ANTÔNIO CLÁUDIO FERREIRA DA COSTA

CHEMICAL WEAPONS OF A TERMITE HOST AND ITS SIGNIFICANCE TO ITS INQUILINE

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

VIÇOSA
MINAS GERAIS - BRASIL
2017

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

C837c
2017

Costa, Antônio Cláudio Ferreira da, 1965-
Chemical weapons of a termite host and its significance to its inquiline / Antônio Cláudio Ferreira da Costa. - Viçosa, MG, 2017.
vi, 103f. : il. ; 29 cm.

Inclui apêndices.
Orientador: Eraldo Rodrigues de Lima.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Térmita. 2. Insetos - Comportamento. 3. Armas dos animais. 4. Defesas animais. I. Universidade Federal de Viçosa. Departamento de Biologia Animal. Programa de Pós-graduação em Entomologia. II. Título.

CDD 22 ed. 595.736

ANTÔNIO CLÁUDIO FERREIRA DA COSTA

CHEMICAL WEAPONS OF A TERMITE HOST AND ITS SIGNIFICANCE TO ITS INQUILINE

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

APROVADA: 9 de fevereiro de 2017.

Paulo Fellipe Cristaldo
(Coorientador)

Og Francisco Fonseca de Souza

Weyder Cristiano Santana

Ângela Alves de Almeida

Eraldo Rodrigues de Lima
(Orientador)

Aos meus pais,

*Antônio Cezário Martins da Costa (**in memoriam**) e*

*Iolanda Ferreira da Costa (**in memoriam**).*

AGRADECIMENTOS

A Deus, que sempre tem me acompanhado em todos os momentos da minha vida.

À Universidade Federal de Viçosa e ao Programa de Pós-Graduação em Entomologia, pela oportunidade de realizar o Doutorado.

À Empresa Agropecuária de Minas Gerais, EPAMIG que, por intermédio do Programa de Capacitação de Recursos Humanos, tornou possível a realização desse curso.

Ao professor Eraldo de Lima pela orientação, pelo apoio e pela iniciação à Ecologia Química dos insetos.

Ao professor Paulo Cristaldo, pela coorientação e pelo apoio ao longo dos fascinantes caminhos dos cupins.

Aos professores do Departamento de Entomologia da Universidade Federal de Viçosa, pela formação recebida.

À Eliane, da secretaria do curso de Pós-graduação em Entomologia, pelas informações e orientações.

À minha família, principalmente à minha esposa Lourdes Aparecida Melino da Costa pelo apoio e carinho.

Aos amigos do Laboratório de Semioquímicos e Comportamento de Insetos e de outros Laboratórios: Hernane, Sandra Mara, Carla Arce, Mateus, Morgana, Juliana, Rebeca, Diego, Josie, Manuel, Anyela, Laura Marcela, Amália Vitória, Nataly, Natália, Diogo, Vinícius, Verônica, Evandro, Raul, Elder, Julieth, Daniela Anita, Aline e Fernanda, agradeço a amizade e o apoio, principalmente nos momentos difíceis.

Aos amigos da EPAMIG Norte, em especial Polyanna Mara, Heloisa, Ariane, Alniusa, Maria Geralda, Ana Rosa, Ermínia, Mário Sérgio, João Batista, Nívio, Luciana, Edison José e Rubens Roberto, agradeço a amizade e a ajuda.

Ao Comitê de pós-graduação da EPAMIG, agradeço o apoio principalmente nos momentos difíceis.

Aos estagiários Patiely, Gabriela, Guilherme Steferson, Anderson, Heydelberg, Thiago, Felipe e Matheus, agradeço a ajuda nos trabalhos de campo.

SUMÁRIO

Página

ABSTRACT

COSTA, Antônio Cláudio Ferreira da, D.Sc., Universidade Federal de Viçosa, February, 2017. **Chemical weapons of a termite host and its significance to its inquiline** Adviser: Eraldo Rodrigues de Lima. Co-adviser: Paulo Fellipe Cristaldo.

The chemical defense is an important resource for termites deal with the conflictual interactions to which they are exposed, such as predator-prey, parasite-host and competition for food and shelter. There is a vast array of poisonous substances produced by the frontal gland of termite soldiers, which is the main exocrine gland responsible for defensive secretions of the phylogenetically derived Rhinotermitidae, Serritermitidae and Termitidae. In termite enemies these defensive secretions act as irritants, topical poisons, antihealing greases, immobilizer glues, repellents, unpalatability agents and inhibitors of fungal and bacterial grouwth. Even though, such impressive weaponry is circumvented by *Inquilinitermes microcerus* Silvestri, 1901 (Termitidae [Termitinae]), an obligatory inquiline of the nest of *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae [Nasutitermitinae]). The present work demonstrated that the frontal gland extract of *C. cyphergaster* causes no toxic or repelent effect on *I. microcerus*. These results suggest that *I. microcerus* has a detoxication mechanism comparable to that employed by *C. cyphergaster* conspecifics to avoid the toxic consequences of the defensive secretion from their own soldiers. This is the first record of the occurrence of detoxication in an obligatory inquiline against the defensive sectretion of its host.

RESUMO

COSTA, Antônio Cláudio Ferreira da, D.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Armas químicas de um cupim hospedeiro e a significância delas para o seu inquilino.** Orientador: Eraldo Rodrigues de Lima. Coorientador: Paulo Fellipe Cristaldo.

A defesa química é um importante recurso para os cupins lidarem com as interações conflituosas às quais eles estão expostos, tais como predador-presa, parasita-hospedeiro e competidores por alimento e abrigo. Há um vasto aparato de substâncias nocivas produzidas pela glândula frontal dos soldados dos cupins, a qual é a principal glândula exócrina responsável pelas secreções defensivas das famílias filogeneticamente derivadas, Rhinotermitidae, Serritermitidae e Termitidae. Nos inimigos dos cupins essas secreções defensivas atuam como irritantes, venenos de contato, géis anti-curativos, colas imobilizadoras, repelentes, agentes de impalatabilidade e inibidores de crescimento fúngico e bacteriano. Ainda assim, esse impressionante arsenal é mitigado por *Inquilinitermes microcerus* Silvestri, 1901 (Termitidae [Termitinae]), um inquilino obrigatório dos ninhos de *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae [Nasutitermitinae]). O presente trabalho demonstrou que o extrato da glândula frontal de soldados de *C. cyphergaster* não causa efeito tóxico ou repelente em *I. microcerus*. Esses resultados sugerem que *I. microcerus* possui um mecanismo de detoxificação comparável ao utilizado por coespecíficos de *C. cyphergaster* para não ser suscetível aos efeitos tóxicos da secreção de defesa dos seus próprios soldados. Esse é o primeiro registro da ocorrência de detoxificação em um cupim inquilino obrigatório contra a secreção de defesa do seu hospedeiro.

1 General Introduction

The insects that reached advanced levels of social life (e.g. bees, wasps, ants, and termites) are probably equivalent to more than half the biomass and are the most abundant of terrestrial arthropods (Hölldobler & Wilson, 2009). Helped by the benefits of colonial life, social insects were able to occupy the most convenient nest sites and defensible foraging places, despite that they represent only 2% of the approximately 900.000 known insect species in the world. On the other hand, solitary insects, such as cockroaches, grasshoopers, and beetles were displaced to the peripheral, remote and transient of living spaces (Hölldobler & Wilson, 2009).

Because of their greater abundance, social insects are more exposed to predators than solitary insects. Nevertheless, cooperative group behaviour in the defense of the colony also helps social insects to achieve environmental domination. So, individual casulties incurred in confrontations with enemies during foraging and nest defense does not affect the safety of the other members of the colony and lost combatants are replaced after a short time (Hölldobler & Wilson, 2009).

The evolutionary success of the termites was mostly because of the defensive strategies that they employ to protect the colony against predators (Prestwich, 1984a) and to challenge intra and interspecific competition for nesting and foraging areas (Thorne, 1982; Binder, 1988; Thorne & Haverty, 1991; Korb & Linsenmair, 2001; Thorne et al., 2003). These tactics comprise passive and active components. The passive element consists in the nest while the active one comprise the behavioral and anatomical defensive mechanisms (Cristaldo et al., *in press*).

The nest is composed by an intricate inner system of tunnels and galleries that work as a barrier, making the track of termites a very difficult task for their enemies. It is

designed to work as a fortification that protects the vulnerable inhabitants of the colony against their enemies and climate variations (Noirot & Darlington, 2000). These elaborated architectural configurations are considered as the most complex structures built by animals (Noirot, 1970; Noirot & Darlington, 2000).

The second set of strategies, the active elements, comprise the behavioral and anatomical defensive apparatus of soldiers and workers. Notwithstanding workers outnumber soldiers in a termite' colony, the the defense is the main task of the soldier caste which is supplied with an efficient collection of mechanical and chemical defensive adaptations. Soldiers of primitive species employ mainly mechanical defense and have strongly sclerotized head capsules harboring powerful mandibles, used to bite or snap (Deligne et al., 1982; Prestwich, 1984a). On the other hand, chemical defense is found in phylogenetically derived species where soldiers are well supplied with exocrine glands, among which, the frontal gland is the chief in this role (Prestwich, 1984a).

The frontal gland is an unpaired organ, present only in termites (Noirot, 1969), which is located in the soldier' head of the phylogenetically derived Rhinotermitidae, Serritermitidae and Termitidae families (Šobotník et al., 2010a). In some Termitidae (Nasutiterminae), soldiers produce a viscous secretion composed primarily by terpenoids which communicates alarm messages responsible for recruitment of nestmates (Roisin et al., 1990; Šobotník et al., 2008; Cristaldo et al., 2016), but they also act as repellents, irritants and toxic agents for a range of vertebrate and invertebrate species (e.g. Redford, 1984; Kuldová et al., 1999). Additionally, they also inhibit the development of fungi and bacteria, helping the control of nest microbes (Rosengaus et al., 2000).

Despite the efficient termite weaponry, some termite species can enter unnoticed and inhabit other termite nests (Cristaldo et al., 2012; Florencio et al., 2013; Cristaldo et al., 2016). The degree of interaction seems restricted to cohabitation without any attempt of the inquiline to make direct contact with the host. This association occurs in nests of *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae: Nasutitermitinae) found in the Brazilian *cerrado* which are frequently cohabited by one species of *Inquilinitermes*, either *I. fur* or *I. microcerus* (Termitidae: Termitinae) (Mathews, 1977). Interestingly, *I. microcerus* is able to follow *C. cyphergaster* trails (Cristaldo et al., 2014) as well as decode and and keep away from its alarm pheromone (Cristaldo et al., 2016). However, how the defensive secretion of the frontal gland of the host affects the inquiline remains unknown.

The work developed in this doctoral thesis aimed to analyse the harmful effects of the frontal gland secretion of termite soldiers. The first chapter is concerned with the literature review of the effects that the frontal gland secretions of termite soldiers have in their antagonists. In the second chapter, it was studied the toxic and repellent effects of the defensive secretion produced by *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae: Nasutitermitinae) on its obligatory inquiline *Inquilinitermes microcerus* Silvestri, 1901 (Termitidae: Termitinae). It was verified that the survivorship and repellence of inquiline groups were not significantly affected by the the frontal gland extracts of *C. cyphergaster* soldiers.

Chapter 1

Effect of chemical weapons from termites soldiers on vertebrates and invertebrates enemies: a review

Antônio C. F. Costa, Paulo F. Cristaldo & Eraldo R. Lima

Abstract

The chemical defence is an important resource that help termites to deal with the conflictual interactions to which they are exposed. There is a vast array of poisonous substances available from the frontal gland of termite soldiers, the main exocrine gland responsible for defensive secretions of the phylogenetically derived Rhinotermitidae, Serritermitidae and Termitidae. The combat strategies employed by the users of these chemical weapons range from one-to-one contact fights of few large soldiers to distant combat hold by numerous smaller soldiers and are associated with the configurations of the discharge system of the frontal gland secretion. The defensive secretions may act in the enemies by different ways. Irritants, promote grooming behaviours, that lead the contender to stop its offensive, topical poisons are found among highly reactive compounds and are very active topically, anti healing greases make the wounds that the mandibles cause difficult to heal, immobilizer glues are viscous substances that lead to the entanglement of the opponent, limiting or stopping their movements as well as obstructing their sensilla and spiracles, repellents force the opponent either to leave the conflictive area or stop its feeding, unpalatability agents modify the behavior of the receptor in order to discourage its feeding and inhibitors of fungal and bacterial growth are used by termites against microbial and invertebrate pathogens and parasites that they may find due their nesting and feeding habits.

Additionally, termites are also provided with detoxification routes in order to avoid casualties due to “friendly fire” from their own nestmate soldiers. Such impressive chemical weaponry allowed termites to leave the ancestor nesting type restricted to dead woods to colonize different ecological niches above the ground, build epigeous nests and explore a broader variety of food resources.

Key words: frontal gland, toxicity, defense

1 Introduction

Termites are among the most abundant and dominant insect group in terrestrial ecosystem in tropical regions and therefore constitute a possible abundant supply of food for predators (Traniello & Leuthold, 2000). The evolutionary success of termites is in large part due to their ability to digest lignocellulose and their efficient defensive adaptations that minimize the negative interactions with competitors and predators (ants and some specialized mamals and reptiles) (Thorne, 1982; Prestwich, 1984a, Binder, 1988; Thorne & Haverty, 1991; Hölldobler & Wilson, 1994; Korb & Linsenmair, 2001; Thorne et al., 2003).

The protection of termites against the attacks of ants, their main predator (Hölldobler & Wilson, 1994), as well as the intra and interspecific competition for nesting and foraging sites (Thorne, 1982; Binder, 1988; Thorne & Haverty, 1991; Korb & Linsenmair, 2001; Thorne et al., 2003) were the selective pressures that influenced the defensive adaptations of termites (Šobotník et al., 2010a).

The defensive strategies of termites consist of the physical barrier of the nest structure (passive defenses) combined with morphological and behavioral weapons employed by workers and soldiers (active defenses) (Cristaldo et al. *in press*).

1.1 The nest

The nest is the lifeless part of the colony, consisting in the structures that termites build, in order to live inside (Eggleton, 2011). There is a wide variety of nesting type which probably evolved together with social behavior and also connected to defense strategy as well as the creation of a controled microclimate for the colony (Noirot & Darlington, 2000). The primitive nest type was probably a dead wood, which the

colony uses as both shelter and food source as verified in Termopside (Eggleton, 2000; Kambhampati & Eggleton, 2000) one of the most primitive termite families (Higashi et al., 2000) (e.g. *Zootermopsis* [Termopsinae]) (Noirot & Darlington, 2000; Inward et al., 2007), Kalotermitidae and a few Rhinotermitidae and Termitidae (Kambhampati & Eggleton, 2000). As the colony grows older, its size and longevity are limited by the availability of the wood. An intermediate-type nesting consists in a net of subterranean galleries that connect diferent pieces of wood. This system allows the expansion of the colony to new pieces of wood, but the source of food still works as the nest (Noirot & Darlington, 2000; Inward et al., 2007) and is possible to have evolved separately in Mastotermitidae and most Rhinotermitidae (Kambhampati & Eggleton, 2000) (e.g. some *Schedorhinotermes* species [Rhinotermitinae]) (Noirot & Darlington, 2000; Inward et al., 2007). According the main tendency in the evolution of termite nest types, the most derived condition consists in the complete distinction between the nest and the food source, where the nest is built at a distance from the feeding substrate (Noirot & Darlington, 2000; Inward et al., 2007), what is observed in Hodotermitidae, a few Rhinotermitidae and most Termitidae (Kambhampati & Eggleton, 2000) (e.g. *Constrictotermes* [Nasutermitinae]).

1.2 The castes

The living part consist of the individuals that live inside the colony, comprising the castes of reproductives, workers and soldiers. The queen, the king and the alates belong to the reproductive caste. The queen is usually the only inhabitant of the colony which is able to lay eggs (Korb, 2008). The king is her life partner and seems to have the only function of mate with the queen at definite intervals of time. The alates are reproductives supplied with wings, produced in large numbers, that leave the nest in masse. After the flight, they land on the ground or on a dead wood and form a couple:

a male (the king) and one female (the queen) to constitute a new colony. The royal pair then breed and the queen start producing workers to care of all inhabitans of the colony, to build colony structures and to search for food. Soldiers will be produced a bit later, accordingly with the development of the colony. Alates will be produced again in the future when the recently founded colony will achieve maturity, continuing the life cicle (Eggleton, 2011).

1.2.1 The worker caste

Workers are the most abundant caste in the colony (Traniello & Leuthold, 2000) which are only found in species with either intermediate or separate-type nests (Inward et al., 2007) being absent in the primitive one-piece nest Termopsidae and Kalotermitidae as well as in a few Rhinotermitidae and Termitidae (Eggleton, 2011). This sterile caste is in charge for the care of the other castes of the colony, leaving the nest only to bring food and water (Eggleton, 2011). Additionally, workers also participate in the defense by aggression (biting), emergency repairing and building of the colony structures as well as removing the offspring to a safer place (Traniello & Leuthold, 2000).

1.2.2 The soldier caste

Soldiers belong to a sterile caste that evolved before workers (Thorne et al., 2003). Their major function is the defense of the colony, specifically the queen and the king (Eggleton, 2011), against predators, inter- and intraspecific competitors (Haverty & Howard, 1981; Thorne & Haverty, 1991; Roux & Korb, 2004), but may also take part in the search for new foraging areas, recruitment of workers to recently found resources (Traniello & Leuthold, 2000) and also egg care (Hanus et al., 2005). However, soldiers do not feed themselves and require to be tended by worker caste (Noirot & Darlington, 2000). The presence of sterile soldiers in all termite families,

exhibiting a wide variety of morphology shows the great significance of defense in the social evolution of termites. However, there was a derived condition of a secondary loss of the soldier caste in two subfamilies of Termitidae. In some soil-feeding species of Apicotermitinae (Higashi et al., 2000) and in three genera of Termitinae (Noirot & Darlington, 2000).

The weaponry of termite soldiers contains mechanical and chemical defensive resources. The mechanical weapons consist in primitive powerful mandibles derived from the worker mandibular type (Šobotník et al., 2010a). They are connected to a robust and well sclerotized head, which are found in the phylogenetically primitive families Termopsidae, Hodotermitidae and Kalotermitidae (Noirot & Darlington, 2000). However, it was also observed that mandibles can be reduced and non-functional in a few Rhinotermitidae and most of Nasutitermitinae (Noirot & Darlington, 2000). The chemical defenses are provided by the salivary or labial glands and the frontal gland. The labial glands exist in all castes of all termites. However, the frontal gland is peculiar among insects and was the main evolutionary novelty in termites, consisting in a synapomorphy of the phylogenetically derived families Rhinotermitidae, Serritermidae and Termitidae (Noirot & Darlington, 2000). This defensive gland produces an enormous diversity of harmful compounds and may be atrophied in workers, diminute in imagoes and greatly expanded in soldiers (Noirot & Darlington, 2000). The defensive substances produced by the frontal gland are employed exclusively as in some Termitidae (e.g. *Nasutitermes*, *Subulitermes* and *Constrictotermes* [Nasutitermitinae]) or combined with the mechanical defense of the soldier mandibles such as in Rhinotermitidae (e.g. *Coptotermes* [Coptotermitinae] and *Rhinotermes* [Rhinotermitinae]), Termitidae (e.g. *Macrotermes* [Macrotermitinae],

Syntermes and *Rhynchotermes* [Nasutitermitinae], *Cubitermes*, *Microcerotermes* and *Termes* [Termitinae]) (Noirot & Darlington, 2000).

