mediated by chemical signals – mainly hydrocarbons – present in the cuticle of these insects and that present essential compounds for the recognition (Blomquist and Bagnères 2010). The chemical composition of cuticular hydrocarbons (CHCs) is controlled by genetic factors. However, environmental factors (e.g. diet, nest material) also contribute significantly to the composition of these compounds (Howard and Blomquist 2005). During encounters with other individuals, recognition signals are perceived via antennation and compared with the template previously stored in the nervous system of the insect (Van Zweden and D’Ettorre 2010). An incompatibility between these signs usually results in aggressive behaviors, such as biting and fighting (Blomquist and Bagnères 2010; Howard and Blomquist 2005).

Although it is an efficient system against intruders, the energy expenditure with constant intercolonial conflicts among neighboring colonies could provoke a reduction in the fitness of colonies. The Dear Enemy Hypothesis predicts less aggression among individuals from neighboring colonies than among individuals from distant territories. The mechanisms behind this friendly behavior among individuals from neighboring colonies are mediated by adjustments in the recognition system (Hölldobler and Carlin

1987), resulting of habituation to allocolonial odors (i.e., mix of two odors) as consequence of frequent encounters (Langen et al. 2000), use of similar food resource (Liang and Silverman 2000) or a higher genetic similarity among neighboring colonies (Suarez et al. 1999; Dimarco et al. 2010). The reduction of animal response as consequence of repeated occurrence of a regular signal is considered one of the simplest form of learning (Glanzman 2011). A range of studies have been demonstrated the ability of ants to become habituated to chemical cues from neighboring colonies, exhibiting less aggressiveness compared to individuals from distant colonies (Nowbahari 2007; Foubert and Nowbahari 2008). Similarly, the decrease in aggressiveness among individuals of ant colonies using the same food resource has already been observed (Buczowski et al. 2005). In termites, however, the role of habituation and diet on intercolonial aggression is still poorly understood (but see Florane et al. 2004; Olugbemi 2013).

Termites are organisms that live in cryptic environments due to the low sclerotization of their cuticles and almost all species are blind. Thus, the recognition system is particularly important to these insects (Wilson 1971). The strategies for modulating intercolonial aggressiveness in termites should play a key role in population and community dynamics, with effects in the spatial distribution of colonies as well as in the overlapping of foraging areas, especially in species that are central-place foragers and have delimited territories. *Nasutitermes* aff. *coxipoensis* (Homgren) (Termitidae: Nasutitermitinae) is an advanced termite species widely distributed in the Neotropical regions. Colonies of this species forage through trails that are converted into galleries when food is found (Almeida *et al.* 2016), which minimizes their foraging costs (Almeida *et al.* 2018). The intercolonial aggressiveness between neighboring colonies of this species is modulated mainly by the resource offer (Ferreira et al. 2018).

In the present study, we analyzed the effect of previous exposure to allocolonial odors, time of exposure to these odors and type of food resource consumed on the intercolonial aggression and the choice of chemical cues in colonies of *N. aff. coxipoensis*. Specifically, we tested the following hypotheses: (i) individuals from colonies previously exposed to allocolonial odors present less aggressiveness than individuals from colonies without previous exposure to allocolonial odors, (ii) individuals from colonies previously exposed to allocolonial odors remain a greater proportion of time in a treated area with allocolonial odor than in untreated areas and (iii) individuals from colonies that consume same food resources decrease intercolonial aggressiveness compared to individuals from colonies that consume different food resources.

METHODS

Study site and ethics statement

Nests of *N. aff. coxipoensis* ($N= 16$) were collected at Biological Reserve of Santa Isabel (10°43'29.2" S, 36°50'24.9"W), municipality of Pirambu- SE, Brazil. The regional vegetation consists of grasslands (grasses and sedges) and post-beach, sandbank, palm trees, wetlands and marshes. The sampling site consists of grasslands, with homogeneous coverage in all its extension. The climate is characterized as humid megathermal and sub-humid, with average annual temperature around 26 °C and average annual rainfall of 1400 mm (Nimer 1972), with dry (October-March) and rainy seasons (April-September) well defined.

The nests were completely removed from the field in January 2018 and were kept at the Laboratory of Ecological Interactions of the Federal University of Sergipe, São Cristóvão, Sergipe. The permit for termite sampling was provided by

ICMBio/IBAMA (no. 47652-1). No specific permits were required for the described laboratory studies, which have been carried out using a species that is neither endangered nor protected. The colonies were kept at rest for, at least, 24 hours prior to the bioassays.

Experimental setup

Effect of previous exposure to allocolonial odors on aggressiveness and choice of chemical cues

Colonies ($N= 6$) were fragmented in equal parts, which were kept in plastic pots (2 L) with or without intercolonial contact for eight days. The openings of the pots containing the fragments of colonies were isolated with organza and adhesive tape. Pairs of pots were joined in the region of their openings to allow the aerial passage of odors, but without physical contact between the individuals of different colonies (see Fig. 1A). Experiments were conducted in two sets (blocks), each one consisting of the combination of three colonies of *N. aff. coxipoensis*. The treatments were established considering a complete factorial including all combinations between individuals of tested colonies. The treatment with previous exposure to allocolonial odor consisted of the union of pots containing fragments of distinct colonies ($N= 6$; 3 pots/block). In the control (without previous contact with allocolonial odors), pots containing fragments of the same colony were joined ($N = 6$; 3 pots/block) (Fig. 1A).