The chemical defenses have probably evolved associated with the diversification of the nest habits of Isoptera (Traniello & Leuthold, 2000). The arising of derived separate-piece nesters obligated workers to forage outside the nest and in the open space, therefore becoming more exposed to predation (Noirot & Darlington, 2000). It is reasonable to think that epigeic foraging demanded continuous improvement of soldier defensive strategies in response to selective forces, evolving from one-to-one contact combats employed by robust biting soldiers to long distance smaller and abundant soldiers releasing noxious secretions produced by the frontal gland (Šobotník et al., 2010a). As a consequence, termites became able to explore new food sources from different ecossistems, less patchly distributed than dead wood (Inward et al., 2007).

This work comprises the information available in scientific articles and text books up to February 2017, concerning the defensive secretions produced by the soldier frontal gland of termite species and its effects over opponents such as predators, parasites and competitors for resources.

2 The source of soldier chemical weapons: the frontal gland

The frontal gland is an unpaired organ present only in termite species (Noirot, 1969). The representative structure of this defensive gland in the soldier is a single saclike reservoir located in its head connected to an opening in the frons, the fontanelle. The defensive secretion produced is then accumulated in the reservoir until a threat demands its use (Šobotník et al., 2010a).

The volume of the frontal gland reservoir varies from exclusively cephalic to extended until the abdomen (Noirot & Darlington, 2000). In Serritermitidae (e.g. *Serritermes*) and Rhinotermitidae (e.g. *Rhinotermes* [Rhinotermitinae], *Schedorhinotermes* [Rhinotermitinae], *Prorhinotermes* [Prorhinotermitinae] and *Coptotermes formosanus* [Coptotermitinae]) it may fill up to two-thirds of the abdominal cavity with the amount of secretion constituting over one third of the fresh body weight (Waller & La Fage, 1987) whereas in some Termitidae (e.g. *Cubitermes* [Termitinae], some *Macrotermes* [Macrotermitinae] and *Nasutitermes* [Nasutitermitinae]) it is limited to the head capsule and the amount of secretion achieves no more than one tenth of the soldier body weight (Prestwich, 1977).

There is a great diversity in the secretions produced by the frontal gland, in terms of their biochemical source, physical attributes and molecular structure. The soldier defensive chemicals can be grouped into three categories: Terpenoids (monoterpenes, sesquiterpenes and diterpenes), Acetate-derived (quinones, macrocyclic lactones, alkanes, alkenes, nitroalkenes, vinil ketones and ketoaldehydes), Proteins and Mucopolysaccharides (Prestwich, 1984a).

The information about the existence of the frontal gland in soldiers is widely recognized whereas there is scarce data about it in imagoes, presoldiers and workers (Quennedey, 1984; Šobotník et al., 2010a, c, d; Kutalová et al., 2013). Under the functional point of view, it is a secretory organ present in other castes than in soldiers (Kutalová et al., 2013) and two evidences suggest a defensive role for their secretions: First, in Rhinotermitidae, imagoes of *Prorhinotermes* [Prorhinotermitinae] exhibit the same noxious nitroolefins as those found in soldiers (Piskorski et al., 2009). Second, the defensive secretion occurs only in the dispersal flight and in the initial phase of the establishment of the colony, while the termites are especially vulnerable to predation.

After this, the production of the secretion is interrupted as soon as the first soldiers emerge (Piskorski et al., 2009).

The great variation in the size of the frontal gland in imagoes of Rhinotermitidae and Serritermitidae not always corresponds to the soldiers. So, it is probable that in these families the selective forces operated in the frontal gland of the imagoes rather than in the soldiers (Šobotník et al., 2010d). This is likely to occur because when imagoes go outside the nest they are are exposed to a wider variety of threats than soldiers.

In Rhinotermitidae, the shape of the frontal gland in presoldiers of *Prorhinotermes simplex* and *P. inopinatus* is a simple tube reaching the third abdominal segment. In this caste, the typical features of this gland were verified whereas the occurrence of the defensive secretion (E-1-nitropentadecene) was not detected (Šobotník et al., 2004).

Among some Termitidae subfamilies, the frontal gland of imagoes is deprived of reservoir in Foraminitermitinae and Macrotermitinae whereas it occurs in Sphaerotermitinae, Apicotermitinae, Termitinae, Syntermitinae and Nasutitermitinae. It was also verified that the size of the frontal gland was variable to each of these subfamilies as well as within them. Despite it had not been possible to detect substances produced by the frontal gland without reservoir, there are proofs that at least a small amount of secretion is produced. Besides, it is possible that they may have a different role from those glands with reservoir, acting as antibacterial or antifungal (Kutalová et al., 2013).

In the Apicotermitinae soldierless species, the frontal gland is well-developed and characterized as a functional secretory organ, despite of the absence of the reservoir. It is located in the head of workers and connected to the muscles in the same way as

in soldiers and imagoes, indicating that it is homologous to these castes. Nevertheless, its defensive function is still to be demonstrated (Šobotník et al., 2010c).

2.1. Mechanisms responsible for discharge of frontal gland secrections

One of the most extraordinary aspects about these chemical weapons is the diversity of adaptative attributes responsible for their discharge, which contribute to improve their effectiveness and reduce their wastage. The discharge mechanism of the secretion produced and stored in the frontal gland is composed by fontanelle, frons, clypeus and labrum. These morphological structures were subjected to a great diversification of their configurations, according the variety of the substances produced and their mode of action in order tho achieve the most efficient way to guide them to the target (Šobotník et al., 2010a).

The fontanelle is the outer opening of the frontal gland and it is located in the frons. In the simplest design of the discharging mechanisms the fontanelle is located at the level of the head surface as a small and almost unnoticeable frontal pore, through which the secretion simply exudes into the enemy (Fig. 1A). This primitive condition is found in some Rhinotermitidae (e.g. *Termitogeton* [Termitogetoninae]) and Termitidae (e.g. *Macrotermes* [Macrotermitinae] (Quennedey & Deligne, 1975; Deligne et al., 1982) and *Foraminitermes* [Termitinae] (Prestwich, 1984a)).

The assemblage of the frons, clypeus and labrum may also constitute a small channel by which the secretion flows from the fontanelle to the contender, as found in some Rhinotermitidae (e.g. *Psammotermes* [Psammotermitinae]) and Termitidae (e.g. *Amitermes* [Termitinae]) (Quennedey & Deligne, 1975; Deligne et al., 1982). Still in Rhinotermitidae, the advanced rhinotermitines exhibit the channel in the frons

modified into a marked groove along an extended labrum bearing a group of setae in the tip, in a form of a paintbrush (e.g. *Schedorhinotermes*, *Rhinotermes* and *Dolichorhinotermes* [Rhinotermitinae]) (Fig. 1B). The exudation of the secretion is then guided from the fontanelle to the extremity of the labrum and applied topically onto the rival (Quennedey & Deligne, 1975).

The fontanelle may also be repositioned toward the forepart of the head, still at its surface level or at the tip of a protuberance. The first configuration is found in some Rhinotermitidae (e.g. *Coptotermes* [Coptotermitinae]) and Termitidae (e.g. *Cubitermes* (Quennedey & Deligne, 1975; Deligne et al., 1982 and *Noditermes* [Termitinae] (Prestwich, 1984a)). The second configuration is found in some Termitidae where the projection may assume either a turret-like shape, as in some Macrotermitinae (e.g. medium and minor soldiers of *Acanthotermes*) (Deligne et al., 1982), or a conspicuous prominence (Fig. 1C) in some Termitinae bearing either symmetrical (e.g. *Cavitermes*, *Inquilinitermes*, *Termes*) or asymmetrical snapping mandibles (e.g. *Cornicapritermes*, *Dihoplotermes*) (Quennedey, 1984). In spite of the snapping mechanism be considered the foremost defensive system of these soldiers (Deligne et al., 1982), the existence of a frontal projection strongly suggests that these groups possess an operative frontal gland (Šobotník et al., 2010a), as verified in *Cavitermes tuberosus* (Termitidae [Termitinae]) (see Kyjaková et al., 2015).

Termite soldiers exhibiting the fontanelle as a frontal pore or located in a frontal projection employ hydrostatic pressure to release the defensive secretion, which is considered the ancestor evolutionary state. The increasing in the haemolimph pressure is promoted by the contraction of mandibular muscles during the biting process. The small reservoir of the frontal gland is then compressed and the secretion is oozed from the fontanelle (Kaji et al., 2016).

The efficiency of the chemical defenses of termites received notable improvements with the ability to expel forcibly the defensive secretion and to aim the discharge toward the contender. The arrangement of the morphological elements that allowed the accomplishment of these functionalities was the relocation of the fontanelle to the extremity of a duct constituted by the rostrum showing a variable extent. The advantages promoted by these novelties are remarkable. The defensive fluid been ejected under pressure increases the range of the chemical defense thus raising the chances that the enemy be reached before it causes injuries in the soldier (Eisner & Meinwald, 1966). The capacity to aim the discharge also contributes to reduce the wastage of the secretion and raise the precision to hit the target, thus enhancing the effectiveness of the defense (Eisner & Meinwald, 1966).

In some Termitidae, this defense mechanism occurs either combined with mechanical defenses, as in Syntermitinae (e.g. *Syntermes*) (Fig. 1D) or employed exclusively as in Nasutitermitinae (e.g. *Nasutiterme*s), which have the manbibles extremely reduced or vestigial (Šobotník et al., 2010b) (Fig. 1E). The Syntermitinae soldiers use their well-developed mandibles to pierce and trap the body of the enemy while the frontal gland secretion is released from the more or less developed nasus (Šobotník et al., 2010b). The large frontal gland is then emptied by direct pressure of mandibular muscles that expand during the bite. This configuration is considered an intermediate state in the evolutionary sequence of the releasing mechanism of the frontal gland secretion (Kaji et al., 2016).

The Nasutitermitinae soldiers, the nasutes, exhibits a “pear-shaped” or ampulae like head, almost entirely filled by the frontal gland, responsible for the production of a viscous and smelly secretion which is squirted in the contender when it is perceived. In this group the mandibles were decreased, simultaneously with the improvement of

a defensive strategy relying exclusively on expelled terpenoids (Emerson, 1961; Deligne et al., 1982; Prestwich, 1982; Prestwich, 1984a) and distant combat. The release of the defensive secretion in these soldiers is achieved by the compression of highly modified mandibular muscles that surround the enlarged frontal gland, consisting in the most derived configuration (Kaji et al., 2016).

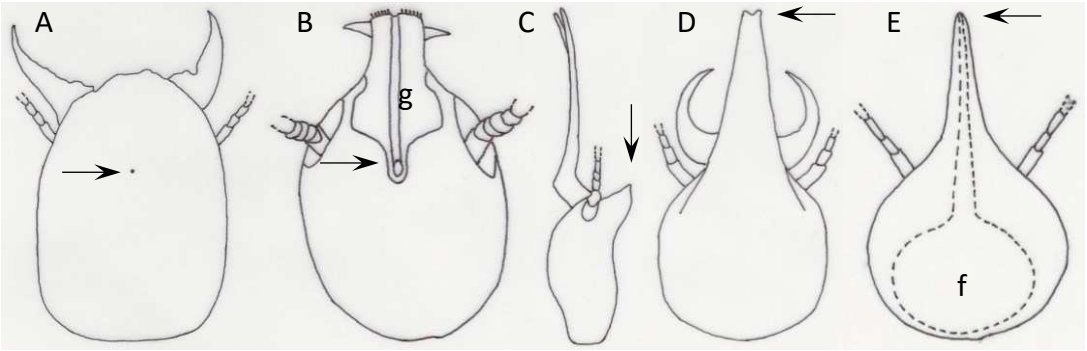


Figure 1. Termite soldier heads showing some discharge systems of the frontal gland secretion (arrow indicates the fontanelle). (A) Small and undetecteable frontal pore. (B) Groove (g) connecting the fontanelle to the tip of the labrum. (C) Fontanelle located in a frontal projection or (D) and (E) in the extremity of the rostrum. Dashed line in E depicts the limits of the frontal gland (f) and its conection with the fontanelle. (Redrawn from Quennedey et al. (1973) (B) and from fotos by Sobotník (C and E) and Cristaldo (A and D)).

A peculiar strategy to discharge the toxic secretion of the frontal gland is the suicidal defensive behavior where a sticky defensive secretion is liberated by the breaching of the soldier body at a specific site. This defense is carried out by some Rhinotermitidae (e.g. *Glossotermes* [Psammotermitinae]), Termitidae (e.g. *Apilitermes* and *Globitermes sulphureus* [Termitinae]), Serritermitidae (e.g. *Serritermes serrifer*). Interestingly, the region of the termite's body where the fracture occurs varies from species to species (Costa-Leonardo & Kitayama, 1991; Bordereau et al., 1997; Deligne

and DeConinck, 2006; Šobotník et al., 2010a). The secretion is produced by a modified frontal gland, which fills a large part of the abdomen and the thorax and has no outside opening. Strong contractions of the abdominal wall breach the gland and the integument at a frailty region of the thoracic sternum, exactly before the fore coxae, allowing a yellowish secretion to flow slowly to the lower position of the head capsule instead of being thrown onto the opponent. Soldiers in this condition are capable of combat for some minutes, thus preventing the scape of the intruder once the secretion quickly becomes rigid when exposed to air (Bordereau et al., 1997).

3 Effects of the substances secreted by the soldier frontal gland

The defensive secretions of the frontal gland can act in the enemies as irritants, topical poisons, anti healing greases, immobilizer glues, repellents, unpalatability agents and inhibitors of fungal and bacterial growth (Table 1).

Substances showing irritant effects are produced by a great number of biting, slashing or piercing mandibulated termites which consist in volatile fatty acid and terpenoid compounds with 10 to 20 carbon atoms, among which sesquiterpenes and monoterpenes occur more frequently. They may also cause repellence, deterrence or disorientation in the enemies dissuading them from attack independently of noxious or physical handicapping effects, also present in the defensive secretion (e.g. elicit a grooming behavior in ants which leads to the cessation of the offensive) (Prestwich, 1984a).

Some compounds also perform a supplementary role also as irritants as the monoterpenes in Rhinotermitidae (Prorhinotermitinae) (e.g. farnesene in *Prorhinotermes*) (Prestwich, 1984a) and mellein, in some Termitidae (Syntermitinae)

(e.g. farnesene in *Cornitermes*) (Blum et al., 1982) (Table 1). Mellein is a volatile phenolic compound that, besides its irritant effect, have a noxious activity against *Calliphora erytrocephala* (Diptera [Caliphoridae]) (Claydon et al., 1979). It is also one of the components of the trail pheromone of the ant *Lasius fuliginosus* (Hymenoptera [Formicidae]) (Kern et al., 1997) and a defense substance of the thrips *Haplothrips leucanthemi* (Thysanoptera: Phlaeothripidae) against *Solenopsis invicta* (Hymenopera [Formicidae]) (Blum et al., 1992). Additionally, (-)-mellein is also known to have larvicidal effect on *Aedes aegypti* (Diptera [Culicidae]) (Kendagor et al., 2013).

Table 1. Effects of chemical substances presents in the frontal gland secretions of termite species verified on heterospecifics

Family	**Subfamily**	**Species**	**Chemical substance**	**Effect**	**Organism tested**	**Reference**
Rhinotermitidae	Prorhinotermitinae	*Prorhinotermes simplex*	(E-1-nitropentadec-1-ene)	Contact poisons	*Musca domestica* (Diptera: Muscidae)	Hrdý et al., 1977; Kuldová et al., 1999
Rhinotermitidae	Prorhinotermitinae	*Prorhinotermes simplex*	(E-1-nitropentadec-1-ene)	Contact poison	Workers of *Schedorhinotermes lamanianus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)	Spanton & Prestwich, 1981
Rhinotermitidae	Prorhinotermitinae	*Schedorhinotermes putorius*	Ketones and β-ketoaldehydes	Contact poison	*Formica rufa*, *Lasius niger*, *Myrmica rubra* and *Leptothorax tuberum* (Hymenoptera; Formicidae)	Quennedey et al., 1973

(Continued)

Table 1 (Continued)

Family	Subfamily	Species	Chemical substance	Effect	Organism tested	Reference
Rhinotermitidae	Heterotermitinae	*Reticulitermes santonensis*, *R. grassei* and *R. banyulensis*	Geranylinalool	Contact poison	Predatory ants (*Pheidole pallidula*, *Hypoponera eduardi*, *Leptothorax unifasciatus*, *L. nylanderi*, *L. parvulus*, *Myrmica rugulosa*, *Aphaenogaster subterranea*, *Crematogaster scutellaris* and *Monomorium* sp. (Hymenoptera: Formicidae)), ant competitors (*Leptothorax lichensteni*, *L. racovitzae* and *Lasius emarginatus* (Hymenoptera: Formicidae) and fifth-instar larvae of *Schistocerca gregaria* (Orthoptera: Cyrtacanthacridinae)	Lemaire et al., 1990

(Continued)

Table 1 (Continued)

Family	Subfamily	Species	Chemical substance	Effect	Organism tested	Reference
Rhinotermitidae	Rhinotermitinae	*Schedorhinotermes lamanianus*	Vinil ketones	Contact poison	Workers of *Prorhinotermes simplex* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)	Spanton & Prestwich, 1982
Rhinotermitidae	Rhinotermitinae	*Schedorhinotermes putorius*	Vinil ketones	Irritation, Paralization and death	*Formica rufa*, *Lasius niger*, *Myrmica rubra* and *Leptothorax tuberum* (Hymenoptera; Formicidae)	Quennedey et al., 1973
Rhinotermitidae	Coptotermitinae	*Coptotermes formosanus* Shiraki	Naphthalene	Inhibition of growth	Common nest saprophytic fungus *Mucor* sp.	Wiltz et al., 1998
Termitidae	Macrotermitinae	*Ancistotermes sp.*	Dialdehyde and furanoid sesquiterpenes	Contact poison or olfactory deterrent	*Megaponera sp.* (Hymenoptera; Formicidae)	Prestwich, 1984a
Termitidae	Nasutitermitinae	*Velocitermes velox*	Diterpenes	Contact poison	*Musca domestica* (Diptera: Muscidae)	Valterová et al., 1988

(Continued)

Table 1 (Continued)

Family	Subfamily	Species	Chemical substance	Effect	Organism tested	Reference
Termitidae	Nasutitermitinae	*Nasutitermes exitiosus*	Monoterpenes α and β-pinene	Irritation	*Musca domestica* (Diptera: Muscidae) and *Periplaneta americana* (Blatodea: Blatellidae)	Eisner et al., 1976
Termitidae	Nasutitermitinae	*Nasutitermes corniger* (Motschulsky), *N. nigriceps* (Haldeman) and *N. costalis* (Holmgren)	Monoterpenes α-pinene and ß-pinene	Irritation	Neotropical anteaters *Tamandua mexicana* (Saussure) and *T. tetradactyla* (L.) (Edentata: Myrmecophagidae)	Lubin & Montgomery, 1981
Termitidae	Nasutitermitinae	*Nasutitermes corniger* (Motschulsky), *N. nigriceps* (Haldeman) and *N. costalis* (Holmgren)	Monoterpenes α-pinene and ß-pinene	Unpalatability	Neotropical anteaters *Tamandua mexicana* (Saussure) and *T. tetradactyla* (L.) (Edentata: Myrmecophagidae)	Lubin & Montgomery, 1981

(Continued)

Table 1 (Continued)

Family	**Subfamily**	**Species**	**Chemical substance**	**Effect**	**Organism tested**	**Reference**
Termitidae	Macrotermitinae	*Macrotermes subhyalinus, M. michelseni*	Unbranced alkenes with 23 to 35 carbon atoms and (Z)-9 alkenes with 27, 29 and 31 carbon atoms	Anti healing	*Megaponera foetens* (Hymenoptera; Formicidae)	Prestwich et al., 1977; Meinwald et al., 1978
Termitidae	Syntermitinae	*Cornitermes sp.*	Mellein and phenyl-acetaldehyde	Irritation, deterrency, long-lasting cleaning and wiping behavior	Ants (Hymenoptera; Formicidae)	Blum et al., 1982

There is a great diversity in the irritant compounds exhibited among termite species according their geographical ocurrence, as exemplified by the genus *Amitermes* (Termitidae [Termitinae]). Many of the irritants produced by Neotropical species (*A. wheeleri* and *A. excellens*) are related to African species (*A. unidentatus*, *A. messinae* and *A. evuncifer*). On the other hand, the monoterpenes produced by Australian species are absent in the Neotropical and African groups mentioned before (Prestwich, 1984a).

The contact toxicity is frequently found among the defensive secretion of some Rhinotermitidae species. In Prorhinotermitinae, the nitroalkenes (or nitro olefins) produced are highly reactive lipophilic compounds and, therefore, very active topically (e.g. (E)-1-nitropentadec-1-ene constitutes more than 90% of the secretion of *Prorhinotermes simplex*) (Vrkoč & Ubik, 1974; Hanus et al., 2006; Piskorski et al., 2007). The defense strategy employed by these mandibulate soldiers combines biting and smearing the frontal gland secretion in the injuries inflicted to the victims (Spanton & Prestwich, 1981).

The representatives of the Rhinotermitinae secrete predominantly ketones and β-ketoaldehydes, which also work as strong topical poisons (Prestwich & Collins, 1980; 1982; Chuah et al., 1990). These substances are also responsible from 35 to 50 % of the dry weight of the abundant and fast pace small soldiers of *Schedorhinotermes*, *Rhinotermes*, *Dolichorhinotermes* and *Acorhinotermes* (Quennedey et al., 1973; Prestwich & Collins, 1980; 1982) and promoted the development of a specialized minor soldier caste with a labral brush (Prestwich & Collins, 1982; Prestwich, 1983). These nasutoid soldiers use their longer labral brush to quickly apply the frontal secretion containing contact poisons and olfactory toxins more chemically reactive and thus more powerful (Prestwich, 1984a).

In Termitidae, the contact toxicity is provided by high amount of monoterpenes (Baker et al., 1981; Prestwich, 1984a; Everaerts et al., 1988) and diterpenes (Valterová et al., 1988; Lemaire et al., 1990) in the viscous secretion of some Nasutitermitinae. Interestingly, the diterpenes synthetized by termites seem to be an ability unique in insects (Everaerts et al., 1988)

As important as the existing toxicity of a defensive secretion is its capacity to diffuse through the enemy integument, otherwise, unless promptly effective, it may produce no practical protection. It does not seem to be a great difficult regarding vertebrates once that these animals begin the attack exposing the permeable and high sensitive mucous surfaces of their head, thus having high chances to be reached and quickly affected by the defensive compounds (Eisner & Meinwald, 1966). This may be the likely explanation for the reactions of the medium-sized Neotropical anteaters to Nasutitermitinae. While these predators were attacking their nests, they showed typical behaviors as brushing their nose, grooming their fur with the foreclaws, scratching themselves with the hind feet and sneezing (Lubin & Montgomery, 1981).

But, in the case of arthropods, the mechanism that allows defensive compounds to trespass the cuticle is to match the polarity between the components of the secretion and the epicuticle. The major cuticular layer in arthropods is the epicuticle and it is resistant to the flow of hydrophilic substances. It is a thin external covering, containing lipoproteins and a high proportion of wax, which is responsible for turning the cuticle impervious to water. As a result, it can restrict the displacement of wax insoluble compounds through the cuticle. On the other hand, the nitroalkenes produced by soldiers of Prorhinotermitinae, due to their lipophilic properties may have free transit through the epicuticle. Since the defensive secretion is generally composed by more than one substance, it is also possible that some lipophilic secondary toxic components

also work as an adjuvant to interfere with the epicuticle, helping the penetration of the main toxicant through the tegument of the victim (Eisner & Meinwald, 1966). Therefore, chemical affinity between the defensive secretion and the epicuticle is probably one of the key factors for the effectiveness of these contact poisons.

The obstacle imposed by the exoskeleton to the penetration of the defensive secretion can be overcome also by physical action. It can be mechanically damaged by the slashing or piercing mandibles of soldiers and a defensive substance specific to this situation is applied in the wounded area: the anti-healing greases. They are relatively nonvolatile and non-polar substances with 20 carbon atoms and up, that are semisolids at ambient temperatures, consisting in alkenes, polyunsaturaed diterpene hidrocarbons and oily lipophilic macrocyclic lactones.

These defensive secretions are commonly associated with the slashing mandibles of the Termitidae and make the poisoned injuries more difficult to heal (Prestwich, 1984a). It seems that the long-chain saturated and monounsaturated hydrocarbons and other lipophilic compounds as lactones present in the secretion prevent the healing of these wounds due to their similarity with the also long-chain hidrocarbons of the cuticular lipids. As a consequence, the contender is led to death by dehydratation (Meinwald et al., 1978). This strategy is adopted by some Termitidae such as in Macrotermitinae (e.g. *Macrotermes subhyalinus* and *M. michelseni*) and Syntermitinae (e.g. *Armitermes* and *Rhynchotermes*), smearing their antihealing greases in the wounds made by their mandibles (Prestwich et al., 1977; 1982).

Glues are viscous substances that lead to the entanglement of the opponent. This chemical defense is suitable for use in a great variety of enemies (Prestwich, 1979) once it may act mechanically by limiting or stopping their movements (e.g.

entanglement of ants) (Prestwich, 1984a) as well as obstructing their sensilla and spiracles (Šobotník et al., 2010a).

In some Rhinotermitidae (Coptotermitinae), one of these glues are composed by aqueous lipid-glycoprotein-mucopolisaccharidae mixtures present in the defensive secretion (e.g. *Coptotermes*). In some Termitidae (Nasutitermitinae), the diterpenes are believed to cause the solidification of the defensive secretion in by polymerization, when it gets in contact with the air (Prestwich, 1979).

Besides causing impairment consequences, glues may also have convenient additional effects as adjuvants in the defensive secretion, which is generally composed by a mixture of two or more components. First, they can work as wetting agents (Prestwich, 1979) contributing to spread the secretion widely over the surface of the cuticle of the contender, thus expanding the area of action of other toxicant components (Eisner & Meinwald, 1966). Second, they can also help to keep the stickiness of the secretion for a longer period of time (Prestwich, 1979).

Both beneficial consequences can be verified in the combination of monoterpenes and diterpenes in the defensive secretion of some Termitidae (Nasutitermitinae). The hydrophobic monoterpene hydrocarbons, present in high proportion in the viscous secretion of these soldiers, work as a good wetting agent spreading well throughout the lipophilic surface of the rival. Also, the preservation of the viscosity of the secretion is achieved by the reduction in the rates of evaporation of the monoterpenes, generated by the presence of small concentration of diterpenes (Prestwich, 1979).

An effective repellent must force the contender (competitor or predator) either to leave the conflictive area or to stop its feeding (Eisner & Meinwald, 1966).

The unpalatability agents are substances that modify the behavior of the receptor in order to discourage its feeding (e.g. differential preferrence of castes as preys). They are likely to affect vertebrates as anteaters (Lubin & Montgomery, 1981; Cunha et al., 2015), lizards (Fuller et al., 2003) and invertebrates (Waller & La Fage, 1987).

The nesting and feeding habits of termites make them to be at risk of contact with microbial and invertebrate pathogens and parasites. Pathogenic and competitor fungi are commonly found associated with termites and their nests. Additionally, termite life history can cause recurrent increasing susceptibility to parasites, and sociality may elevate transmission rates of infection within colonies (Rosengaus et al., 2011). However, the detection of compounds in the frontal gland secretion that have antifungal and antibacterial properties shows that natural selection enabled termites to tackle with this threat such as Naphthalene (Chen et al., 1998a, b; Wiltz et al., 1998; Wright et al., 2000; Zhang et al., 2006) the monoterpenes α-pinene and limonene (Rosengaus et al., 2000) amongst other substances (Lutikova, 1990; Fuller, 2007).

4 Autodetoxification

The use of chemical weapons by organisms necessarily requires the availability of detoxification routes in order to avoid casualties among themselves due to “friendly fire”, as verified in the determination of interspecific toxicities of the defensive secretions of some Rhinotermitidae (e.g. *Prorhinotermes simplex* (Hagen) [Prorhinotermitinae]) and *Schenorhinotermes lamanianus* [Rhinotermitinae]) on workers of both species and on that of *Reticulitermes flavipes* (Rhinotermitidae [Heterotermitinae]). The results showed no noxious effect of the two secretions on conspecifics whereas heterospecifics were severely affected. That is because nestmate

workers are able to detoxify these poisonous nitroalkenes through a biochemical pathway mediated by a specific reductase, resulting in a non-toxic product (Spanton & Prestwich, 1981).

Also, In Termitidae, the Nasutitermitinae are not vulnerable to the defensive secretion of their conspecifics soldiers most likely because of autodetoxification mechanisms (Spanton & Prestwich, 1981; 1982; Thorne, 1982; Lefeuve & Bordereau, 1984; Thorne & Haverty, 1991).

5 Conclusion

The chemical defence is an important novel weapon that help termites to deal with negative interactions to which they are exposed. There is a vast array of defensive substances available from the frontal gland of termite soldiers, the main exocrine gland responsible for defensive secretions in Neoisoptera group (Rhinotermitidae, Serritermitidae and Termitidae). It allowed the transition from the ancestor nesting type restricted to dead woods and under the soil surface to the derived epigeous nesting and open air foraging.

The primitive strategy employed to release the defensive secretion is found where the secretion produced by a small frontal gland gradually flows during the soldier biting. It is used in one-to-one combats and the soldier have to trap the opponent time enough to allow the secretion reach the target. Additionally, the secretion has to enter the wounds and the poisoning and dehydratation effects do not take the opponent out of combat imediately.

A larger frontal gland combined with mandibular defenses is situated in an intermediate evolutionary state of chemical defense. A greater amount of defensive

secretion is released by smaller soldiers but the combat strategy still relies on contact fights.

In some Termitidae (Nasutitermitinae), soldiers are supplied with a very expanded frontal gland connected to a long tube with the fontanelle located at its point. This configuration consists in an evolutionary derived morphology specialized to eject defensive secretions enabling these abundant and small soldiers to combat the enemies at a safe distance. Therefore, the mandibular decreasing verified in the nasute soldiers, was associated to the development of the most advanced chemical secretions that promote in the enemies the combined effects of irritants, topical poisons, anti-healing greases, immobilizer glues, repellents, unpalatability agents and inhibitors of fungus growing. Undoubtely, these chemical defensive attributes made possible to forage in open air and helped the Nasutitermitinae to be evolutionarily well succeeded, as they comprise more than 20% of all termite species and successfully colonize different kinds of ecological niches.

6 References

Baker, R., Edwards, M., Evans, D. A., & Walmsley, S. (1981). Soldier-specific chemicals of the termite *Curvitermes strictinasus* Mathews (Isoptera, Nasutitermitinae). *Journal of Chemical Ecology*, 7, 127–133.

Binder, B. F. (1988). Intercolonial aggression in the subterranean termite *Heterotermes aureus* (Isoptera: Rhinotermitidae). *Psyche*, 95, 123-137.Blum, M. S., Jones, T. H., & Howard, W. L. (1982). Overall Biochemistry of termite defenses: *Coptotermes*, *Rhinotermes* and *Cornitermes* species. *Comparative Biochemistry and Physiology* B, 71, 731-733.

Blum, M. S., Foottit R., & Fales, H.M. (1992). Defensive chemistry and function of the anal exudate of the thrips *Haplothrips leucanthemi*. *Comparative Biochemistry and Physiology*, 102C, 209-211.

Bordereau, C., Robert, A., van Tuyen, V., & Peppuy, A. (1997). Suicidal defensive behaviour by frontal gland dehiscence in *Globitermes sulphureus* Haviland soldiers (Isoptera). *Insectes Sociaux*, 44, 289-297.

Calaby, J. H. (1960). Observations on the banded ant-eater, *Myrmecobius f. fasciatus* Waterhouse (Marsupialia), with particular reference to its food habits. *Proceedings of the Zoological Society of London*, 135, 183-207.

Chen, J., Henderson, G., Grimm, C. C., Lloyd, S. W., & Laine, R. A. (1998a). Naphthalene in Formosan subterranean termite carton nests. *Journal of Agricultural and Food Chemistry*, 46, 2337-2339.

Chen, J. A., Henderson, G., Grimm, C. C., Lloyd, S. W., & Laine, R. A. (1998b). Termites fumigate their nests with naphthalene. *Nature*, 392, 558.

Chuah, C. H., Goh, S. H., & Tho, Y. P. (1990). Chemical defense secretions of some species of Malaysian Rhinotermitidae (Isoptera, Rhinotermitidae. *Journal of Chemical Ecology*, 16, 685-692.

Claydon, N., Grove, J. F., & Pople, M. (1979). Insecticidal secondary metabolic products from the entomogenous fungus *Fusarium larvarum. Journal of Invertebrate Pathology*, 33*,* 364-367.

Costa-Leonardo, A. M., & Kitayama, K. (1991). Frontal gland dehiscence in the Brazilian termite *Serritermes serrifer* (Isoptera: Serritermitidae). *Sociobiology*, 19, 333-338.

Cristaldo, P. F., Araújo, A. P. A., Rosa, C. S., Florencio, D. F., Marins, A., & DeSouza, O. Defensive behaviour in two termite species (Blattodea: Isoptera): the role of workers and nest's structure. Brazilian Journal of Biology (*in press*).

Cristaldo, P. F., Rosa, C. S., Florencio, D. F., Marins, A., & DeSouza, O. (2012). Termitarium volume as a determinant of invasion by obligatory obligatory termitophiles and inquilines in the nests of *Constrictotermes cyphergaster* (Termitidae, Nasutitermitinae). *Insectes Sociaux*, 59, 541-548.

Cristaldo, P. F., Rodrigues, V. B., Elliot S. L., Araújo, A. P. A., & DeSouza, O. (2016). Heterospecific detection of host alarm cues by an inquiline termite species (Blattodea: Isoptera: Termitidae). *Animal Behaviour*, 120, 43-49.

Cunha, H., Costa, D. A., Santo-Filho, K., & Brandão, D. (2003). Relationship between *Constrictotermes cyphergaster* and inquiline termites in the Cerrado (Isoptera: Termitidae). *Sociobiology*, 42, 1-10.

Cunha, H. F., Carrijo, T. F., Prestes, A. C., Arruda, L. S., Rezende, P. B., Santos, T., & Brandão, D. (2015). Food preference of giant anteater and collared anteater (Pilosa, Myrmecophagidae) regarding the termite defense strategies. *Bioscience Journal*, *31*(1), 234-241.

Deligne, J., Quennedey, A., & Blum, M. S. (1982). The enemies and defense mechanism of termites. In: Hermann, H. R. (Ed.). *Social Insects*. Vol. II. Academic Press, New York, 491p.

Deligne, J., DeConinck, E. (2006), Suicidal defence through a dehiscent frontal weapon in *Apilitermes longiceps* soldiers (Isoptera: Termitidae). *Belgian Journal of Entomology*, 8, 3-10.

Eggleton, P. (2000). Global patterns of tetrmite diversity p. 25-51 In: Abe, T., Bignell, D. E. & Higashi, M. (Ed.). *Termites:* Evolution, sociality, symbioses, ecology. Kluwer Academic Publishers, London, pp. 25-51.

Eggleton, P. (2011). An introduction to Termites: Biology, Taxonomy and Functional Morphology In: Bignell, D. E., Roisin, Y., & Lo, N. (Ed.). *Biology of termites*. London: Springer. pp. 1-26.

Eisner, T., & Meinwald, J. (1966). Defensive secretions of arthropods. *Science*, *153*(3742), 1341-1350.

Eisner, T., Kriston, I. & Aneshansley, D. J. (1976). Defensive behaviour of a termite (*Nasutitermes exitiosus*). *Behavioural Ecology and Sociobiology*, 1, 83-125.

Emerson, A. E., (1961). Vestigial characters of termites and processes of regressive evolution. *Evolution*, 15, 115-131.

Everaerts, C., Pasteels, J. M., Roisin, Y., & Bonnard, O. (1988). The monoterpenoid fraction of the defensive secretion in Nasutitermitinae from Papua New Guinea. *Biochemical and Systematic Ecology*, 16, 437-444.

Florencio, D. F., Marins, A., Cristaldo, P. F., Araujo, A. P. A., Silva, I. R., & DeSouza, O. (2013). Diet segregation between cohabiting builder and inquiline termite species. *PLoS ONE*, 8, e66535.

Fuller, C. A., Rock, P., & Philips, T. (2003). Behavior, color changes, and predation risk induced by Acanthocephalan parasitism in the Caribbean Termite *Nasutitermes acujutlae*. *Caribbean Journal of Science*, *39*(1), 128-135.

Fuller, C. A., (2007). Fungistatic activity of freshly killed termite, *Nasutitermes acajutlae*, soldiers in the Caribbean. *Journal of Insect Science*, 7, 1-8.

Hanus, R., Šobotník, J. & Cizek, L. (2005). Egg care by termite soldiers. *Insectes Sociaux*, 52, 357–359.

Hanus, R., Šobotník, J., Valterová, I., & Lukáš, J. (2006). The ontogeny of soldiers in *Prorhinotermes simplex* (Isoptera, Rhinotermitidae). *Insectes Sociaux*, 53, 249-257.

Haverty M. I. & Howard, R. W. (1981). Production of soldiers and maintenance of soldier proportions by laboratory experimental groups of *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks) (Isoptera: Rhinotermitidae). *Insectes Sociaux* 28, 32-39.

Higashi, M., Yamamura, N. & Abe, T. (2000). Theories on the sociality of termites In: Abe, T., Bignell, D. E. & Higashi, M. (Ed.). *Termites:* Evolution, sociality, symbioses, ecology. Kluwer Academic Publishers, London, pp. 169-187.

Hölldobler, B. & Wilson, E. O. (1994). *The ants*. The Belknap Press of Harvard University Press Cambridge, Massachusetts.

Hölldobler, B. & Wilson, E. O. (2009). *The superorganism:* The beauty, elegance, and strangeness of insect societies. W. W. Norton & Company, NY, 522 pp.

Hrdý, I., Křeček, J. & Vrkoč, J. (1977). Biological activity of soldier secretions in the termite *Nasutitermes ripertii*, *N. costalis* and *Prorhinotermes simplex*. In: Proceedings, VIIIth Congress IUSSI, Wageningen, p. 303.

Inward, D. J. G., Vogler A. P. & Eggleton P. (2007). A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Molecular Phylogenetics and Evolution* 44: 953–967.

Kaib, M. (1982). Disruption of ant recruitment by the frontal gland secretion of a termite: a chemical defense strategy. In: Breed, M. D., Michener, C. D., & Evans, H. E. (Ed.). *The Biology of Social Insects*. Westview Press, Boulder, Colorado, pp. 385-389.

Kaji, T., Keiler, J., Bourguignon, T., & Miura, T. (2016). Functional transformation series and the evolutionary origin of novel forms: evidence from a remarkable termite defensive organ. *Evolution & Development* 18(2), 78-88.