After exposure of the termites to the treatments, aggression tests (Fig. 1B) and choice of chemical cues bioassays (Fig. 1C) were performed for all combinations between colonies. The intercolonial aggressive tests were conducted following procedures described in section 2.3. We carried out three repetitions per paired combination (3 combination/treatment), each using different individuals per nest,

totalizing 36 tests and 12 true replicates. To analyze the effect of previous exposure to allocolonial odors on the choice of chemical cues and intercolonial aggressiveness, cuticular compounds of 50 workers/colony were extracted using hexane, following the procedure described in Ferreira et al. (2018). Then, five workers were placed in Petri dishes (5.5 cm diameter and 1.5 cm high) covered with filter paper. Half of the filter paper was treated with 5 μ l of colony odor extract to which individuals were maintained (with or without previous exposure to allocolonial odor) and the other half was treated with 5 μ l of hexane (solvent) (Fig. 1C). Petri dishes containing the individuals were video-recorded using a camera (Panasonic SD5 Superdynamics - WV-CP504), equipped with Spacecom lens (1/3" 3-8 mm) coupled in a computer. The behaviors were captured with Ethovision[®] XT software (version 8.5; Noldus Integration System, Sterling, VA) and later the videos were analyzed in Studio 9 software (Pinnacle Systems, Mountain View, CA). The proportion of time in which individuals remained on each side of the Petri dish (treated or untreated) was counted. We carried out three repetitions per paired combination, each using different individuals per nest, totalizing 144 tests and 48 true replicates.

Effect of exposure time to allocolonial odor on aggressiveness

The treatments consisted of the pairwise combination of individuals with and without exposure to allocolonial odor for 2h or 12h. The experiment was conducted in a Petri dish (5.5 x 1.5 cm) containing the bottom covered with filter paper totally treated with 5 μ l of odor of colony or with 5 μ l of hexane (control) (Fig. 2). Five individuals (four workers and one soldier/colony) were placed into each treated arena, sealed with plastic film and placed in a biochemical oxygen demand incubator (B.O.D) at 26°C in the absence of light for a period of 2 or 12 hours. After these times, the pairwise

combination of the individuals exposed to the odors were made with five other individuals, considering all combinations between the colonies tested. The intercolonial aggressive tests were conducted following procedures described in section 2.3. We carried out three repetitions per paired combination (6 combination/treatment/time), each using different individuals per nest, totalizing 72 tests and 48 true replicates.

*Effect of food resource type consumed by *N. aff. coxipoensis* on aggressiveness*

The experiment was conducted using nests of *N. aff. coxipoensis* ($N= 10$) kept during 25 days under offer of different types of food resources (sugarcane [$N= 5$ nests] and dung [$N= 5$ nests]). The amount of food resource offered was 380 g/colony for both types of resources (sugarcane and dung), which were renewed continuously. Intercolonial aggression tests (see section 2.3) consisted in the pairing of individuals from colonies kept in the same food resource ($N= 20$; 10 combination/resource type) and in different type of food resource (sugarcane \times dung; $N= 25$ combinations). Bioassays were conducted after 14 and 25 days of treatment establishment. For each combination, three repetitions were performed, totaling 270 repetitions and 90 true replicates.

Intercolonial aggression tests

Intercolonial aggressive tests were performed considering a complete factorial that consisted of all combinations of individuals from the tested colonies in each bioassay described above. For this, 10 individuals (four workers and one soldier/colony) were placed at the same time on opposite side of Petri dish (7 x 1.5 cm) covered with filter paper. To discriminate intercolonial behaviors, individuals of each colony were previously marked in the pronotum region with a mixture of gouache and glue (white or

black) (Marins et al. 2018). Petri dishes with pairwise individuals were video-recorded during 2 min/dishes. Videos from aggressive bioassays were analyzed for quantification of intercolonial interactions among all individuals in the Petri dishes, considering positive behaviors (trophallaxis, antennation and allogrooming), negative (biting and fighting) and vibration (alert). Intracolony behaviors were not evaluated. The counting of the numbers of behaviors were performed at intervals of 15s, by two observers simultaneously. Then, the index of intercolonial aggressiveness was calculated considering the number of negative behaviors/total number of behaviors. All data were recorded using blinded methods to minimize observers bias.

Statistical analyzes

Data were analysed in R software (R Development Core Team 2016), using Linear Generalized Models (GLM) followed by residual analyses to check the suitability of the model and the error distribution. Model simplification, when necessary, was conducted by extracting explanatory terms from the initial model and evaluating the subsequent change in deviance. Contrast analyses were performed to check the differences in the variations among different treatment levels (Crawley 2012).

To check whether the intercolonial behaviours (*y-axis*) were affected by treatments (with or without previous exposure to allocolonial cues) (*x-axis*), data were submitted to Deviance Analyses (ANODEV) under normal error distribution. Similar model was performed to test the effects of treatments on the time remained in each side of arena (treated or untreated), however, under binomial error distribution. The response variable was considered the proportion of time of individuals on each side of arena/total time.

Data from exposure time bioassays were submitted to ANODEV under negative binomial error distribution to test whether intercolonial behaviours (*y-axis*) were affected by the effect of treatments (*x-axis₁*), time of exposure to allocolonial cues (*x-axis₂*) and interactions between these variables (*time of exposure x allocolonial cues*) (*x-axis₃*).

Data from type of food resource bioassays were submitted to ANODEV under normal error distribution to test whether intercolonial behaviours (*y-axis*) were affected by the type of food resource (*x-axis₁*), time of exposure to these food resources (*x-axis₂*) and interactions between these variables (*consumed resource x time of exposure*) (*x-axis₃*).

RESULTS

Effect of previous exposure to allocolonial odor on aggressiveness and choice to chemical cues

The index of intercolonial aggressiveness of *N. aff. coxipoensis* was not significantly affected by treatments (with and without previous exposure to allocolonial odor) ($F_{1,10} = 0.018$, $P = 0.89$). Similarly, none of the intercolonial behaviors were significantly affected by treatments (Table 1). The trophallaxis and allogrooming behaviors were never observed among paired individuals during bioassays.

The proportion of time in which individuals of *N. aff. coxipoensis* remained in the arena was significantly affected by area (treated \times untreated) ($Deviance = 79.54$, $d.f. = 48$, $P < 0.001$) and by the interaction between area and treatment (with and without previous exposure to allocolonial odor) ($Deviance = 57.30$, $d.f. = 44$, $P < 0.001$). The proportion of time in which individuals without previous exposure to allocolonial odor remained in each side of arena did not vary significantly, however, individuals with previous

exposure to allocolonial odors remained a greater proportion of time in treated areas compared to untreated areas (Fig. 3).

Effect of exposure time to allocolonial odor on aggressiveness

The index of intercolonial aggressiveness was not significantly affected by treatment (with and without previous exposure to allocolonial odor) (*Deviance*= 30.31, *d.f.*= 48, *P*= 0.98), exposure time (*Deviance*=30.31, *d.f.*= 47, *P*=0.97) and neither by the interaction between treatment and exposure time (*Deviance*= 30.30, *d.f.*= 46, *P*= 0.92). The exposure time significantly decreased the number of antennation (Table 2). The others intercolonial behaviors were not significantly affected by treatments, exposure time and neither by interaction between these variables (Table 2). The trophallaxis and allogrooming behaviors were never observed among paired individuals during bioassays.

Effect of food resource type consumed by *N. aff. coxipoensis* on the aggressiveness

The index of intercolonial aggressiveness was not significantly affected by food resource type (same or different) ($F_{2,86}= 0.14$, *P*= 0.86), exposure time to food resources (14 or 25 days) ($F_{1,88}= 1.16$, *P*= 0.28) and by the interaction between these factors ($F_{2,84}= 0.22$, *P*= 0.80). The exposure time to food resources significantly increased the number of antennation among paired individuals (Table 3). The number of negative behaviors (biting and fighting) were not significantly affected by resource type, exposure time to food resources and neither by interaction between these variables (Table 3). However, the number of vibrations was significantly affected by exposure time to food resources and by the interaction between type of resource and exposure

time to food resources (Table 3). The individuals from colonies that consumed the same food resource (sugarcane or dung) showed a higher number of vibration movements after 25 days compared to 14 days; however, the number of vibrations did not vary in individuals from colonies that consumed different food resource (Fig. 4). The trophallaxis and allogrooming behaviors were never observed among paired individuals during bioassays.

Table 1. Effect of treatments (with or without contact with allocolonial odors) after eight days in the intercolonial behaviors of *Nasutitermes* aff. *coxipoensis*.

Source	<i>d.f.</i>	F	<i>P</i>	
Antennation				
Treatment	15	0.23	0.79	<i>n.s.</i>
Biting				
Treatment	15	3.21	0.06	<i>n.s.</i>
Fighting				
Treatment	15	3.02	0.07	<i>n.s.</i>
Vibration				
Treatment	15	1.51	0.25	<i>n.s.</i>

n.s. = not significant

Table 2. Effect of exposure time (2 and 12 hours) of treatments (with and without contact with allocolonial odor) in the intercolonial behaviors of *Nasutitermes aff. coxipoensis*.

Source	<i>d.f.</i>	F	<i>P</i>	
Antennation				
Treatment (<i>a</i>)	46	2.06	0.15	<i>n.s.</i>
Exposure time (<i>b</i>)	45	4.31	0.04	*
<i>a:b</i>	44	0.04	0.83	<i>n.s.</i>
Biting				
Treatment (<i>a</i>)	46	0.22	0.63	<i>n.s.</i>
Exposure time (<i>b</i>)	45	0.49	0.48	<i>n.s.</i>
<i>a:b</i>	44	0.01	0.88	<i>n.s.</i>
Fighting				
Treatment (<i>a</i>)	46	0.87	0.35	<i>n.s.</i>
Exposure time (<i>b</i>)	45	3.57	0.06	<i>n.s.</i>
<i>a:b</i>	44	0.04	0.82	<i>n.s.</i>
Vibration				
Treatment (<i>a</i>)	46	0.38	0.53	<i>n.s.</i>
Exposure time (<i>b</i>)	45	3.24	0.07	<i>n.s.</i>
<i>a:b</i>	44	0.01	0.98	<i>n.s.</i>

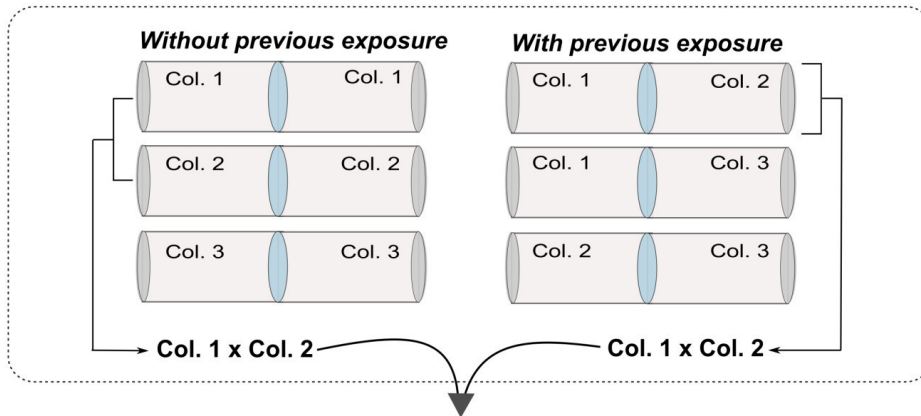
*= $P < 0.05$; *n.s.* = not significant

Table 3. Effect of type of food resource consumed (same or different) and exposure time to treatments (14 and 25 days) in the intercolonial behaviors of *Nasutitermes* aff. *coxipoensis*.