Kambhampati, S. & Eggleton, P. (2000) Taxonomy and Phylogeny of termites In: Abe, T., Bignell, D. E. & Higashi, M. (Ed.). *Termites:* Evolution, sociality, symbioses, ecology. Kluwer Academic Publishers, London, pp. 1-23.

Kendagor, A. C., Langat, M. K, Cheplogoi, P. K, & Omolo, J. O. (2013). Larvicidal activity of mellein from cultures of an ascomycete *Pezicula livida* against *Aedes aegypti*. *International. Journal of Life Sciences Biotechnology & Pharma Research*, 2, 70-80.

Kern, F., Klein, R. W., Janssen, E., Bestmann, H. J., Attygalle, A. B., Schaefer, D., & Maschwitz, U. (1997). Pheromones 103. Mellein, a trail pheromone component of the ant *Lasius fuliginosus*. *Journal of Chemical Ecology*, 23, 779-792.

Korb, J. & Linsenmair, K. E. (2001). The causes of spatial patterning of mounds of a fungus-cultivating termite: results from nearest-neighbour analysis and ecological studies *Oecologia*, 127, 324-333.

Korb, J. (2008). Termites, hemimetabolous diploid white ants? *Frontiers in Zoology* 5:15.

Krishna, K., Grimaldi, D. A., Krishna, V. & Engel, M. S. (2013). Treatise on the Isoptera of the new world. *Bulletin of the American Museum of Natural History*, 377, 1-2704.

Kruuk, H. & Sands, W. A. (1972). The aardwolf (Proteles cristatus Sparrman, 1783) as a predator of termites. *East African Wildlife J*ournal, 10, 211-227.

Kuldová, J., Hrdý, I., & Svatoš, A. (1999). Defense secretion of *Prorhinotermes simplex*: toxicity to insecticide susceptible and resistant house fly. *Journal of Chemical Ecology*, 25, 657-662.

Kutalová, K., Bourguignon, T., Sillam-Dussès, D., Hanus, R., Roisin, Y. & Šobotník, J. (2013). Armed reproductives: Evolution of the frontal gland in imagoes of Termitidae. *Arthropod Structure & Development*, 42, 339-348.

Kyjaková, P., Dolejšová, K., Krasulová, J., Bednárová, L., Hadravová, R., Pohl, R. & Hanus, R. (2015). The evolution of symmetrical snapping in termite soldiers need not lead to reduced chemical defence. *Biological Journal of the Linnean Society*, 115, 818-825.

Lefeuve, P. & Bordereau, C. (1984). Soldier formation regulated by a primer pheromone from the soldier frontal gland in a higher termite, *Nasutitermes lujae. Proceedings of the National Academy of Science*, 81, 7665-7668.

Lelis, A. T. & Everaerts, C. (1993). Effects of juvenile-hormone analogs upon soldier differentiation in the termite *Reticulitermes santonensis* (Rhinotermitidae: Heterotermitinae). *Journal of Morphology*, 217, 239-261.

Lemaire, M., Nagnan, P., Clément, J. L., Lange, C., Peru, L. & Basselier, J. J. (1990). Geranyllinalool (dipterpene alcohol). An insecticidal component of pine wood and termites (Isoptera: Rhinotermitidae) in four European ecossystems *Journal of Chemical Ecology*, 16, 2067-2079.

Lubin, Y. D. & Montgomery, G. G. (1981). Defenses of *Nasutitermes* termites (Isoptera, Termitidae) against *Tamandua* anteaters (Edentata, Myrmecophagidae). *Biotropica*, 13, 66-76.

Lutikova, L. I. (1990). The influence of soil substrates from the *Anacanthotermes ahngerianus* Jac. termite-house on the development of entomopathogenic fungi. *Mycology and Phytopathology*, 24, 520-528.

Mathews, A. G. A. (1977). *Studies on termites from the Mato Grosso State, Brazil*. Rio de Janeiro: Academia Brasileira de Ciências, 267 p.

Meinwald, J., Prestwich, G. D., Nakanishi, K. & Kubo, I. (1978). Chemical ecology: studies from East Africa. *Science*, 199, 1167-1173.

Moura, F. M. S., Vasconcellos, A., Araújo, V. F. P. & Bandeira, A. G. (2006b). Seasonality on foraging behaviour of *Constrictotermes cyphergaster*

(Termitidae, Nasutitermitinae) in the Caatinga of Northeastern Brazil. *Insectes Sociaux*, 53, 472-479.

Noirot, C., (1969). Glands and secretions. In: Krishna, K. & Weesner, F. M. (Ed.). *Biology of Termites*. Vol. I. Academic Press, New York, pp. 89-123.

Noirot, C., (1970). The Nests of Termites. In: Krishna, K. & Weesner, F. M. (Ed.). *Biology of Termites*. V. II. Academic Press, New York, pp. 73-125.

Noirot, C. & Darlington, J. P. E. C. (2000). Termite nests: architecture, regulation and defence. In: Abe, T., Bignell, D. E. & Higashi, M. (Ed.). *Termites: Evolution, sociality, symbioses, ecology*. Kluwer Academic Publishers, London, pp. 121-139.

Piskorski, R., Hanus, R., Vašíčková, S., Cvačka, J., Šobotník, J., Svatoš, A. & Valterová, I. (2007). Nitroalkenes and sesquiterpene hydrocarbons from the frontal gland secretion of three *Prorhinotermes* termite species. *Journal of Chemical Ecology*, 33, 1787-1794.

Piskorski, R., Hanus, R., Kalinová, B., Valterová, I., Křeček, J., Bourguignon, T., Roisin, Y. & Šobotník, J. (2009). Temporal and geographic variations in the morphology and chemical composition of the frontal gland in imagoes of *Prorhinotermes* species (Isoptera: Rhinotermitidae). *Biological Journal of the Linnean Society,* 98, 384-392.

Prestwich, G. D. (1977). Chemical composition of the soldier secretions of the termite-*Trinervitermes gratiosus*. *Insect Biochemistry*, 7, 91-94.

Prestwich, G. D., Bierl, B. A., Devilbiss, E. D. & Chaudhury, M. F. B. (1977). Soldier frontal glands of the termite *Macrotermes subhyalinus*: morphology, chemical composition, and use in defense. *Journal of Chemical Ecology*, 3, 579-590.

Prestwich, G. D. (1979). Interspecific variation in the defence secretions of *Nasutitermes* soldiers. *Biochemical and Systematic Ecology*, 7, 211-221.

Prestwich, G. D. & Collins, M. S. (1980). A novel enolic beta-ketoaldehyde in the defense secretion of the termite *Rhinotermes hispidus*. *Tetrahedron Letters*, 21, 5001-5002.

Prestwich, G. D. (1982). From tetracycles to macrocycles: chemical diversity in the defense secretions of Nasute termites. *Tetrahedron*, 38, 1911-1919.

Prestwich, G. D. & Collins, M. S. (1982). Chemical defense secretions of the termite soldiers of *Acorhinotermes* and *Rhinotermes* (Isoptera, Rhinotermitinae): ketones, vinyl ketones, and b-ketoaldehydes derived from fatty acids. *Journal of Chemical Ecology*, 8, 147-161.

Prestwich, G. D. (1983). Chemical systematics of termite exocrine secretions. *Annual Review of Ecology and Systematics*, 14, 287-311.

Prestwich, G. D. (1984a). Defense mechanisms of termites. *Annual Review of Entomology*, 29, 201-232.

Prestwich, G. D. (1984b). Interspecific variation of diterpene composition of *Cubitermes* soldier defense secretions. *Journal of Chemical Ecology*, 10, 1219-1231.

Quennedey, A., Brulé, G., Rigaud, J., Dubois, P. & Brossut, R. (1973). La glande frontale des soldats de *Schedorhinotermes putorius* Sjöstedt (Isoptera, Rhinotermitidae): analyse chimique et fonctionnement. *Insect Biochemistry*, 3, 67-74.

Quennedey, A. & Deligne, J. (1975). Larme frontale des soldats de termites. I. Rhinotermitidae. *Insectes Sociaux*, 22, 243-267.

Quennedey, A. (1984). Morphology and ultrastructure of termite defense glands. In: Hermann, H. R. (Ed.). *Defensive mechanisms in social insects*. Praeger, New York, pp. 151-200.

Redford, K. (1984). Mammalian predation on termite: tests with burrowing mouse (*Oxymycterus roberti*) and its prey. *Oecologia*, 65, 145-152.

Roisin, Y., Everaerts, C., Pasteels, J. M. & Bonnard, O. (1990). Caste-dependent reactions to soldier defensive secretion and chiral alarm/recruitment pheromone in *Nasutitermes princeps*. *Journal of Chemical Ecology*, 16, 2865-2875.

Rosengaus, R. B., Lefebvre, M. L. & Traniello, J. F. A. (2000). Inhibition of fungal spore germination by *Nasutitermes*: evidence for a possible antiseptic role of soldier defensive secretions. *Journal of Chemical Ecology*, 26, 21-39.

Rosengaus, R. B., Traniello, J. F. A. & Bulmer, M. S. (2011). Ecology, behavior and evolution of disease resistance in Termites. In: Bignell, D. E., Roisin, Y., & Lo, N. (Ed.). *Biology of termites*. London: Springer. pp. 165-191.

Roux, E. A. & Korb, J. (2004). Evolution of eusociality and the soldier caste in termites: a validation of the intrinsic benefit hypothesis. *Journal of Evolutionary Biology* 17, 869–875.

Šobotník, J., Weyda, F., Hanus, R., Kyjaková, P. & Doubský, J. (2004). Ultrastructure of the frontal gland in *Prorhinotermes simplex* (Isoptera: Rhinotermitidae) and quantity of the defensive substance. *European Journal of Entomology*, 101, 153-163.

Šobotník, J., Hanus, R. & Roisin, Y. (2008). Agonistic behaviour of the termite *Prorhinotermes canalifrons* (Isoptera: Rhinotermitidae). *Journal of Insect Behaviour*, 21, 521-534.

Šobotník, J., Jirosová, A. & Hanus, R. (2010a). Chemical warfare in termites. *Journal of Insect Physiology*, 56, 1012-1021.

Šobotník, J., Bourguignon, T., Hanus, R., Weyda, F. & Roisin, Y. (2010b). Structure and function of defensive glands in soldiers of *Glossotermes oculatus* (Isoptera: Serritermitidae). *Biological Journal of the Linnean Society*, 99, 839-848.

Šobotník, J., Sillam-Dussès, D., Weyda, F., Dejean, A., Roisin, Y., Hanus, R. & Bourguignon, T. (2010c). The frontal gland in workers of neotropical soldierless termites. *Naturwissenschaften*, 97, 495-503.

Šobotník, J., Bourguignon, T., Hanus, R., Sillam-Dussès, D., Pflegerová, J., Weyda, F., Kutalová, K., Vytisková, B. & Roisin, Y. (2010d). Not only soldiers have weapons: evolution of the frontal gland in imagoes of the termite families Rhinotermitidae and Serritermitidae. *PLoS ONE*, 5(12), e15761.

Spanton, S. G. & Prestwich, G. D. (1981). Chemical self-defense by termite workers: prevention of autotoxication in two rhinotermitids. *Science*, 214, 1363-1365.

Spanton, S. G. & Prestwich, G. D. (1982). Chemical defense and self-defense: biochemical transformations of contact insecticides produced by soldier termites. *Tetrahedron*, 38, 1921-1930.

Thorne, B. L. (1982). Termite-termite interactions: workers as an agonistic caste. *Psyche*, 89, 133-150.

Thorne, B. L. & Haverty, M. I. (1991). A review of intracolony, intraspecific, and interspecific agonism in termites. *Sociobiology*, 19, 115-145.

Thorne, B. L., Breisch, N. L. & Muscedere, M. L. (2003). Evolution of eusociality and the soldier caste in termites: influence of intraspecific competition and accelerated inheritance. *Proceedings of the National Academy of Sciences*, 100, 12808-12813.

Traniello, J. F. A. & Leuthold, R. H. (2000). Behavior and ecology of foraging in termites. In: Abe, T., Bignell, D. E. & Higashi, M. (Ed.). *Termites: Evolution, sociality, symbioses, ecology*. Kluwer Academic Publishers, London, pp. 141-168.

Valterová, I., Křeček, J. & Vrkoč, J. (1988). Chemical composition of frontal gland secretion in soldiers of *Velocitermes velox* (Isoptera, Termitidae) and its biological activity. *Acta Entomologica Bohemoslovaca*, 85, 241-248.

Vasconcellos, A., Araujo, V. F. P., Moura, F. M. S. & Bandeira, A. G. (2007). Biomass and populational structure of *Constrictotermes cyphergaster* (Silvestri) (Isoptera, Termitidae) in the dry forest of Caatinga, Northeastern Brazil. *Neotropical Entomology*, 36, 693-698.

Vrkoč, J. & Ubik, K. (1974). 1-Nitro-trans-1-pentadecene as the defensive compound of termites. *Tetrahedron Letters*, 15, 1463-1464.

Waller, D. A. & La Fage, J. P. (1987). Unpalatability as a passive defense of *Coptotermes formosanus* Shiraki soldiers against ant predation. *Journal of Applied Entomology*, 103, 148-153.

Wiltz, B. A., Henderson, G. & Chen, J. (1998). Effect of naphthalene, butylated hydroxytoluene, dioctyl phthalate, and adipic dioctyl ester, chemicals found in the nests of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) on a Saprophytic *Mucor* sp. (Zygomycetes: Mucorales). *Environmental Entomology*, 27, 936-940.

Wright, M. S., Lax, A. R., Henderson, G. & Chen, J. (2000). Growth response of *Metarhizium anisopliae* to two Formosan subterranean termite nest volatiles, naphthalene and fenchone. *Mycologia*, 42-45.

Zhang, S., Mo, J., Teng, L., Cheng, M., & Cheng, J. (2006). Inter-colonial variation in the compositions of the frontal gland secretion of *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Sociobiology*, 47, 553-561.

Chapter 2

Toxic and behavioral effect of *Constrictotermes cyphergaster* (Blattodea: Termitidae: Nasutitermitinae) frontal gland secretion on its obligatory inquiline *Inquilinitermes microcerus* (Blattodea: Termitidae: Termitinae)

Antônio C. F. Costa, Carla C. M. Arce, Og DeSouza, Paulo F. Cristaldo & Eraldo R. Lima

Abstract

Monoterpenes and diterpenes found in the frontal gland secretion of Nasutitermitinae species have been reported to act as repellent, irritant and toxic in a range of vertebrate and invertebrate species. However, the effect of defensive secretion on termite species that share a common nest is still open to investigation. Here, we tested whether the frontal gland secretions of *Constrictotermes cyphergaster* (Termitidae [Nasutitermitinae]) act as toxic and repellent on its obligatory inquiline *Inquilinitermes microcerus* (Termitidae [Termitinae]). For this, behavioural assays with two doses of *C. cyphergaster* frontal gland extracts (FGE) were conducted in order to study its effects on repellency and survival of *I. microcerus*. Our results showed that FGE of *C. cyphergaster* is not toxic and show no repellent effect in *I. microcerus*. Such results suggest that this obligatory inquiline is provided with a detoxification mechanisms comparable to that employed by *C. cyphergaster* to avoid poisoning by the defensive secretion of their own soldiers. This is the first record of a detoxication mechanism exhibited by an obligatory inquiline termite against the defensive secretion of its host.

Key words: defense, nest-sharing, inquilinism, toxicity, repellency

1 Introduction

Colonies of social insects are known to be a fortress in which nestmates are prompt to defend their resources from non-nestmates through a complex and sophisticated variety of defensive strategies (Wilson, 1971). In termite species, these strategies range from mechanical to chemical mechanisms that are used to deter non-nestmates that are harmful to their colonies. These are effective weapons to face not only predation but also competition for resources. The phylogenetically lower termite soldiers rely mainly on mechanical defence of crushing or slashing provided by powerful mandibles connected heavily sclerotized head capsules (Prestwich, 1984). On the other whereas soldiers of higher termite species rely on chemical defence either exclusively or combined with mechanical weapons (Prestwich, 1983; 1984).

In Termitidae, soldiers of the phylogenetically derived Nasutiterminae subfamily, adopt exclusively the chemical defence provided by the viscous secretion of the frontal gland. This secretion is composed primarily by terpenoids that also play a role in the alarm communication and in the recruitment of nestmates (e.g. α-pinene in *Nasutitermes princeps* (Roisin et al., 1990), α-pinene, myrcene and β-ocimene in *Constrictotermes* cyphergaster (Cristaldo et al., 2016)). Previous studies have showed that frontal gland secretion of termites also acts as repellent, irritant and toxic agent for a variety of vertebrate and invertebrate species (*e.g.* Redford, 1984; Kuldová et al., 1999).

These defensive tactics, on the other hand, lead to the selection of counter-measures by intruders in order to overcome their hosts strategies to hinder invasion (Kilner & Langmore, 2011). Some termite species are able to overcome the defensive system, enter and inhabit other termite nests (Cristaldo et al., 2012; Florencio et al., 2013;

Cristaldo et al., 2016) making use of chemical and/or behavioral strategies that allow the deceiving of host perception and, therefore, avoiding confrontation. Amongst the strategies employed by parasites to escape from host recognition are chemical insignificance, when the invader is deprived of identification signals on its cuticle (Lenoir et al., 2001) and the the production of alarm signals which the host is incapable to detect (Cristaldo et al., 2016).

The obligatory termite inquiline, *Inquilinitermes microcerus* Silvestri, 1901 (Termitidae: Termitinae) employ an additional advantageous strategy to survive with the unfriendly population of its host *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae [Nasutitermitinae]) which is the capacity to decode the alarm message and avoid sites where the alarm pheromone (a mixture of (1S)-α-pinene, myrcene and (E)-β-ocimene (Cristaldo et al., 2015)) is present (Cristaldo et al., 2016). This sensorial ability could possibly be used by *I.* microcerus to avoid the threat represented by the confrontation with its host (Cristaldo et al., 2016). On the other hand, the alarm pheromone and other substances produced by the frontal gland of *C. cyphergaster* could have negative effects on *I. microcerus* such as repellent, toxic or both. 1.1 Study species: termite host and inquiline

The genus *Constrictotermes* Silvestri, 1910 (Termitidae: Nasutitermitinae) is distributed across the Neotropical region and comprises seven living species (Krishna et al., 2013). *Constrictotermes cyphergaster* (Silvestri, 1901) is a common species in savannah regions (“Cerrado”) in the center of Brazil, Paraguay, Bolivia and northern Argentina (Mathews, 1977; Krishna et al., 2013), as well as in the dry tropical forest (“Caatinga”) of northeastern Brazil (Vasconcellos et al., 2007). It feeds on wood in distinct stages of decomposition, on the surface of the bark of trees (Moura et al., 2006a) and on lichen (Bourguignon et al., 2011). This species forage in open air under

the protection of the soldiers (Moura et al., 2006b) and a proportion of 4.5 workers for each soldier was verified by Cunha et al. (2003). The nests of *C. cyphergaster* are arboreal and habitually cohabited by the obligatory inquiline species *Inquilinitermes fur* or *I. microcerus* (Termitidae [Termitinae]) (Mathews, 1977), both occurring in South America (Mathews, 1977). Colonies of *Inquilinitermes* spp. are located in specific parts of the host nest, mainly close to its center (Cunha et al., 2003). Besides shelter, the inquilines obtain their nourishment from the nest material of their hosts (Mathews, 1977) such as accumulated organic products, host feces, dead bodies and the covering of the inside surface of the galleries (Florencio et al., 2013). *Inquilinitermes microcerus* Silvestri, 1901 occurs only in *C. cyphergaster* nests and live in galleries made of dark grey material, separated from those of its host, which have a different color (Mathews, 1977). In the Brazilian "Cerrado" biome, 70% of *C. cyphergaster* nests are inhabited by *I. microcerus* colonies (Cristaldo et al., 2012).