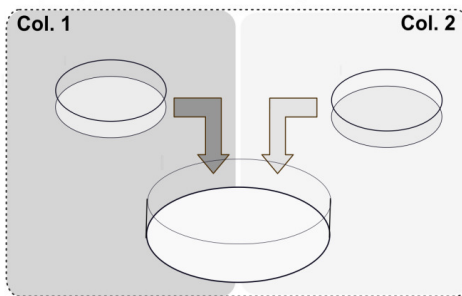
Source	<i>d.f.</i>	F	<i>P</i>	
Antennation				
Type of resource (<i>a</i>)	88	0.40	0.52	<i>n.s.</i>
Exposure time (<i>b</i>)	86	4.44	0.01	*
<i>a:b</i>	84	0.17	0.84	<i>n.s.</i>
Biting				
Type of resource (<i>a</i>)	88	0.05	0.81	<i>n.s.</i>
Exposure time (<i>b</i>)	86	0.92	0.40	<i>n.s.</i>
<i>a:b</i>	84	0.28	0.75	<i>n.s.</i>
Fighting				
Type of resource (<i>a</i>)	88	1.68	0.19	<i>n.s.</i>
Exposure time (<i>b</i>)	86	0.43	0.65	<i>n.s.</i>
<i>a:b</i>	84	0.34	0.70	<i>n.s.</i>
Vibration				
Type of resource (<i>a</i>)	88	6.98	0.01	*
Exposure time (<i>b</i>)	86	1.36	0.26	<i>n.s.</i>
<i>a:b</i>	84	3.23	0.04	*

*= $P < 0.05$; *n.s.* = not significant

A) Establishment of treatments



B) Aggressive bioassay



C) Choice of chemical cues bioassay

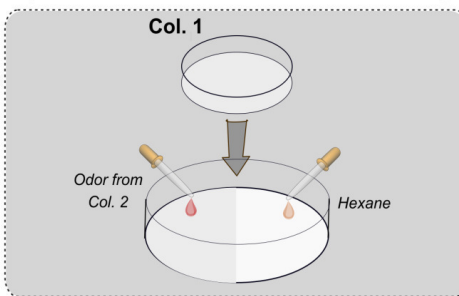


Figure 1. Scheme of experimental design of previous exposure to allocolonial odors experiments. A) Procedure to establishment of treatments; B) Setup of intercolonial aggressive test performed after eight days of establishment of treatments; C) Setup of choice of chemical cues tests performed after eight days of establishment of treatments. See more details in Methods section.

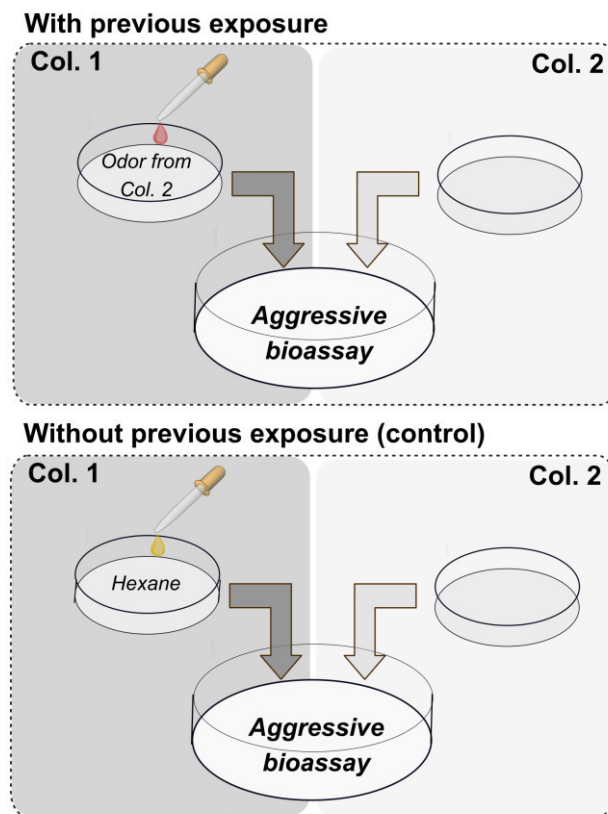


Figure 2. Scheme of experimental design of exposure time to allocolonial odor experiments using solvent (hexane) and allocolonial odor. Experiments were performed after 2 and 12 hours of exposure to allocolonial odors. See more details in Methods section.

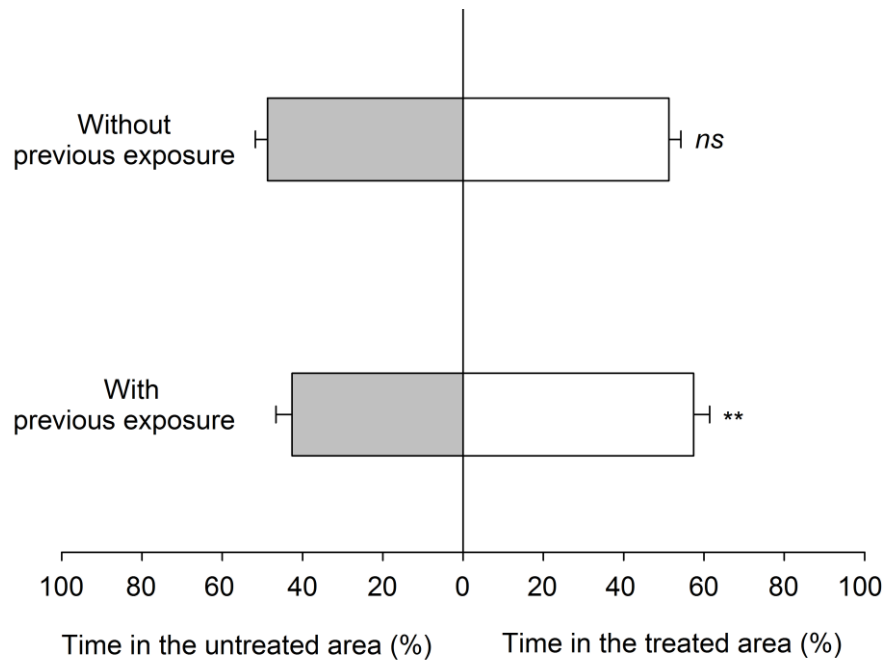


Figure 3. Proportion of time (%) in which individuals of *Nasutitermes aff. coxipoensis* without and with previous exposure to allocolonial odors after eight days remained in the treated and untreated areas. ** means significant differences in each treatment; *n.s.* means no significant differences in each treatment.

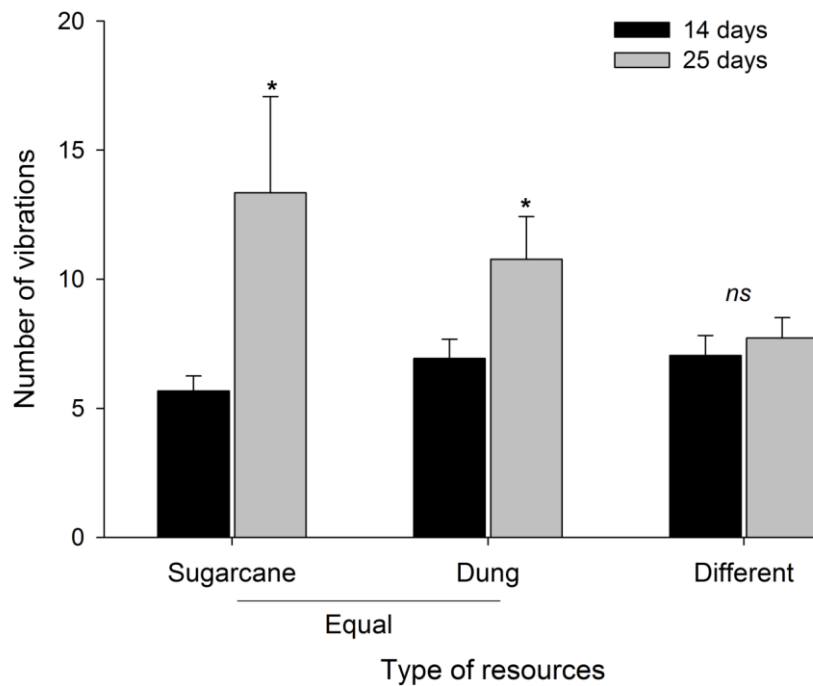


Figure 4. Effect of type of food resource (same vs. different) and time of exposition of food resources (14 or 25 days) in the number of vibration of *Nasutitermes aff. coxipoensis* during intercolonial aggression bioassays. * means significant differences in each treatment; *n.s.* means no significant differences in each treatment.

DISCUSSION

Our results showed that, although the exposure to allocolonial odor cues and the type of food resource does not trigger changes in aggressiveness (Table 1-3), individuals of *N. aff. coxipoensis* were attracted to cues from colonies in which they were previously exposed (Fig. 3) and the consumption of similar food resources increased the number of vibration (Fig. 4).

Frequent exposure to allocolonial odor cues generally triggers acceptance of these cues via habituation with consequent reduction of aggressive behaviors, as already observed in ants (Langen et al. 2000). However, frequent exposure may also facilitate

the recognition of intruders, increasing the aggressiveness of individuals (Thomas et al. 2007, 2005). Nasty behavior among individuals from neighboring colonies is frequently explained due the competition of territorial resources (Leiser 2003). Here, despite the absence of variation in the aggressiveness, individuals of *N. aff. coxipoensis* were attracted to allocolonial odor cues that they were previously exposed (Fig. 3). The use of interindividual/interspecific cues to obtain own benefits, such as access to resource or information about risk, has already been demonstrated for a range of animals (*e.g.* Silverman et al. 2004; Goodale et al. 2010; Binz et al. 2014), including termites (Evans et al. 2009; Cristaldo et al. 2016a, 2016b). Although the use of allocolonial foraging cues is still an unexplored subject in termites (but see Cristaldo 2018), the attraction of allocolonial odor cues observed here strongly indicates that termites could use this cues to access new food sources. In natural situations of food resource scarcity, such behavior could result in both facilitation (*e.g.* joint exploitation of resources and colony fusion) or competition. In termites, it is common to observe the simultaneous use of same food source by several species (Schuurman 2006; Roisin et al. 2006) or even the nest-sharing among different termite species (*e.g.* inquiline) (Costa et al. 2009; Cristaldo et al. 2012). On the other hand, termite species also exhibit defense of their territories (Levings and Adams 1984; Adams and Levings 1987) and a greater number of conflicts with neighboring colonies than distant ones (Dunn and Messier 1999; Ferreira et al. 2018). In addition, some studies have been shown that colony fusion of neighboring colonies, with reduction of agonistic behaviors, is an adaptive strategy based in the cost/benefit balance of local conditions (*e.g.* food resource offer) (Matsuura and Nishida 2001; Deheer and Vargo 2004; Korb and Roux 2012). Thus, the determinant factor to triggers facilitation or competition in termites seems to be mediated by food resource availability at local scale.