The frontal gland secretion of *C. cyphergaster* contains ((1S)-α-pinene, (1S)-β-pinene, camphene, myrcene, (R)-limonene, (Z)-β-ocimene, (E)-β-ocimene), two sesquiterpene and a mixture of diterpenes, from which the alarm pheromone is a combination of (1S)-α-pinene, myrcene and (E)-β-ocimene (Cristaldo et al., 2015). Interestingly, *I. microcerus* not only detects and avoids this alarm cue but also recognizes its different levels (Cristaldo et al., 2016). However, how the alarm pheromone of *C. cyphergaster* affects *I. microcerus* remains unknown. In order to shed light on how the alarm pheromone of the host acts on its obligatory inquiline, we analysed the toxic and repellent effects of *C. cyphergaster* frontal gland secretion on *I. microcerus*. For this, we conducted bioassays with two doses of frontal gland extracts of soldiers of *C. cyphergaster* on soldiers and workers of *I. microcerus* and verified the effect of the extracts on the survivorship and repellency of the inquilines.

2 Material and methods

2.1 Study site and maintenance

Constrictotermes cyphergaster whole nests containing colonies of *I. microcerus (N= 10)* were sampled in a "Cerrado" area (vegetational formation physiognomically, but not floristically, similar to savannas) located in Montes Claros (16º 39' 42.4" S, 43º 44' 18" W) and "Caatinga" area located in Verdelândia (15º 41' 39.8" S, 43º 36' 0.5" W), Minas Gerais, Southeastern Brazil, from May to June 2016. Subsequently, nests were transported to the Entomology and Phytopathology facilities of EPAMIG Norte, settled in Nova Porteirinha (Minas Gerais, Brazil), where they were kept in ambient conditions (aproximately 26 ºC and 57% relative air humidity) prior to its use for preparation of Frontal Gland extracts (FGE) and behavioral bioassays.

2.2 Preparation of FGE

FGE were prepared from soldiers of each *C. cyphergaster* nest (*N= 10*) following procedure described in Cristaldo et al. (2015). Soldiers were immobilized at 0 ºC during 5 min. for dissection the head from the body. 10 Soldier's heads were cut off, crushed with forceps, and placed into glass insert containing 200 µL of hexane. Each sample was extracted overnight at 0 ºC followed by the quantification of the final volume and transferred to another insert inside a vial. All FGE were stored in the freezer at 0 ºC, until their use in the bioassays. For behavioural bioassays, we used the final volume quantified after the extraction to calculate the volume of extract required to provide two (FGE-2Eq) and six (FGE-6Eq) soldier's head.

2.3 Survival and repellency bioassays

To test whether the host frontal gland secretion affects the survival of its obligatory inquiline, bioassays were performed exposing inquilines groups to different stimuli as follows: Control (C), absence of any substance, Control Solvent (CS), hexane; Frontal Gland Extract (FGE) of its host with the doses of two soldiers equivalent (2Eq) and six soldiers equivalent (6Eq).

Bioassay was performed in four arenas made of glass Petri dish (ø 90 mm), lined with a paper filter disc as described in Šobotník et al. (2008) and Cristaldo et al. (2015). This disc was divided in two equal halves by a line drawn with a pencil defining the treated and untreated areas in order to verify the existence of a repellent effect of the frontal gland secretion of the host on the inquilines, according in whose area they were found at the end of the bioassay. Four groups of two soldiers and 20 workers of *I. microcerus* were removed from their colonies in the sampled nests and distributed randomly all over the experimental arenas, prior to the addition of the treatments, in order to ensure their acclimatization. The number of and caste ratio of the inquilines was chosen according to their natural caste proportions (see Cunha et al., 2003; and Cristaldo et al., 2015: 1 soldier: 9.5 workers) and to maximize their interactions and survival (see Miramontes & DeSouza, 1996).

After the acclimatization, the treatments CS and FGE (2 and 6 Equivalents) were loaded onto only one half of the filter paper disc using a Hamilton syringe, prior to the beginning of observations. Each *I. microcerus* group was tested with FGE extract from its host species (*i.e.* both species came from the same nest). Each nest was tested once for each one of the treatments.

After the stimuli were loaded, the experimental arenas were placed in the dark in a

climate chamber (25 °C ± 0.5) and no food or water was provided to termite groups. Observations of behaviors and number of individuals alive in the treated and untreated area of each experimental arena were measured at 30 min., 1, 2, 4, 8, 16, 24 and 48 hours after the beginning of experiments.

2.4 Statistical analyses

In order to verify the toxicity of the FGE to the inquilines, two analyses were performed. Censored Survival Regression Analysis with Weibull model distribution (Crawley, 2007) was used to verify whether survival patterns differed among treatments (*i.e.* C, CS, FGE-2Eq and FGE-6Eq). For the analyses 'Petri Dish' and 'colony' were considered as a blocking factor. Analyses were performed using *survival* package on R Software (R Development Core Team, 2012). Additionally, comparisons of the mean time to death among treatments were conducted through contrast analyses with *F* test, removing non-significant ($P < 0.05$) terms sequentially, starting from the most complex one and then lumping treatments levels together within retained categorical variables. After each model simplification, the effect on deviation was analyzed following Crawley (2007).

To check the repellent effect of each treatment, *I. microcerus* individuals counted along the 10 repetitions in treated and untreated areas 48 hours after the beginning of the experiments were submitted to Analysis of Deviance for Generalised Linear Method (GLM) under Normal distribution, on R Software (R Development Core Team, 2012).

3 Results

3.1 Survival assays

The survival patterns of *I. microcerus* groups did not differ among the treatments C, CS, FGE-2Eq and FGE-6Eq (P=0.2356189) (Fig. 1). However, the analysis of the worker caste exclusively showed significant difference between the Control and the treatments CS, FGE-2Eq and FGE-6Eq (χ^2= 9.537765, *d.f.* = 3, P=0.02293298). On the other hand, no significant difference between CS and FGE-2Eq (P=0.4542878) as well as from both to FGE-6Eq (P=0.3525208) (Fig. 2). Additionally, the mean time to death of the inquiline groups was not significatively affected by the treatments analysed (P=0.1078) (Fig. 3).

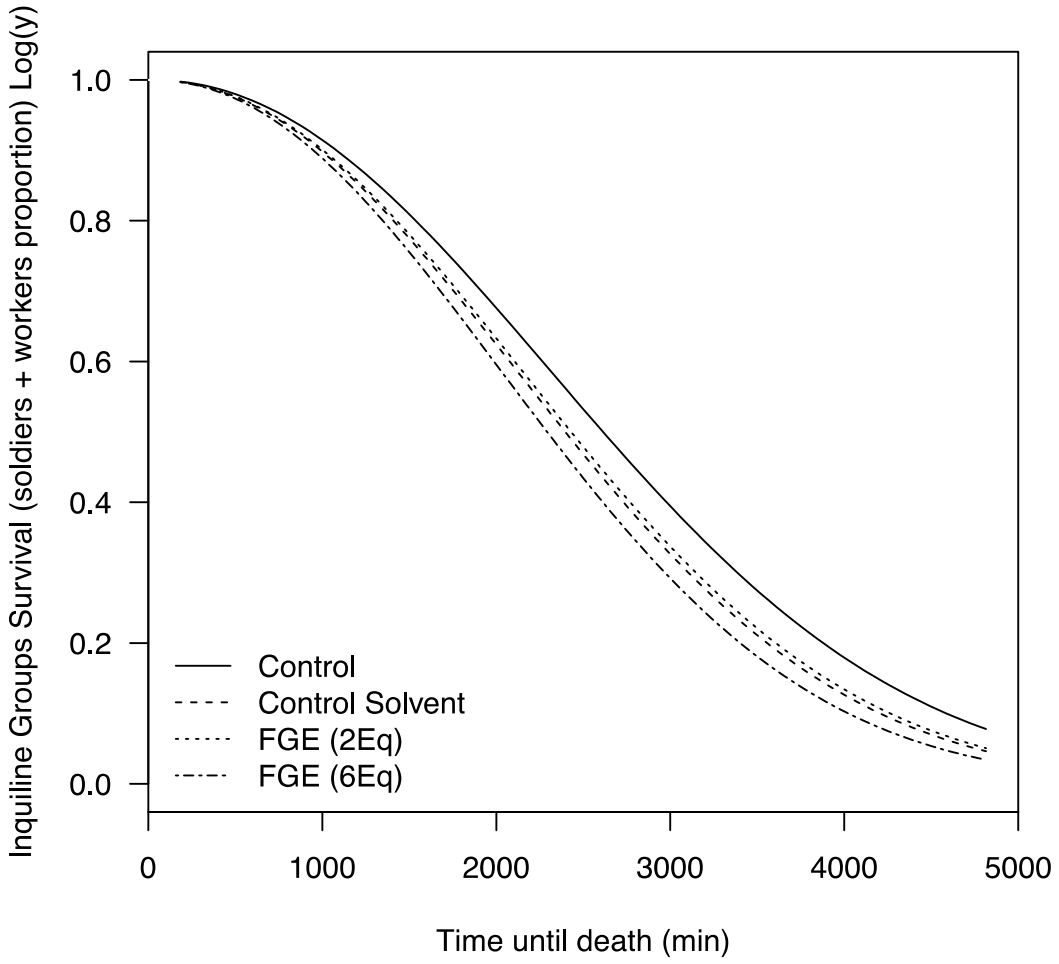


Fig. 1: Survival of *Inquilinitermes microcerus* groups exposed to controls (C), control solvent (CS) and frontal gland extracts (FGE-2Eq and FGE-6Eq) of their host, *Constrictotermes cyphergaster* (Weibull distribution).

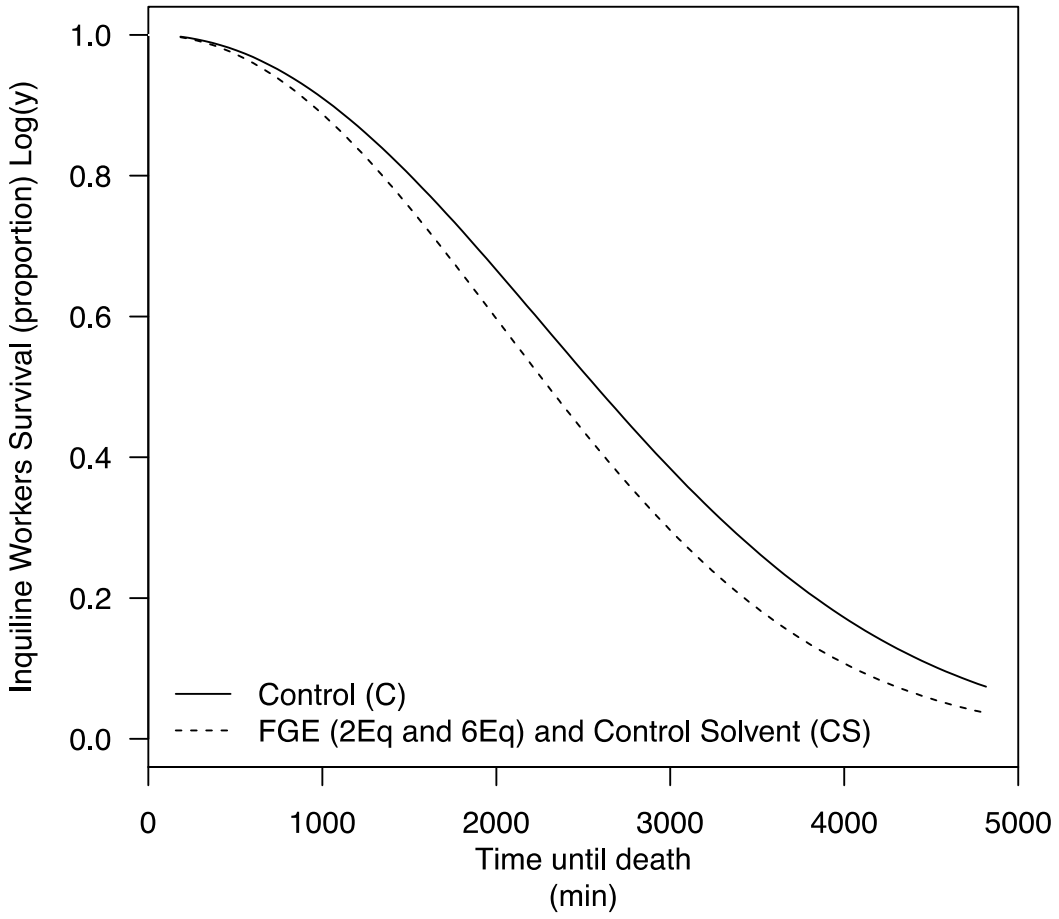


Fig. 2: Survival of *Inquilinitermes microcerus* workers exposed to control (C), as well as frontal gland extracts (FGE-2Eq and FGE-6Eq) and control solvent (CS) of their host, *Constrictotermes cyphergaster* (Weibull distribution).

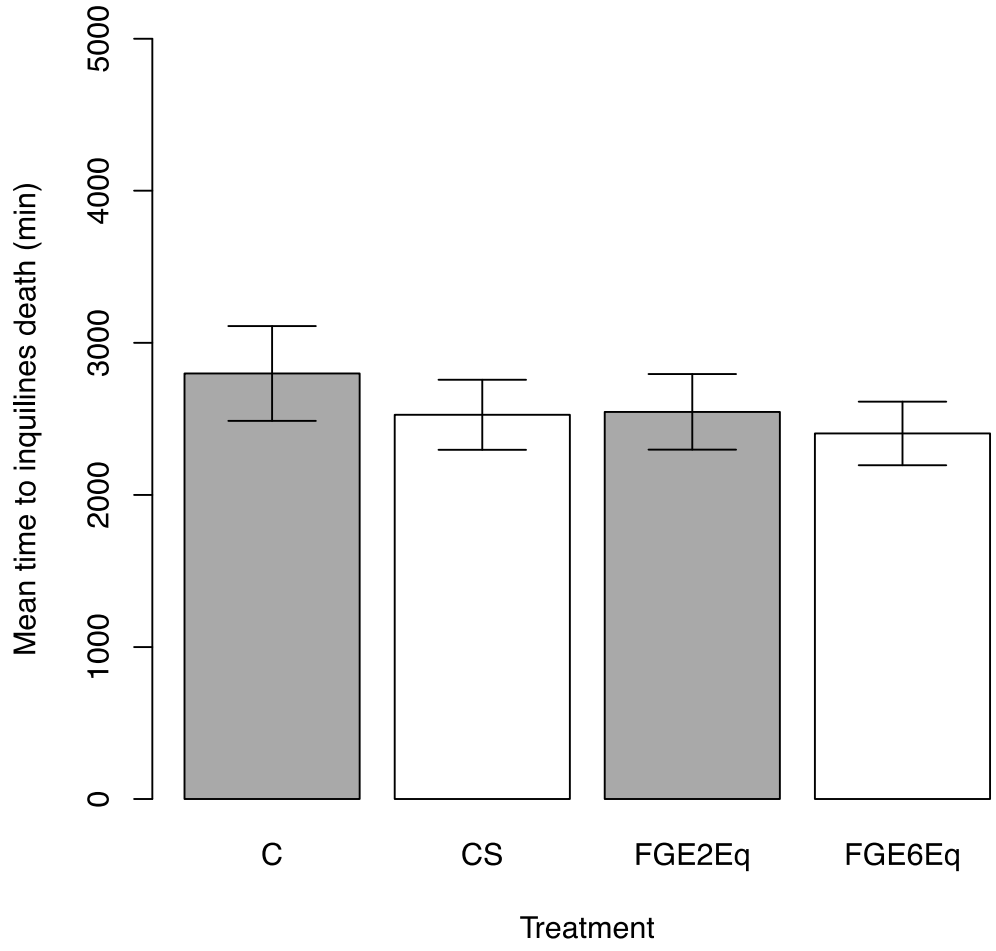


Fig. 3. Mean time to death of *Inquilinitermes microcerus* groups exposed to control (C), control solvent (CS) and frontal gland extracts (FGE-2Eq and FGE-6Eq) of their host, *Constrictotermes cyphergaster*.

3.2 Repellency assays

There was no significant difference between the number of *I. microcerus* individuals observed at the end of the bioassay in the areas treated with C, CS, FGE-2Eq and FGE-6Eq and in the untreated areas (F= 0.1735, P= 0.8417) (Fig. 4).

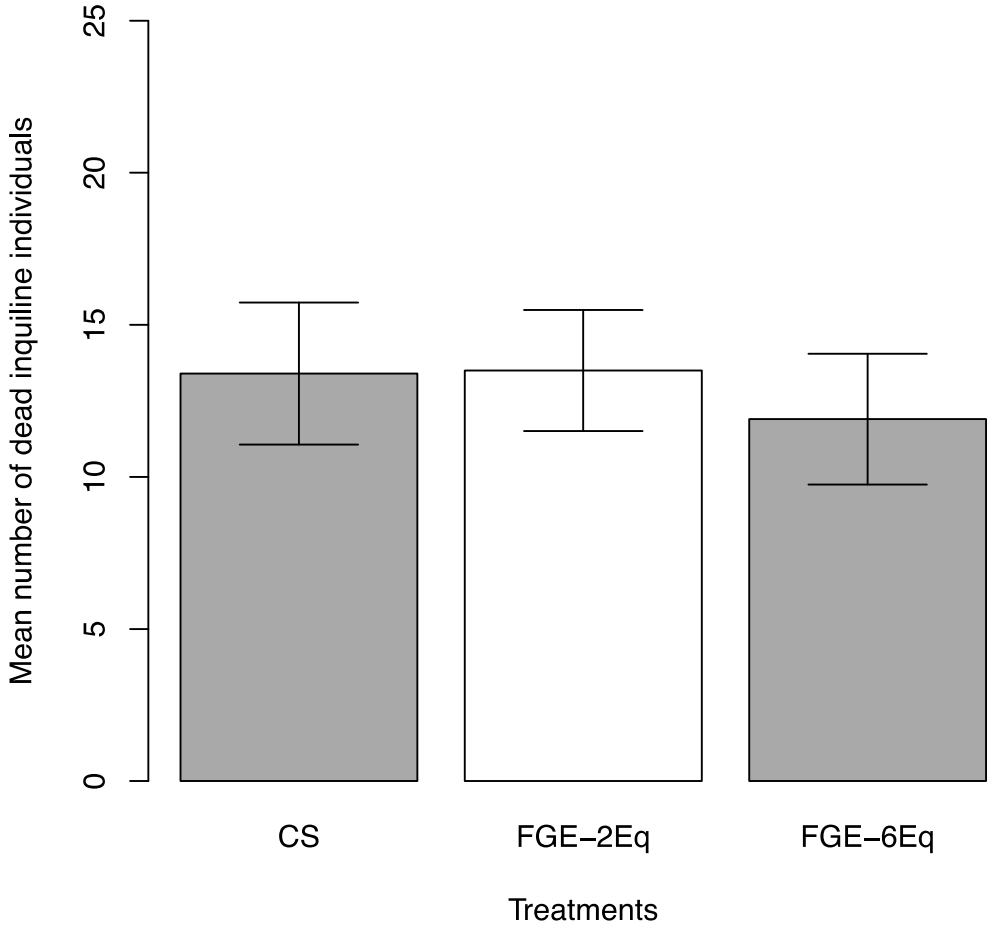


Fig. 4. Mean number of dead *Inquilinitermes microcerus* individuals (soldiers and workers) found in the untreated areas 48 hours after exposed to control solvent (CS) and frontal gland extracts (FGE-2Eq and FGE-6Eq) of their host, *Constrictotermes cyphergaster*.