In social insects, individuals perceive variations in both quantity (Cristaldo et al. 2016a) and type of resources used by other colonies (Buczowski et al. 2005) through allocolonial cues. *Coptotermes formosanus*, for example, behave less aggressive during encounters with individuals of colonies that consumed the same food resource (Florane et al. 2004). In the present study, *N. aff. coxipoensis* increased the number of vibratory movements when confronted with individuals consuming the same food resource (Fig. 4). Vibroacoustic communication is widely diffused among social groups to the emission of warning signals (Hunt and Richard 2013). In termites, this communication is observed from the most basal groups (Delattre et al. 2015) to the most derived ones (Cristaldo et al. 2015). In addition to alert about risks, vibration in termite is also used during foraging activities to indicate nestmates about the amount of food resources found (Evans et al. 2005). In the advanced termite *Constrictotermes cyphergaster*, which has a foraging habit similar to *N. aff. coxipoensis*, the intensity of vibration frequency is modulated according to the stimulus source and the different types of vibration (drumming or shaking) can trigger distinct behaviors from alert to evasion (Cristaldo et al. 2015). In *N. aff. coxipoensis*, the emission of alertness via vibration among individuals consuming similar food resource seems to inform nestmates about the presence of possible competitors. The information of competitor presence via vibratory movements has already been demonstrated in termites. Evans et al. (2009) showed that the emission of vibration signals resulted in attraction among individuals of colony but triggered evasion of individuals from other species (e.g. a weak competitor). The behavior of transmitting the information about the competitor presence via vibration can be more efficient in *N. aff. coxipoensis*, since they forage on the surface of the ground, which could promote a faster propagation of the information among the nestmates.

Individuals of *N. aff. coxipoensis* can perceive the allocolonial odor cues and to choose cues from colonies under more abundant food availability compared to colonies under scarce food availability (Cristaldo et al 2016a). In addition, it has already been shown that *N. aff. coxipoensis* perform more fighting among individuals from neighboring colonies compared to those from distant one, especially when the local supply of resources is reduced (Ferreira et al. 2018). These results, together, allow us to infer that in natural conditions of food scarcity (*e.g.* where neighboring colonies are more likely to consume similar resources), individuals of *N. aff. coxipoensis* increase the number of vibratory movements informing the presence of competitors and consequently there would be a greater number of fighting between such colonies. This mechanism could explain patterns of spatial distribution and co-occurrence of termite species in the natural conditions. The overlapping of foraging areas by different termite species is depend of the local availability of resources, as already showed by Araújo et al. (2017). Thus, the mechanisms responsible for this pattern may be related to the results presented here, which include the capacity of perception and habituation to intercolonial signals and the consequent communication of alarm to nestmates.

5. CONCLUSIONS

In summary, the present study demonstrates that colonies of *N. aff. coxipoensis* can perceive allocolonial cues that were previously exposed and can communicate the presence of potential competitors increasing the number of vibratory movements. Thus, the use of social information can play a key role of the foraging decisions in termite species. Future studies are necessary to assess how widespread this capacity is present among the different Isoptera groups and the consequences of colony recognition odor cues on the use of space by termite species.

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REFERENCES

- Adams ES, Levings SC. 1987. Territory Size and Population Limits in Mangrove Termites. *J Anim Ecol* **56**: 1069–1081. <http://www.jstor.org/stable/4967>.
- Almeida CS, Cristaldo PF, Desouza O, Bacci L, Florencio DF, Cruz NG, Santos AA, Santana AS, Oliveira AP, Lima APS, et al. 2018. Resource density regulates the foraging investment in higher termite species. *Ecol Entomol* **43**: 371-378. <https://doi.org/10.1111/een.12508>.
- Almeida CS, Cristaldo PF, Florencio DF, Cruz NG, Santos AA, Oliveira AP, Santana AS, Ribeiro EJM, Lima APS, Bacci L, et al. 2016. Combined foraging strategy and soldier behaviour in *Nasutitermes* aff. *coxipoensis* (Blattodea: Termitoidea: Termitidae). *Behav Processes* **126**: 76-81. <https://doi.org/10.1016/j.beproc.2016.03.006>.
- Araújo APA, Cristaldo PF, Florencio DF, Araújo FS, DeSouza O. 2017. Resource suitability modulating spatial co-occurrence of soil-forager termites (Blattodea: Termitoidea). *Austral Entomol* **56**: 235-243. <https://doi.org/10.1111/aen.12226>.
- Binz H, Foitzik S, Staab F, Menzel F. 2014. The chemistry of competition: Exploitation of heterospecific cues depends on the dominance rank in the community. *Anim Behav* **94**: 45–53. <http://dx.doi.org/10.1016/j.anbehav.2014.05.024>.
- Blomquist G, Bagnères A. 2010. *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. eds. G. Blomquist and A. Bagnères. Cambridge Univ Press, Cambridge, UK.
- Buczowski G, Kumar R, Suib SL, Silverman J. 2005. Diet-related modification of cuticular hydrocarbon profiles of the argentine ant, *Linepithema humile*, diminishes intercolony aggression. *J Chem Ecol* **31**: 829–843.
- Costa DA, Carvalho RA De, Lima-Filho GF, Brandão D. 2009. Inquilines and Invertebrate Fauna Associated With Termite Nests of *Cornitermes cumulans* (Isoptera, Termitidae) in the Emas National Park, Mineiros, Goias, Brazil. *Sociobiology* **53**: 443–454.
- Crawley MJ. 2012. *The R Book*. John Wiley & Sons, Ltd, Chichester, UK.
- Cristaldo PF. 2018. Trail Pheromones in Termites - Termites and Sustainable Management: Volume 1 - Biology, Social Behaviour and Economic Importance. In (eds. M.A. Khan and W. Ahmad), pp. 145–158, Springer International Publishing, Cham https://doi.org/10.1007/978-3-319-72110-1_7.
- Cristaldo PF, Araújo APA, Almeida CS, Cruz NG, Ribeiro EJM, Rocha ML, Santana AS, Santos AA, Oliveira AP, DeSouza O, et al. 2016a. Resource availability influences aggression and response to chemical cues in the Neotropical termite *Nasutitermes* aff. *coxipoensis* (Termitidae: Nasutitermitinae). *Behav Ecol Sociobiol* **70**: 1257–165.
- Cristaldo PF, Jandák V, Kutalová K, Rodrigues VB, Brothánek M, Jiříček O, DeSouza O, Šobotník J. 2015. The nature of alarm communication in *Constrictotermes cyphergaster* (Blattodea: Termitoidea: Termitidae): the integration of chemical and vibroacoustic signals. *Biol Open* **4**: 1649-1659.