4 Discussion

Our results showed that FGE of *C. cyphergaster* has no effect on the survivorship, mean time to death and repellency of its obligatory inquiline *I. microcerus*. The frontal

gland secretion of *C. cyphergaster*, as in other Nasutitermitinae species, is made of a mixture of monoterpenes ((1S)-α-pinene, (1S)-β-pinene, Camphene, Myrcene, (R)-limonene, (Z)-β-ocimene, (E)-β-ocimene), two sesquiterpene and a mixture of diterpenes hydrocarbons, sesquiterpenes and diterpenes (Cristaldo et al., 2015). The repellent and toxic effect of monoterpenes presents in the frontal gland secretion of termites was already reported on different vertebrates and invertebrate species (see Eisner et al., 1976; Lubin & Montgomery, 1981; Mill, 1983; Redford, 1984; Kuldová et al., 1999; Xie et al., 2014). However, none of these consequences were verified in this study.

The absence of toxic effects of the FGE of *C. cyphergaster* on the survivorship of inquiline groups indicates that natural selection has probably enabled inquilines with countermeasures to deal with the chemical weapons of its host. Chemical defence is intrinsically coupled with a biochemical mechanism that allows conspecifics to avoid intoxication by its defensive substances (Spanton & Prestwich, 1981; 1982). This capacity is known to occur in the polidesmid milipedes *Oxidus gracilis*, which detoxifies HCN, phenol and guaiacol (Duffey & Blum, 1977) whereas *Harpaphe haydeiana*, HCN only (Duffey et al., 1974). Spanton and Prestwich (1981) found that specific oxireductases produced by two species of Rhinotermitidae are able to convert the toxic (E)-1-Nitropentadec-1-ene into the nontoxic 1-nitropentadecane. This detoxication process allows nestmates of these species to survive in the presence of toxic nitroalkene produced by their own soldiers in a way that neither soldiers nor workers are poisoned by the frontal gland secretion from its conspecifics. Considering that *I. microcerus* is an obligatory inquiline, specialized on living only in *C. cyphergaster* nests, it may be expected that a detoxication route had been also selected making it capable to escape from the noxious action of the defensive secretion of its host. Additionally, the two doses of frontal gland extract of the host had no effect on

the mean time to death and in the repellency of inquiline groups

In spite of *I. microcerus* is not harmed by the toxicity of the frontal gland secretion of *C. cyphergaster* soldiers, its stickness also works for defensive purpose, as a viscous glue. It may limit or stop the movements of the opponent (Prestwich, 1984), besides obstructing its sensillae and spiracles (Šobotník et al., 2010). In addition, the combat strategy of *C. cyphergaster* combines the chemical weapons of soldiers with one-to-one contact fights of numerous workers. Then, the opponent made more vulnerable may be crushed by the bites of workers, as verified in *Nasutitermes exitiosus* by Eisner et al. (1976). Therefore, the mechanical effect of the frontal gland secretion of the host combined with the role of the workers in the defence are potential threats to inquilines.

On the one hand, the absence of communication between the nests of *I. microcerus* and *C. cyphergaster* reduces the chance of meeting between these species (Cristaldo et al., 2014), whereas the fact that cohabitation may happen in confined spaces as nests with volumes of 13 litres raises it (Cristaldo et al., 2012). So, as *I. microcerus* is capable of distinguish different intensities in the alarm pheromone of *C. cyphergaster* (Cristaldo et al., 2015), it is likely that this inquiline uses the alarm signals of its host as a warning not only of a coming danger but also of its magnitude, as proposed by Cristaldo et al. (2015). Moreover, this danger sign refers not only to the host but also to common enemies that both species share, because they live in the same nest (Cristaldo et al., 2016).

It is likely that natural selection provided *I. microcerus* with strategies that allow this obligatory inquiline escape host detection such as living in distinct galleries (Mathews, 1977), avoinding detection of its trail (Cristaldo et al., 2014) and alarm cues (Cristaldo et al., 2016) by its host. It is possible that the chemoreception of *I. microcerus* was

selected to recognize the alarm pheromone of *C. cyphergaster* as a message of evasion instead of recruitment.

Then, it leads to the belief that inquiline keep away from the contact with the host numerically superior and supplied with the chemical weapons of the soldiers and the bites of the workers, in order to improve its chance of survival, as also suggested by authors as Cristaldo et al. (2016).

Acknowledgements

The authors are grateful to the staff of EPAMIG Norte, specially for sharing the phytopatology facilities (D. Sc. Mário Sérgio), laboratory assistance (Mrs. Ana Rosa), field work (Mr. Edison José, Mr. Reny, Mr. Rubens and Mrs. Ermínia), institutional support (D. Sc. Polyanna Mara) and exchange of views (D. Sc. Alniusa Maria, D. Sc. Maria Geralda and D. Sc. Ariane).

5 References

Bourguignon, T., Šobotník J., Lepoint, G., Martin, J. M., Hardy, O. J., Dejean, A., & Roisin, Y. (2011). Feeding ecology and phylogenetic structure of a complex Neotropical termite assemblage, revealed by nitrogen stable isotope ratios. *Ecological Entomology*, 36, 261-269.

Crawley, M. J. (2007). *The R Book*. England: John Wiley and Sons, 942 p.

Cristaldo, P. F., Rosa, C. S., Florencio, D. F., Marins, A., & DeSouza, O. (2012). Termitarium volume as a determinant of invasion by obligatory termitophiles and inquilines in the nests of *Constrictotermes cyphergaster* (Termitidae, Nasutitermitinae). *Insectes Sociaux*, 59, 541-548.

Cristaldo, P. F., DeSouza, O., Krasulová, J., Jirošová, A., Kutalová, K., Lima, E. R., Šobotník, J. & Sillas-Dussès, D. (2014). Mutual use of trail-following chemical cues by a termite host and its inquiline. *PLoS ONE*, 9(1): e85315.

Cristaldo, P. F., Jandak, V., Kutalova, K., Rodrigues, V. B., Brothanek, M., Jiricek, O., DeSouza, O., & Sobotnik, J. (2015). The nature of alarm communication in

*Constrictotermes cyphergaste*r (Blattodea: Termitoidea: Termitidae): the integration of chemical and vibroacoustic signals. *Biology Open*, 4, 1649-1659.

Cristaldo, P. F., Rodrigues, V. B., Elliot S. L., Araújo, A. P. A., & DeSouza, O. (2016). Heterospecific detection of host alarm cues by an inquiline termite species (Blattodea: Isoptera: Termitidae). *Animal Behaviour*, 120, 43-49.

Cunha, H., Costa, D. A., Santo-Filho, K., & Brandão, D. (2003). Relationship between *Constrictotermes cyphergaster* and inquiline termites in the Cerrado (Isoptera: Termitidae). *Sociobiology*, 42, 1-10.

Duffey, S. S., & Blum, M. S. (1977). Phenol and guaiacol: Biosynthesis detoxication, and function in a polydesmid milipede *Oxidus gracilis*. *Insect Biochemistry*, 7, 57-65.

Duffey, S. S., Underhill E. W., & Towers, G. H. N. (1974). Intermediates in the biosynthesis of HCN and benzaldehyde by a polydesmid milipede, *Harpape haydeniana* (Wood). *Comparative Biochemistry and Physiology*, 47B, 753-766.

Eggleton, P. (2011). An introduction to termites: biology, taxonomy and functional morphology. In: Bignell, D. E., Roisin, Y., & Lo, N. (Ed.). *Biology of termites*. London: Springer, pp. 1-26.

Eisner, T., Kriston, I., & Aneshansley, D. J. (1976). Defensive behaviour of a termite (*Nasutitermes exitiosus*). *Behavioural Ecology and Sociobiology*, 1, 83-125.

Florencio, D. F., Marins, A., Cristaldo, P. F., Araujo, A. P. A., Silva, I. R., & DeSouza, O. (2013). Diet segregation between cohabiting builder and inquiline termite species. *PLoS ONE*, 8(6): e66535.

Krishna, K., Grimaldi, D. A., Krishna, V., & Engel, M. S. (2013). Treatise on the Isoptera of the new world. *Bulletin of the American Museum of Natural History*, 377, 1-2704.

Kuldová, J., Hrdý, I., & Svatoš, A. (1999). Defense secretion of *Prorhinotermes simplex*: toxicity to insecticide susceptible and resistant house fly. *Journal of Chemical Ecology*, 25, 657-662.

Lenoir, A., D'Ettorre, P., Errard, C., & Hefetz, A. (2001). Chemical ecology and social parasitism in ants. *Annual Review of Entomology*, 46, 573-599.

Lubin, Y. D., & Montgomery G. G. (1981). Defenses of *Nasutitermes* termites (Isoptera, Termitidae) against *Tamandua* anteaters (Edentata, Myrmecophagidae). *Biotropica*, 13, 66-76.

Mathews, A. G. A. (1977). *Studies on termites from the Mato Grosso State, Brazil*. Rio de Janeiro: Academia Brasileira de Ciências, 267 p.

Mill A. E. (1983) Behavioural and toxic effects of termite defensive secretions on ants. *Physiological Entomology*, 8, 413-418.

Miramontes, O., & DeSouza, O. (1996). The nonlinear dynamics of survival and social facilitation in termite. *Journal of Theorical Biology*, 181, 373-380.

Moura, F. M. S., Vasconcellos, A., Araújo, V. F. P., & Bandeira, A. G. (2006a). Feeding habitat of Constrictotermes cyphergaster (Isoptera, Termitidae) in an area of Caatinga, Northeast Brazil. *Sociobiology*, 48, 21-26.

Moura, F. M. S., Vasconcellos, A., Araújo, V. F. P. & Bandeira, A. G. (2006b). Seasonality on foraging behaviour of *Constrictotermes cyphergaster* (Termitidae,

Nasutitermitinae) in the Caatinga of Northeastern Brazil. *Insectes Sociaux*, 53, 472-479.

Prestwich, G. D. (1983). Chemical systematics of termite exocrine secretions. *Annual Review of Ecology and Systematics*, 14, 287-311.

Prestwich, G. (1984). Defense mechanisms of termites. *Annual Review of Entomology*, 29, 201-232.

R Development Core Team (2012). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. URL http://www.R-project.org/.

Redford, K. (1984). Mammalian predation on termite: tests with burrowing mouse (*Oxymycterus roberti*) and its prey. *Oecologia*, 65, 145-152.

Roisin, Y., Everaerts, C., Pasteels, J. M., & Bonnard, O. (1990). Caste-dependent reactions to soldier defensive secretion and chiral alarm/recruitment pheromone in *Nasutitermes princeps Journal of Chemical Ecology*, 16, 2865-2875.

Rosengaus, R. B., Lefebvre, M. L. & Traniello, J. F. A. (2000). Inhibition of fungal spore germination by *Nasutitermes*: evidence for a possible antiseptic role of soldier defensive secretions. *Journal of Chemical Ecology*, 26, 21-39.

Šobotník, J., Jirosová, A., Hanus, R. (2010). Chemical warfare in termites. *Journal of Insect Physiology*, 56, 1012-1021.

Spanton, S. G., & Prestwich, G. D. (1981), Chemical self-defense by termite workers: prevention of autotoxication in two rhinotermitids. *Science*, 214, 1363-1365.

Spanton, S. G., & Prestwich, G. D. (1982). Chemical defense and self-defense: biochemical transformations of contact insecticides produced by soldier termites. *Tetrahedron*, 38, 1921-1930.

Vasconcellos, A., Araujo, V. F. P., Moura, F. M. S., & Bandeira, A. G. (2007). Biomass and populational structure of *Constrictotermes cyphergaster* (Silvestri) (Isoptera, Termitidae) in the dry forest of Caatinga, Northeastern Brazil. *Neotropical Entomology*, 36, 693-698.

Wilson, E. (1971). *The insect societies*. Massachusetts: Harvard University Press, 562 p.

Xie, Y., Wang, K., Huang, Q., & Lei, C. (2014). Evaluation toxicity of monoterpenes to subterran termite, *Reticulitermes chinensis* Synder. *Industrial Crops and Products*, 53, 163-166.

2 General conclusions

The development of the frontal gland in the soldiers of Rhinotermitidae, Serritermitidae and Termitidae allowed the chemical defence in this phylogenetically derived Neoisoptera group. The great variety of defensive substances produced by this exocrine gland can act in heterospecifics as anti healing greases, irritants, topical poisons, immobilizer glues, repellents, unpalatability agents and inhibitors of fungal and bacterial growth. On the other hand, conspecifics are protected against the noxious secretions of their soldiers by biochemical detoxification pathways. The chemical defensive strategy evolved from a combined action of a small frontal gland and robust mandibles to a derived state of the nasutes, where the defense relies exclusively in a large frontal gland and reduced mandibles. This novel form of defense promoted the great evolutionary success of the Nasutitermitinae which is composed by a large part of all termite species and capable to utilize diferent food sources in a wider variety of ecossistems. Despite of this defensive weaponry, *Constrictotermes cyphergaster* nests can be inhabited by *Inquilinitermes microcerus*, an obligatory inquilile. In this study we found that the frontal gland secretion of *C. cyphergaster* soldiers have no toxic or repellent effect on its specialized inquiline *I. microcerus*. Then is probably that this inquiline is supplied with a detoxication mechanism that helps its cohabitation in the nest of its host. As far as we are concerned, it is the first record of a detoxication mechanism exhibited by heterospecific termite.

APÊNDICES

APÊNDICE A

Análise de sobrevivência de soldados e operários de *Inquilinitermes microcerus* expostos ao extrato hexânico da glândula frontal de soldados de *Constrictotermes cyphergaster*

12.07.2017

Resumo

O experimento objetivou verificar se inquilinos (*Inquilinitermes microcerus*) teriam o seu tempo de sobrevivência influenciado pelos exsudatos do construtor (*Constrictotermes cyphergaster*). Para isso, inquilinos foram colocados numa placa de petri (20 operários e 2 soldados) forrada com papel filtro marcado diametralmente em duas metades. Em somente uma das metades da placa aplicou-se a substância sob teste. A outra metade serviu de controle local, uma vez que nada foi adicionado. Foi anotado o número de inquilinos vivos e mortos em cada uma das metades da placa aos 15, 30, 60, 120, 240, 480, 960, 1440 e 2880 minutos após a aplicação da substância sob teste.

1 Preâmbulo

1.1 Tratamentos

Há quatro tratamentos:

1. somente papel (branco)
2. somente solvente (hexano)
3. 2 individuos-equivalentes de exsudatos da glândula frontal
4. 6 individuos-equivalentes de exsudatos da glândula frontal

1.2 Hipótese biológica

Sabe-se que *Inquilinitermes microcerus* reconhece o feromônio de alarme do seu hospedeiro e evita os locais onde essa substância está presente. É plausível supor, portanto, que o feromônio de alarme e as substâncias de defesa produzidas pela glândula frontal de *Constrictotermes cyphergaster* sejam nocivas ao seu inquilino obrigatório. Nesse caso, espera-se que *Inquilinitermes microcerus* morra mais rápido quando exposto a essas substâncias.

1.3 Hipótese estatística

Havendo efeito nocivo, os inquilinos expostos à secreção de defesa do construtor apresentarão o tempo médio até a morte menor do que aqueles que não foram expostos a essa substância. Isso deve ser mais evidente nas placas com maior quantidade de exsudatos (6 equivalentes) do que naquelas onde há menos exsudatos (solvente somente ou 2 equivalentes)

2 Estabelecimento do diretório de trabalho e do arquivo dos dados

```
> setwd("~/ISOP.DSC.AN.SOB13")
> read.table("AnSobEPAMIGdosTXT9.txt",h=T)->dados
```

```
> summary(dados)
```

```
      nest     pDish        indiv          caste      trat          time
 N1     : 88   P1:220   I1     : 40   soldiers: 80   C  :220   Min.   :  30
 N13    : 88   P2:220   I10    : 40   workers :800   CS :220   1st Qu.:1440
 N18    : 88   P3:220   I11    : 40                  Eq2:220   Median :2880
 N23    : 88   P4:220   I12    : 40                  Eq6:220   Mean   :2267
 N26    : 88            I13    : 40                            3rd Qu.:2880
 N28    : 88            I14    : 40                            Max.   :2880
 (Other):352            (Other):640
     censor
 Min.   :0.0000
 1st Qu.:0.0000
 Median :1.0000
 Mean   :0.7045
 3rd Qu.:1.0000
 Max.   :1.0000
```

```
> head(dados)
```

```
  nest pDish indiv    caste trat time censor
1   N1    P1    I1 soldiers    C 1440      1
2   N1    P1    I2 soldiers    C 2880      0
3   N1    P1    I3  workers    C  240      1
4   N1    P1    I4  workers    C  480      1
5   N1    P1    I5  workers    C  480      1
6   N1    P1    I6  workers    C  480      1
```

3 Análises de sobrevivência

3.1 Análise das duas castas conjuntamente (soldados e operários)

```
> attach(dados)
```

M0sdop= modelo nulo
M1sdop= modelo completo (tratamento + ninho)

```
> library(survival)
```

```
> M0sdop<-survreg(Surv(time,censor)~1)
```

```
> M1sdop<-survreg(Surv(time,censor)~trat+nest)
```

```
> anova(M0sdop,M1sdop)
```

```
        Terms Resid. Df    -2*LL Test Df Deviance     Pr(>Chi)
1           1       878 10922.78      NA       NA           NA
2 trat + nest       866 10592.49    = 12 330.2946 1.999373e-63
```

Comparando o modelo nulo (M0sdop) com o modelo completo (M1sdop), a sobrevivência dos grupos de inquilinos (considerando soldados e operários conjuntamente) é explicada pelo modelo completo ($p < 0.001$).

A seguir, será feita a análise para verificar se cada uma das variáveis, separadamente, afeta o modelo.

Criar o modelo M2sdop: nesse a sobrevivência é explicada pelo efeito do ninho (bloco), removendo-se o efeito do tratamento.

```
> M2sdop<-survreg(Surv(time,censor)~nest)
```

Verificar se o tratamento influencia significativamente a sobrevivência dos inquilinos: Comparar o modelo sem o tratamento (M2sdop) (Terms = nest) com o modelo completo (M1sdop) (Terms = trat + nest)

```
> anova(M1sdop,M2sdop)
```

```
        Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1 trat + nest       866 10592.49      NA       NA        NA
2        nest       869 10596.74    = -3 -4.25086 0.2356189
```

A remoção do tratamento do modelo não causa uma mudança significativa. A sobrevivência dos grupos de inquilinos não é influenciada significativamente pelo efeito do tratamento ($p = 0.2356189$). Portanto, o tratamento pode ser removido no modelo.

Criar o modelo M3sdop: nesse a sobrevivência dos grupos de inquilinos é explicada pelo efeito do tratamento, removendo-se o efeito do ninho.

```
> M3sdop<-survreg(Surv(time,censor)~trat)
```

```
> anova(M1sdop,M3sdop)
```

```
        Terms Resid. Df    -2*LL Test Df  Deviance     Pr(>Chi)
1 trat + nest       866 10592.49      NA        NA           NA
2        trat       875 10916.45    = -9 -323.9626 2.134663e-64
```

A sobrevivência dos grupos de inquilinos é influenciada significativamente pelos ninhos ($p < 0.001$). Portanto apenas o ninho deve ser mantido no modelo.

Calcular o tempo médio até a morte em cada tratamento.

```
> sort(tapply(predict(M1sdop),list(trat),mean))
```

```
     Eq6      Eq2       CS        C
2872.727 2933.086 2953.404 3146.639
```

```
> levels(trat)
```

```
[1] "C"   "CS"  "Eq2" "Eq6"
```

4 Elaboração dos gráficos para os 4 tratamentos

4.1 Elaboração do gráfico do modelo M3sdop (nesse a sobrevivência dos inquilinos é explicada pelo efeito do tratamento)

M3sdop<-survreg(Surv(time,censor) trat):

A equação da curva de sobrevivência é:

curve (exp(-mu^-alpha)*(x^$alpha$), $from = 0 to = 5000$).

```
> summary(M3sdop)
```

```
Call:
survreg(formula = Surv(time, censor) ~ trat)
              Value Std. Error      z        p
(Intercept)  8.0403     0.0386 208.34 0.00e+00
tratCS      -0.0869     0.0541  -1.61 1.08e-01
tratEq2     -0.0730     0.0536  -1.36 1.74e-01
tratEq6     -0.1312     0.0533  -2.46 1.38e-02
Log(scale)  -0.7579     0.0365 -20.77 8.07e-96

Scale= 0.469

Weibull distribution
Loglik(model)= -5458.2   Loglik(intercept only)= -5461.4
        Chisq= 6.33 on 3 degrees of freedom, p= 0.097
Number of Newton-Raphson Iterations: 5
n= 880
```

O valor de Scale (0.469) que aparece no Summary do modelo M3sdop é necessário para calcular o alpha:

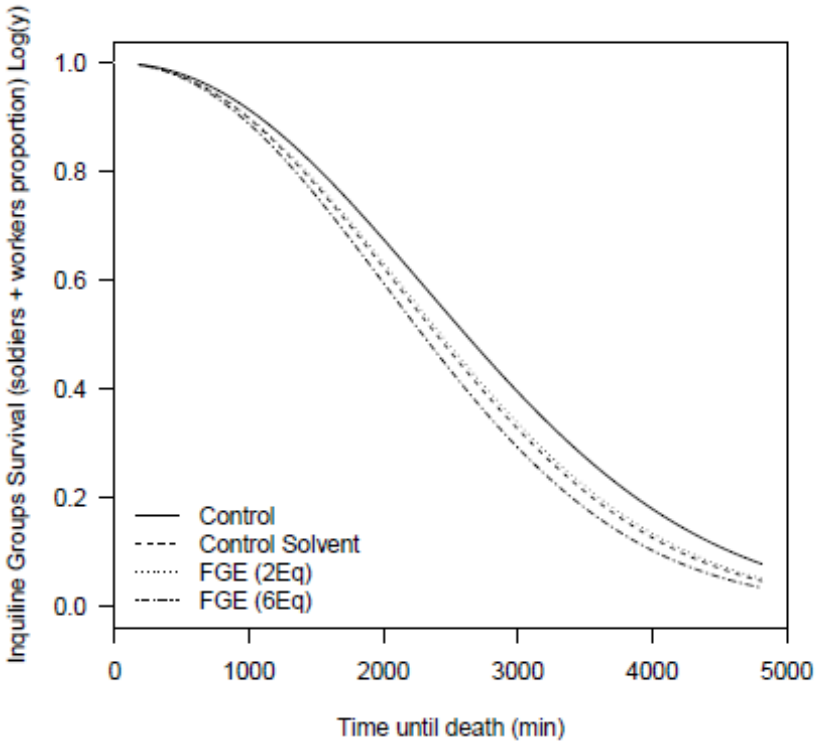
alpha=1/scale

Cálculo do alfa para as 4 curvas de sobrevivência, utilizando-se o modelo M3sdop: O valor de alpha é o mesmo para todas as curvas.

```
> alfaM3sdop<-1/M3sdop$scale
> alfaM3sdop
[1] 2.1338
```

Cálculo do mu para cada uma das 4 curvas de sobrevivência dos tratamentos:

```
> muControl<-tapply(predict(M3sdop),list(trat),mean)[1]
> muControl
       C
3103.637
> muCS<-tapply(predict(M3sdop),list(trat),mean)[2]
> muCS
      CS
2845.424
> muFGE2Eq<-tapply(predict(M3sdop),list(trat),mean)[3]
> muFGE2Eq
     Eq2
2885.215
> muFGE6Eq<-tapply(predict(M3sdop),list(trat),mean)[4]
> muFGE6Eq
     Eq6
2721.967
> par(las=1)
> with(dados,plot(survfit(Surv(time,censor)~trat),xlab = "Time until death (min)",
+ ylab = "Inquiline Groups Survival (soldiers + workers proportion) Log(y)",col = "white", ylim = c(0.0,1.0),
+ xlim = c(0,5000)))
> curve(exp((-muControl^(-alfaM3sdop))*(x^alfaM3sdop)),col="black",lty=1,add = T)
> curve(exp((-muCS^(-alfaM3sdop))*(x^alfaM3sdop)),col="black",lty=2,add = T)
> curve(exp((-muFGE2Eq^(-alfaM3sdop))*(x^alfaM3sdop)),col="black",lty=3,add = T)
> curve(exp((-muFGE6Eq^(-alfaM3sdop))*(x^alfaM3sdop)),col="black",lty=6,add = T)
> legend("bottomleft",c("Control","Control Solvent","FGE (2Eq)","FGE (6Eq)"),
+        col=c("black","black","black","black"),
+ lty=c(1,2,3,6),bty="n")
```



text(1900,0.6,"***")

5 Análise de sobrevivência para a casta dos operários

5.1 Cortar a tabela para a casta dos operários

```
> #detach(tabsd)
> rm(list = ls())
> read.table("AnSobEPAMIGdosTXT9.txt",h=T)->dados
> subset(dados, caste=="workers")->tabop
> droplevels(tabop)->tabop
> summary(tabop)
      nest     pDish        indiv         caste      trat          time
 N1     : 80   P1:200   I10    : 40   workers:800   C  :200   Min.   :  30
 N13    : 80   P2:200   I11    : 40                 CS :200   1st Qu.:1440
 N18    : 80   P3:200   I12    : 40                 Eq2:200   Median :2880
 N23    : 80   P4:200   I13    : 40                 Eq6:200   Mean   :2237
 N26    : 80            I14    : 40                           3rd Qu.:2880
 N28    : 80            I15    : 40                           Max.   :2880
 (Other):320            (Other):560
     censor
 Min.   :0.00
 1st Qu.:0.00
 Median :1.00
 Mean   :0.73
```

```
 3rd Qu.:1.00
 Max.   :1.00
```

```
> head(tabop)
```

```
  nest pDish indiv   caste trat time censor
3   N1    P1    I3 workers    C  240      1
4   N1    P1    I4 workers    C  480      1
5   N1    P1    I5 workers    C  480      1
6   N1    P1    I6 workers    C  480      1
7   N1    P1    I7 workers    C 1440      1
8   N1    P1    I8 workers    C 2880      1
```

```
> attach(tabop)
```

5.2 Criar os modelos para os operários

M0op= modelo nulo M1op= modelo completo

```
> library(survival)
```

```
> M0op<-survreg(Surv(tabop$time,tabop$censor)~1)
```

```
> M1op<-survreg(Surv(tabop$time,tabop$censor)~tabop$trat+tabop$nest)
```

5.3 ANOVA entre os modelos M0op e M1op

A anova entre os modelos M0op (modelo nulo) e M1op (modelo completo) tem por objetivo verificar se alguma das variáveis consideradas possuem efeito na sobrevivência dos operários.

```
> anova(M0op,M1op)
```

```
                     Terms Resid. Df     -2*LL Test Df Deviance     Pr(>Chi)
1                        1       798 10236.022      NA       NA           NA
2 tabop$trat + tabop$nest       786  9892.701    = 12 343.3218 3.593864e-66
```

Comparando-se o modelo nulo (M0op) com o modelo completo (M1op), constatou-se que a sobrevivência dos operários é explicada pelo modelo completo (M1op) ($p< 0.001$)

5.4 Criação do modelo M2op

No modelo M2op a sobrevivência dos operários é explicada pelo efeito do ninho (bloco).

```
> library(survival)
```

```
> M2op<-survreg(Surv(tabop$time,tabop$censor)~tabop$nest)
```

5.5 ANOVA entre os modelos M2op e M1op

A anova entre os modelos M2op e M1op tem o objetivo de verificar se os tratamentos possuem efeito sobre a sobrevivência dos operários.

```
> anova(M2op,M1op)
```

```
                     Terms Resid. Df    -2*LL Test Df Deviance   Pr(>Chi)
1              tabop$nest       789 9902.238      NA       NA         NA
2 tabop$trat + tabop$nest       786 9892.701    =  3 9.537765 0.02293298
```

Comparando-se o modelo M1op (modelo completo) com o modelo M2op (modelo considerando apenas o efeito do ninho), constatou-se que a sobrevivência dos operários foi afetada significativamente pelo efeito do tratamento (p=0.02293298).

5.6 Criação do modelo M3op

No modelo M3op a sobrevivência dos operários é explicada pelo efeito do tratamento.

```
> library(survival)
```

```
> M3op<-survreg(Surv(tabop$time,tabop$censor)~tabop$trat)
```

5.7 ANOVA entre os modelos M3op e M1op

```
> anova(M3op,M1op)
```

```
                    Terms Resid. Df     -2*LL Test Df Deviance     Pr(>Chi)
1              tabop$trat       795 10228.299      NA       NA           NA
2 tabop$trat + tabop$nest       786  9892.701     =  9 335.5988 7.175846e-67
```

Comparando-se o modelo M1op (modelo completo) com o modelo M3op (modelo considerando apenas o efeito do tratamento), constatou-se que a sobrevivência dos operários foi afetada significativamente pelo efeito do ninho ($p< 0.001$).

Portanto, ambas as variáveis (ninho e tratamento) influenciam a sobrevivência dos inquilinos e devem ser mantidas.

A seguir será feita a análise de contrastes a fim de verificar se é possível amalgamar os tratamentos.

5.8 Calculando o tempo médio até a morte dos operários

Quais são os tempos médios até a morte para os tratamentos que compõem o M3op?

```
> sort(tapply(predict(M3op),list(tabop$trat),mean))
```

```
     Eq6       CS      Eq2        C
2655.242 2717.034 2831.502 3062.532
```

```
> levels(trat)
```

```
[1] "C"   "CS"  "Eq2" "Eq6"
```

[1]"C"[2]"CS"[3]"Eq2"[4]"Eq6"

5.9 Amalgamando os níveis C e CS

Renomeando trat para tratOpCCS

```
> tratOpCCS<-trat
```

```
> levels(tratOpCCS)[1]<-"OpCCS"
> levels(tratOpCCS)[2]<-"OpCCS"
```

Verificar os níveis do tratOpCCS:

```
> levels(tratOpCCS)
```

```
[1] "OpCCS" "Eq2"   "Eq6"
```

5.10 Construção do modelo mOpCCS

No modelo mOpCCS os tratamentos "C"e "CS"estão amalgamados(tratOpCCS)

```
> library(survival)
```

```
> mOpCCS<-survreg(Surv(tabop$time,tabop$censor)~tratOpCCS)
```

5.11 ANOVA entre os modelos M3op e mOpCCS

```
> anova(M3op,mOpCCS)
```

```
       Terms Resid. Df    -2*LL Test Df  Deviance   Pr(>Chi)
1 tabop$trat       795 10228.30      NA        NA         NA
2  tratOpCCS       796 10232.86    = -1 -4.561706 0.03269448
```

Comparando os modelos M3op (modelo completo= C, CS, 2Eq e 6Eq) e mOpCCS (modelo com C e CS amalgamados), constatou-se que há diferença significativa entre os mesmos (p= 0.03269448). Portanto, aceita-se o modelo completo(M3op) e rejeita-se o modelo mais simples (mOpCCS). Assim, não é possível amalgamar os tratamentos "C"e "CS".

5.12 Amalgamando os níveis CS e Eq2

Quais são os tempos médios até a morte para os tratamentos que compõem o M3op?

```
> sort(tapply(predict(M3op),list(tabop$trat),mean))
```

```
     Eq6       CS      Eq2        C
2655.242 2717.034 2831.502 3062.532
```

```
> levels(trat)
```

```
[1] "C"   "CS"  "Eq2" "Eq6"
```

Portanto, renomear trat para tratOpEq2CS

```
> tratOpEq2CS<-trat
```

Verificando os níveis do tratOp2

```
> levels(tratOpEq2CS)
```

```
[1] "C"   "CS"  "Eq2" "Eq6"
```

Os tratamentos [2] "CS"e [3]"Eq2"serão amalgamados sob nome de "OpEq2CS"

```
> levels(tratOpEq2CS)[2]<-"OpEq2CS"
> levels(tratOpEq2CS)[3]<-"OpEq2CS"
```

Verificando os níveis do tratOpEq2CS com [2]"CS"e [3]"Eq2"amalgamados

```
> levels(tratOpEq2CS)
```

```
[1] "C"       "OpEq2CS" "Eq6"
```

5.13 Construindo o modelo mOpEq2CS

O modelo mOpEq2CS apresenta os tratamentos "CS"e "Eq2"amalgamados. Construindo o modelo com tratOpEq2CS:

```
> mOpEq2CS<-survreg(Surv(tabop$time,tabop$censor)~tratOpEq2CS)
```

5.14 ANOVA entre os modelos M3op e mOpEq2CS

```
> anova(M3op,mOpEq2CS)
```

```
       Terms Resid. Df    -2*LL Test Df   Deviance  Pr(>Chi)
1  tabop$trat       795 10228.30      NA         NA        NA
2 tratOpEq2CS       796 10228.86    = -1 -0.5599315 0.4542878
```

Comparando-se os modelos M3op("C", "CS", "Eq2"e "Eq6") e mOpEq2CS ("COpEq2CS"e "Eq6") constatou-se que não há diferença significativa entre os mesmos (p= 0.4542878). Portanto, é possível amalgamar os tratamentos CS e Eq2.

5.15 Amalgamando os níveis "OpEq2CS"e "Eq6"

A seguir, será feita a tentativa de amalgamar os tratamentos "OpEq2CS"e "Eq6". Portanto, renomear tratOpEq2CS para tratOpEq26CS

```
> levels(tratOpEq2CS)
[1] "C"        "OpEq2CS" "Eq6"
> tratOpEq26CS<-tratOpEq2CS
```

Verificando os níveis do tratOpEq26CS

```
> levels(tratOpEq26CS)
[1] "C"        "OpEq2CS" "Eq6"
```

Os tratamentos [2]"OpEq2CS"e [3]"Eq6"serão amalgamados sob nome de "OpEq26CS"

```
> levels(tratOpEq26CS)[2]<-"OpEq26CS"
> levels(tratOpEq26CS)[3]<-"OpEq26CS"
```

Verificando os novos níveis do tratOpEq26CS com s tratamentos [2]"OpEq2CS"e [3]"Eq6"amalgamados.

```
> levels(tratOpEq26CS)
[1] "C"         "OpEq26CS"
```

5.16 Construindo o modelo mOpEq26CS

O modelo mOpEq26CS apresenta os níveis [2]"OpEq2CS"e [3]"Eq6"amalgamados.

```
> mOpEq26CS<-survreg(Surv(tabop$time,tabop$censor)~tratOpEq26CS)
```

5.17 ANOVA entre os modelos mOpEq2CS e mOpEq26CS

```
> anova(mOpEq2CS,mOpEq26CS)
         Terms Resid. Df    -2*LL Test Df   Deviance  Pr(>Chi)
1  tratOpEq2CS       796 10228.86      NA         NA        NA
2 tratOpEq26CS       797 10229.72    = -1 -0.8643623 0.3525208
```

Comparando-se os modelos mOpEq2CS("C", "OPEq2CS"e "Eq6") e mOpEq26CS ("C"e "OpEq26CS") constatou-se que não há uma diferença significativa entre os mesmos(p= 0.3525208). Portanto, Aceita-se o modelo mais simples (mOpEq26CS). Assim, é possível amalgamar os tratamentos "OPEq2CS"e "Eq6".

A seguir, o modelo mOpEq26CS será utilizado para calcular o tempo médio de sobrevivência em cada tratamento, destinado à confecção do gráfico com as curvas de sobrevivência para os tratamentos "C"e "OpEq26CS".

5.18 Curvas de sobrevivência dos operários dos inquilinos para os tratamentos amalgamados("C"e "OpEq26CS")

Obtendo informações do modelo mOpEq26CS (com os níveis "C"e "OpEq26CS") por intermédio do summary:

```
> summary(mOpEq26CS)

Call:
survreg(formula = Surv(tabop$time, tabop$censor) ~ tratOpEq26CS)
                      Value Std. Error      z        p
(Intercept)           8.027     0.0403 199.31 0.00e+00
tratOpEq26CSOpEq26CS -0.113     0.0461  -2.46 1.41e-02
Log(scale)           -0.749     0.0374 -20.01 4.45e-89

Scale= 0.473
```

```
Weibull distribution
Loglik(model)= -5114.9   Loglik(intercept only)= -5118
        Chisq= 6.3 on 1 degrees of freedom, p= 0.012
Number of Newton-Raphson Iterations: 5
n= 800
```

O valor de Scale é necessário para calcular o alpha: alpha=1/scale do mOpEq26CS

```
> alfamOpEq26CS<-1/mOpEq26CS$scale

> alfamOpEq26CS

[1] 2.114293
```

O valor do alpha é o mesmo para todas as curvas

O valor de mu é específico para cada curva Cálculo do valor de mu para a curva de sobrevivência do tratamento "C":

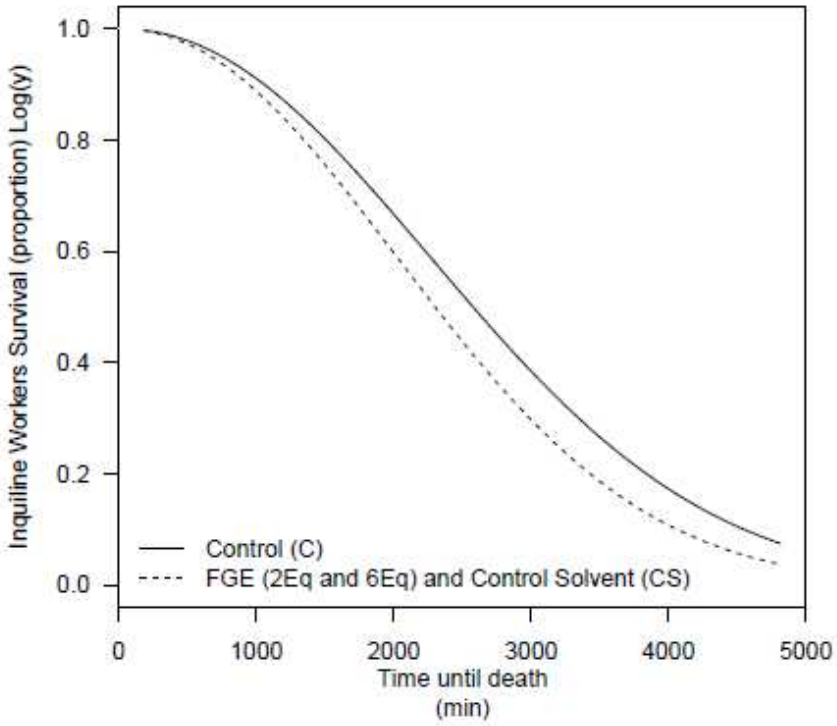
```
> muC<-tapply(predict(mOpEq26CS),list(tratOpEq26CS),mean)[1]

> muC

       C
3062.741
```