<http://bio.biologists.org/content/early/2015/10/31/bio.014084.abstract>.

- Cristaldo PF, Rodrigues VB, Elliot SL, Araújo APA, DeSouza O. 2016b. Heterospecific detection of host alarm cues by an inquiline termite species (Blattodea: Isoptera: Termitidae). *Anim Behav* **120**: 43–49. <http://www.sciencedirect.com/science/article/pii/S0003347216301518>.
- Delattre O, Sillam-Dussès D, Jandák V, Brothánek M, Rucker K, Bourguignon T, Vytisková B, Cvačka J, Jiříček O, Šobotník J. 2015. Complex alarm strategy in the most basal termite species. *Behav Ecol Sociobiol* **69**: 1945–1955. <http://dx.doi.org/10.1007/s00265-015-2007-9>.
- Dimarco RD, Farji-Brener AG, Premoli AC. 2010. Dear enemy phenomenon in the leaf-cutting ant *Acromyrmex lobicornis*: behavioral and genetic evidence. *Behav Ecol* **21**: 304–310. <http://dx.doi.org/10.1093/beheco/arp190>.
- Dunn R, Messier S. 1999. Evidence for the Opposite of the Dear Enemy Phenomenon in Termites. *J Insect Behav* **12**: 461–464. <https://doi.org/10.1023/A:1020958505815>.
- Evans T, Inta R, Lai J, Prueger S, Foo N, Fu E, Lenz M. 2009. Termites eavesdrop to avoid competitors. *Proc R Soc Lond B* **276**: 4035–4041.
- Evans TA, Lai JCS, Toledano E, McDowall L, Rakotonarivo S, Lenz M. 2005. Termites assess wood size by using vibration signals. *Proc Natl Acad Sci United States Am* **102**: 3732–3737. <http://www.pnas.org/content/102/10/3732.abstract>.
- Florane C, Bland J, Husseneder C, Raina A. 2004. Diet-mediated inter-colonial aggression in the Formosan subterranean termite *Coptotermes formosanus*. *J Chem Ecol* **30**: 2559–2574. <http://dx.doi.org/10.1007/s10886-004-7950-2>.
- Foubert E, Nowbahari E. 2008. Memory span for heterospecific individuals' odors in an ant, *Cataglyphis cursor*. *Learn Behav* **36**: 319–326.
- Glanzman DL. 2011. Olfactory habituation: Fresh insights from flies. *Proc Natl Acad Sci* **108**: 14711–14712. <http://www.pnas.org/cgi/doi/10.1073/pnas.1111230108>.
- Goodale E, Beauchamp G, Magrath RD, Nieh JC, Ruxton GD. 2010. Interspecific information transfer influences animal community structure. *Trends Ecol Evol* **25**: 354–361. <http://www.sciencedirect.com/science/article/pii/S0169534710000182>.
- Hölldobler B, Carlin NF. 1987. Anonymity and specificity in the chemical communication signals of social insects. *J Comp Physiol A* **161**: 567–581. <https://doi.org/10.1007/BF00603662>.
- Howard RW, Blomquist GJ. 2005. Ecological, Behavioral, and Biochemical Aspects of Insect Hydrocarbons. *Annu Rev Entomol* **50**: 371–393. <http://www.annualreviews.org/doi/10.1146/annurev.ento.50.071803.130359>.
- Hunt JH, Richard FJ. 2013. Intracolony vibroacoustic communication in social insects. *Insectes Soc* **60**: 403–417.
- J. DC, L. VE. 2004. Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. *Mol Ecol* **13**: 431–441. <https://doi.org/10.1046/j.1365-294X.2003.2065.x>.

- Korb J, Roux EA. 2012. Why join a neighbour: fitness consequences of colony fusions in termites. *J Evol Biol* **25**: 2161–2170. <http://doi.wiley.com/10.1111/j.1420-9101.2012.02617.x>.
- Langen TA, Tripet F, Nonacs P. 2000. The red and the black: Habituation and the dear-enemy phenomenon in two desert Pheidole ants. *Behav Ecol Sociobiol* **48**: 285–292.
- Leiser JK. 2003. When are neighbours ‘dear enemies’ and when are they not? The responses of territorial male variegated pupfish, *Cyprinodon variegatus*, to neighbours, strangers and heterospecifics. *Anim Behav* **65**: 453–462. <http://www.sciencedirect.com/science/article/pii/S0003347203920872>.
- Leonhardt SD, Menzel F, Nehring V, Schmitt T. 2016. Ecology and Evolution of Communication in Social Insects. *Cell* **164**: 1277–1287. <http://dx.doi.org/10.1016/j.cell.2016.01.035>.
- Levings SC, Adams ES. 1984. Intra- and Interspecific Territoriality in *Nasutitermes* (Isoptera: Termitidae) in a Panamanian Mangrove Forest. *J Anim Ecol* **53**: 705–714. <http://www.jstor.org/stable/4653>.
- Liang D, Silverman J. 2000. “You are what you eat”: Diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* **87**: 412–416. <https://doi.org/10.1007/s001140050752>.
- Marins A, Cristaldo PF, L. R. Paiva, Miramontes O, de Souza OFF. 2017. A new approach to mark termites (*Cornitermes cumulans* (Kollar) Blattodea: Isoptera) for laboratory bioassays. *Braz J Biol* impress.
- Matsuura K, Nishida T. 2001. Colony fusion in a termite: What makes the society “open”? *Insectes Soc* **48**: 378–383. <https://doi.org/10.1007/PL00001795>.
- Nimer E. 1972. Climatologia da região Nordeste do Brasil. Introdução à climatologia dinâmica. *Rev Bras Geogr* **34**: 3–51.
- Nowbahari E. 2007. Learning of colonial odor in the ant *Cataglyphis niger* (Hymenoptera; Formicidae). *Learn Behav* **35**: 87–94. <http://www.springerlink.com/index/10.3758/BF03193043>.
- Olugbemi BO. 2013. Intra- and Inter-colonial Agonistic Behavior in the Termite, *Microcerotermes fuscotibialis* Sjostedt (Isoptera: Termitidae: Termitinae). *J Insect Behav* **26**: 69–78.
- R Development Core Team. 2016. *R: A Language and Environment for Statistical Computing*. The R Foundation for Statistical Computing. ISBN: 3-900051-07-0, Vienna, Austria.
- Roisin Y, Dejean A, Corbara B, Orivel J, Samaniego M, Leponce M. 2006. Vertical stratification of the termite assemblage in a neotropical rainforest. *Oecologia* **149**: 301–311. <http://www.ncbi.nlm.nih.gov/pubmed/16791633>.
- Schuurman G. 2006. Foraging and Distribution Patterns in a Termite Assemblage Dominated by Fungus-Growing Species in Semi-Arid Northern Botswana. *J Trop Ecol* **22**: 277–287. <http://www.jstor.org/stable/4092047>.
- Silverman ED, Veit RR, Nevitt GA. 2004. Nearest neighbors as foraging cues: *Mar Ecol*

Prog Ser **277**: 25–35. <http://www.jstor.org/stable/24867724>.

- Suarez A V, Tsutsui ND, Holway DA, Case TJ. 1999. Behavioral and Genetic Differentiation Between Native and Introduced Populations of the Argentine Ant. *Biol Invasions* **1**: 43–53. <https://doi.org/10.1023/A:1010038413690>.
- Thomas ML, Payne-Makrisâ CM, Suarez A V, Tsutsui ND, Holway DA. 2007. Contact between supercolonies elevates aggression in Argentine ants. *Insectes Soc* **54**: 225–233. <https://doi.org/10.1007/s00040-007-0935-8>.
- Thomas ML, Tsutsui ND, Holway DA. 2005. Intraspecific competition influences the symmetry and intensity of aggression in the Argentine ant. *Behav Ecol* **16**: 472–481. <http://dx.doi.org/10.1093/beheco/ari014>.
- Van Zweden JS, D’Ettorre P. 2010. Nestmate recognition in social insects and the role of hydrocarbons. *Chem Commun* 222–243.
- Wilson E. 1971. *The Insect Societies*. Belknap Press of Harvard University Press, Cambridge, MA.

CONSIDERAÇÕES FINAIS

- A disponibilidade de recursos é um fator chave na modulação da agressividade de *N. aff. coxipoensis*. Em situações de escassez de recursos esses cupins exibiram maior número de lutas com vizinhos e seguiram menos os sinais de outras colônias do que sob maior oferta de recursos.
- A exposição prévia aos sinais de odor intercolonial não modulou a agressividade, no entanto os indivíduos se tornaram mais atraídos por estes sinais. Este resultado sugere que cupins poderiam utilizar pistas intercoloniais para exploração de fontes de recursos.
- O uso de recurso similar por diferentes colônias aumentou o número de vibrações dos indivíduos. Este aumento de sinais de alerta pode ser uma forma de indicar a presença de potenciais competidores.
- Assim, pode-se esperar que sob baixa oferta de recursos, onde as colônias possuem maior chance de utilizarem recursos similares, haja maior agressividade entre vizinhos.
- Estes resultados podem contribuir para a compreensão dos mecanismos responsáveis pela modulação da agressividade intercolonial, possibilitando assim o entendimento de padrões de coocorrência de cupins em diferentes escalas (ex. dentro e no entorno do ninho).