Cálculo do valor de mu para a curva de sobrevivência do tratamento "CSEq26":

```
> muOpEq26CS<-tapply(predict(mOpEq26CS),list(tratOpEq26CS),mean)[2]

> muOpEq26CS

OpEq26CS
2735.263
```

Gráfico do mOpEq26CS (com os níveis "C"e "OpEq26CS):

```
> par(las=1)
> with(dados,plot(survfit(Surv(tabop$time,tabop$censor)~tratOpEq26CS),xlab = "Time until death
+ (min)",ylab = "Inquiline Workers Survival (proportion) Log(y)",col = "white",
+ ylim = c(0.0,1.0),xlim = c(0,5000)))
> curve(exp((-muC^(-alfamOpEq26CS))*(x^alfamOpEq26CS)),col="black",lty=1,add = T)
> curve(exp((-muOpEq26CS^(-alfamOpEq26CS))*(x^alfamOpEq26CS)),col="black",lty=2,add = T)
> legend("bottomleft",c("Control (C)","FGE (2Eq and 6Eq) and Control Solvent (CS)"),col=c("black","black"),
+ lty=c(1,2),bty="n")
```

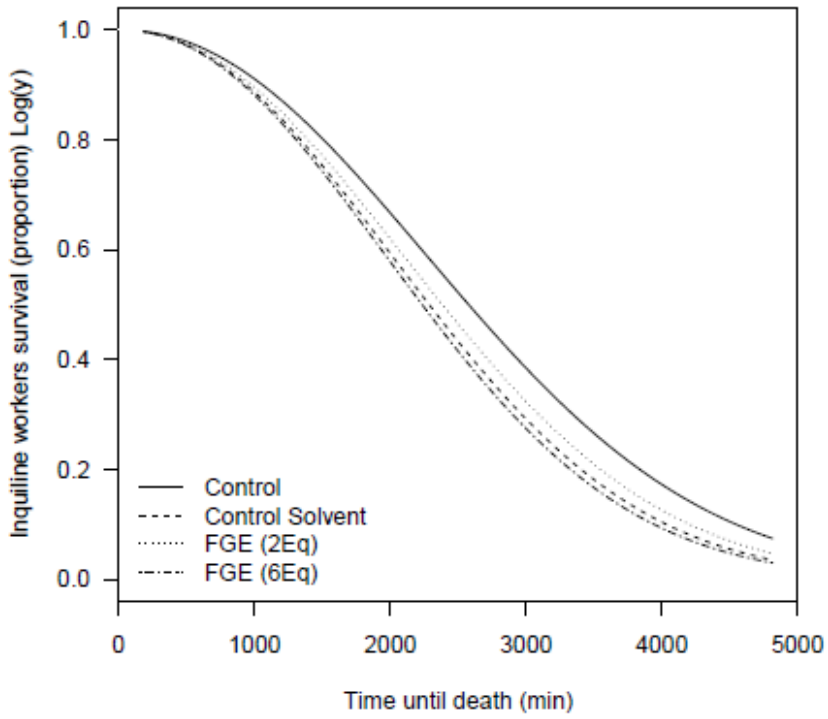


5.19 Curvas de sobrevivência dos operários dos inquilinos para os tratamentos "C", "CS", "FGE-2Eq"e "FGE-6Eq

Obtendo informações do modelo M3op por intermédio do summary:

```
> summary(M3op)

Call:
survreg(formula = Surv(tabop$time, tabop$censor) ~ tabop$trat)
                Value Std. Error      z        p
(Intercept)    8.0270     0.0402 199.45 0.00e+00
tabop$tratCS  -0.1197     0.0561  -2.13 3.28e-02
tabop$tratEq2 -0.0784     0.0559  -1.40 1.60e-01
tabop$tratEq6 -0.1427     0.0557  -2.56 1.04e-02
Log(scale)    -0.7494     0.0374 -20.04 2.50e-89

Scale= 0.473

Weibull distribution
Loglik(model)= -5114.1   Loglik(intercept only)= -5118
        Chisq= 7.72 on 3 degrees of freedom, p= 0.052
Number of Newton-Raphson Iterations: 5
n= 800

> alfaOp<-1/M3op$scale
> alfaOp

[1] 2.115809
```

```
> muOpC<-tapply(predict(M3op),list(tabop$trat),mean)[1]
> muOpC
       C
3062.532
> muOpCs<-tapply(predict(M3op),list(tabop$trat),mean)[2]
> muOpCs
      CS
2717.034
> muOpEq2<-tapply(predict(M3op),list(tabop$trat),mean)[3]
> muOpEq2
     Eq2
2831.502
> muOpEq6<-tapply(predict(M3op),list(tabop$trat),mean)[4]
> muOpEq6
     Eq6
2655.242
> par(las=1)
> with(dados,plot(survfit(Surv(time,censor)~trat),xlab = "Time until death (min)",
+ ylab = "Inquiline workers survival (proportion) Log(y)",col = "white", ylim = c(0.0,1.0),
+ xlim = c(0,5000)))
> curve(exp((-muOpC^(-alfaOp))*(x^alfaOp)),col="black",lty=1,add = T)
> curve(exp((-muOpCs^(-alfaOp))*(x^alfaOp)),col="black",lty=2,add = T)
> curve(exp((-muOpEq2^(-alfaOp))*(x^alfaOp)),col="black",lty=3,add = T)
> curve(exp((-muOpEq6^(-alfaOp))*(x^alfaOp)),col="black",lty=6,add = T)
> legend("bottomleft",c("Control","Control Solvent","FGE (2Eq)","FGE (6Eq)"),
+ col=c("black","black","black","black"),lty=c(1,2,3,6),bty="n")
```



6 Cálculo do tempo até a morte para cada ninho

```
> levels(nest)
```

```
 [1] "N1"  "N13" "N18" "N23" "N26" "N28" "N29" "N3"  "N31" "N32"
```

6.1 "N1"

```
> subset(dados, nest=="N1")->timeN1
> droplevels(timeN1)->timeN1
> summary(timeN1)
```

```
 nest    pDish       indiv         caste     trat         time
 N1:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 240
         P2:22   I10    : 4   workers :80   CS :22   1st Qu.:1440
         P3:22   I11    : 4                 Eq2:22   Median :2880
         P4:22   I12    : 4                 Eq6:22   Mean   :2258
                 I13    : 4                          3rd Qu.:2880
                 I14    : 4                          Max.   :2880
                 (Other):64
     censor
 Min.   :0.0000
 1st Qu.:1.0000
 Median :1.0000
 Mean   :0.8295
 3rd Qu.:1.0000
 Max.   :1.0000
```

```
> m0N1<-survreg(Surv(timeN1$time, timeN1$censor)~1)
> m1N1<-survreg(Surv(timeN1$time, timeN1$censor)~timeN1$trat)

> anova(m0N1,m1N1)

        Terms Resid. Df    -2*LL Test Df Deviance Pr(>Chi)
1           1        86 1248.032      NA       NA       NA
2 timeN1$trat        83 1243.207    =  3 4.825424 0.185036

> summary(m1N1)

Call:
survreg(formula = Surv(timeN1$time, timeN1$censor) ~ timeN1$trat)
                  Value Std. Error      z        p
(Intercept)      7.9509     0.097 81.978 0.00e+00
timeN1$tratCS   -0.0802     0.135 -0.593 5.53e-01
timeN1$tratEq2   0.0415     0.135  0.307 7.59e-01
timeN1$tratEq6  -0.2301     0.133 -1.736 8.26e-02
Log(scale)      -0.9166     0.105 -8.719 2.80e-18

Scale= 0.4

Weibull distribution
Loglik(model)= -621.6   Loglik(intercept only)= -624
        Chisq= 4.83 on 3 degrees of freedom, p= 0.19
Number of Newton-Raphson Iterations: 6
n= 88

> alfaN1<-1/m1N1$scale
> alfaN1

[1] 2.500693

> muCtrlN1<-tapply(predict(m1N1),list(timeN1$trat),mean) [1]
> muCtrlN1

       C
2838.177

> trmCtrlN1<-muCtrlN1*gamma(1+(1/alfaN1))
> trmCtrlN1

       C
2518.229

> muCSN1<-tapply(predict(m1N1),list(timeN1$trat),mean) [2]
> muCSN1

      CS
2619.427

> trmCSN1<-muCSN1*gamma(1+(1/alfaN1))
> trmCSN1

      CS
2324.139

> muFGE2EqN1<-tapply(predict(m1N1),list(timeN1$trat),mean) [3]
> muFGE2EqN1

     Eq2
2958.501

> trmFGE2EqN1<-muFGE2EqN1*gamma(1+(1/alfaN1))
> trmFGE2EqN1
```

```
     Eq2
2624.988
> muFGE6EqN1<-tapply(predict(m1N1),list(timeN1$trat),mean) [4]
> muFGE6EqN1
     Eq6
2254.713
> trmFGE6EqN1<-muFGE6EqN1*gamma(1+(1/alfaN1))
> trmFGE6EqN1
     Eq6
2000.539
```

6.2 "N3"

```
> subset(dados, nest=="N3")->timeN3
> droplevels(timeN3)->timeN3
> summary(timeN3)
 nest    pDish       indiv         caste     trat         time
 N3:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 960
         P2:22   I10    : 4   workers :80   CS :22   1st Qu.:1440
         P3:22   I11    : 4                 Eq2:22   Median :2880
         P4:22   I12    : 4                 Eq6:22   Mean   :2171
                 I13    : 4                          3rd Qu.:2880
                 I14    : 4                          Max.   :2880
                 (Other):64
     censor
 Min.   :0.0000
 1st Qu.:1.0000
 Median :1.0000
 Mean   :0.8864
 3rd Qu.:1.0000
 Max.   :1.0000
> m0N3<-survreg(Surv(timeN3$time, timeN3$censor)~1)
> m1N3<-survreg(Surv(timeN3$time, timeN3$censor)~timeN3$trat)
> anova(m0N3,m1N3)
        Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1           1        86 1287.528      NA       NA        NA
2 timeN3$trat        83 1276.384    =  3 11.14397 0.0109723
> summary(m1N3)
Call:
survreg(formula = Surv(timeN3$time, timeN3$censor) ~ timeN3$trat)
                 Value Std. Error       z        p
(Intercept)     7.9549     0.0723 110.075 0.00e+00
timeN3$tratCS  -0.2846     0.1023  -2.783 5.39e-03
timeN3$tratEq2 -0.0496     0.1050  -0.473 6.36e-01
timeN3$tratEq6 -0.2491     0.1043  -2.388 1.69e-02
Log(scale)     -1.1295     0.0936 -12.067 1.58e-33

Scale= 0.323

Weibull distribution
Loglik(model)= -638.2   Loglik(intercept only)= -643.8
        Chisq= 11.14 on 3 degrees of freedom, p= 0.011
Number of Newton-Raphson Iterations: 6
n= 88
```

```
> alfaN3<-1/m1N3$scale
> alfaN3

[1] 3.094141

> muCtrlN3<-tapply(predict(m1N3),list(timeN3$trat),mean) [1]
> muCtrlN3

       C
2849.473

> trmCtrlN3<-muCtrlN3*gamma(1+(1/alfaN3))
> trmCtrlN3

       C
2548.074

> muCSN3<-tapply(predict(m1N3),list(timeN3$trat),mean) [2]
> muCSN3

      CS
2143.603

> trmCSN3<-muCSN3*gamma(1+(1/alfaN3))
> trmCSN3

      CS
1916.867

> muFCE2EqN3<-tapply(predict(m1N3),list(timeN3$trat),mean) [3]
> muFCE2EqN3

     Eq2
2711.499

> trmFCE2EqN3<-muFCE2EqN3*gamma(1+(1/alfaN3))
> trmFCE2EqN3

     Eq2
2424.695

> muFCE6EqN3<-tapply(predict(m1N3),list(timeN3$trat),mean) [4]
> muFCE6EqN3

     Eq6
2221.162

> trmFCE6EqN3<-muFCE6EqN3*gamma(1+(1/alfaN3))
> trmFCE6EqN3

     Eq6
1986.222
```

6.3 "N13"

```
> subset(dados, nest=="N13")->timeN13
> droplevels(timeN13)->timeN13
> summary(timeN13)

  nest     pDish       indiv          caste      trat          time
 N13:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 480
          P2:22   I10    : 4   workers :80   CS :22   1st Qu.:1440
          P3:22   I11    : 4                 Eq2:22   Median :2880
          P4:22   I12    : 4                 Eq6:22   Mean   :2198
                  I13    : 4                          3rd Qu.:2880
                  I14    : 4                          Max.   :2880
```

```
                (Other):64
    censor
 Min.   :0.0000
 1st Qu.:1.0000
 Median :1.0000
 Mean   :0.8977
 3rd Qu.:1.0000
 Max.   :1.0000
```

```
> m0N13<-survreg(Surv(timeN13$time, timeN13$censor)~1)
> m1N13<-survreg(Surv(timeN13$time, timeN13$censor)~timeN13$trat)
```

```
> anova(m0N13,m1N13)
```

```
         Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1            1        86 1325.069      NA       NA        NA
2 timeN13$trat        83 1323.326    =  3 1.742895 0.6274398
```

```
> summary(m1N13)
```

```
Call:
survreg(formula = Surv(timeN13$time, timeN13$censor) ~ timeN13$trat)
                   Value Std. Error       z        p
(Intercept)      7.84925     0.0863 90.9193 0.00e+00
timeN13$tratCS   0.00325     0.1233  0.0264 9.79e-01
timeN13$tratEq2 -0.09817     0.1204 -0.8151 4.15e-01
timeN13$tratEq6  0.06118     0.1233  0.4961 6.20e-01
Log(scale)      -0.95515     0.1000 -9.5550 1.24e-21

Scale= 0.385

Weibull distribution
Loglik(model)= -661.7   Loglik(intercept only)= -662.5
        Chisq= 1.74 on 3 degrees of freedom, p= 0.63
Number of Newton-Raphson Iterations: 7
n= 88
```

```
> alfaN13<-1/m1N13$scale
> alfaN13
```

```
[1] 2.599054
```

```
> muCtrlN13<-tapply(predict(m1N13),list(timeN13$trat),mean) [1]
> muCtrlN13
```

```
       C
2563.803
```

```
> trmCtrlN13<-muCtrlN13*gamma(1+(1/alfaN13))
> trmCtrlN13
```

```
       C
2277.172
```

```
> muCSN13<-tapply(predict(m1N13),list(timeN13$trat),mean) [2]
> muCSN13
```

```
      CS
2572.154
```

```
> trmCSN13<-muCSN13*gamma(1+(1/alfaN13))
> trmCSN13
```

```
      CS
2284.589
```

```
> muFGE2EqN13<-tapply(predict(m1N13),list(timeN13$trat),mean) [3]
> muFGE2EqN13
     Eq2
2324.066
> trmFGE2EqN13<-muFGE2EqN13*gamma(1+(1/alfaN13))
> trmFGE2EqN13
     Eq2
2064.238
> muFGE6EqN13<-tapply(predict(m1N13),list(timeN13$trat),mean) [4]
> muFGE6EqN13
     Eq6
2725.557
> trmFGE6EqN13<-muFGE6EqN13*gamma(1+(1/alfaN13))
> trmFGE6EqN13
     Eq6
2420.842
```

6.4 "N18"

```
> subset(dados, nest=="N18")->timeN18
> droplevels(timeN18)->timeN18
> summary(timeN18)
  nest    pDish       indiv         caste     trat         time
 N18:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 480
          P2:22   I10    : 4   workers :80   CS :22   1st Qu.:2880
          P3:22   I11    : 4                 Eq2:22   Median :2880
          P4:22   I12    : 4                 Eq6:22   Mean   :2804
                  I13    : 4                          3rd Qu.:2880
                  I14    : 4                          Max.   :2880
                  (Other):64
     censor
 Min.   :0.0000
 1st Qu.:0.0000
 Median :0.0000
 Mean   :0.2955
 3rd Qu.:1.0000
 Max.   :1.0000
> m0N18<-survreg(Surv(timeN18$time, timeN18$censor)~1)
> m1N18<-survreg(Surv(timeN18$time, timeN18$censor)~timeN18$trat)
> anova(m0N18,m1N18)
         Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1            1        86 472.4294      NA       NA        NA
2 timeN18$trat        83 466.4656     =  3 5.963728 0.1133884
> summary(m1N18)

Call:
survreg(formula = Surv(timeN18$time, timeN18$censor) ~ timeN18$trat)
                Value Std. Error      z       p
(Intercept)     8.056     0.0464 173.60 0.00e+00
timeN18$tratCS  0.123     0.0827   1.49 1.36e-01
timeN18$tratEq2 0.089     0.0764   1.17 2.44e-01
timeN18$tratEq6 0.199     0.1036   1.92 5.44e-02
```

```
Log(scale)      -1.906     0.1955  -9.75 1.92e-22

Scale= 0.149

Weibull distribution
Loglik(model)= -233.2   Loglik(intercept only)= -236.2
        Chisq= 5.96 on 3 degrees of freedom, p= 0.11
Number of Newton-Raphson Iterations: 7
n= 88
> alfaN18<-1/m1N18$scale
> alfaN18
[1] 6.724716
> muCtrlN18<-tapply(predict(m1N18),list(timeN18$trat),mean) [1]
> muCtrlN18
       C
3151.649
> trmCtrlN18<-muCtrlN18*gamma(1+(1/alfaN18))
> trmCtrlN18
      C
2941.97
> muCSN18<-tapply(predict(m1N18),list(timeN18$trat),mean) [2]
> muCSN18
     CS
3565.11
> trmCSN18<-muCSN18*gamma(1+(1/alfaN18))
> trmCSN18
      CS
3327.923
> muFGE2EqN18<-tapply(predict(m1N18),list(timeN18$trat),mean) [3]
> muFGE2EqN18
     Eq2
3445.151
> trmFGE2EqN18<-muFGE2EqN18*gamma(1+(1/alfaN18))
> trmFGE2EqN18
     Eq2
3215.946
> muFGE6EqN18<-tapply(predict(m1N18),list(timeN18$trat),mean) [4]
> muFGE6EqN18
     Eq6
3846.733
> trmFGE6EqN18<-muFGE6EqN18*gamma(1+(1/alfaN18))
> trmFGE6EqN18
    Eq6
3590.81
```

6.5 "N23"

```
> subset(dados, nest=="N23")->timeN23
> droplevels(timeN23)->timeN23
> summary(timeN23)
```

```
  nest     pDish       indiv         caste      trat         time
 N23:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 480
          P2:22   I10    : 4   workers :80   CS :22   1st Qu.:2880
          P3:22   I11    : 4                 Eq2:22   Median :2880
          P4:22   I12    : 4                 Eq6:22   Mean   :2591
                  I13    : 4                          3rd Qu.:2880
                  I14    : 4                          Max.   :2880
                  (Other):64
     censor
 Min.   :0.000
 1st Qu.:0.000
 Median :0.000
 Mean   :0.375
 3rd Qu.:1.000
 Max.   :1.000
```

```
> m0N23<-survreg(Surv(timeN23$time, timeN23$censor)~1)
> m1N23<-survreg(Surv(timeN23$time, timeN23$censor)~timeN23$trat)
```

```
> anova(m0N23,m1N23)
```

```
         Terms Resid. Df    -2*LL Test Df Deviance   Pr(>Chi)
1            1        86 627.0526      NA       NA         NA
2 timeN23$trat        83 618.4532    =  3 8.599459 0.03511871
```

```
> summary(m1N23)
```

```
Call:
survreg(formula = Surv(timeN23$time, timeN23$censor) ~ timeN23$trat)
                 Value Std. Error      z        p
(Intercept)      8.611      0.226 38.083 0.00e+00
timeN23$tratCS  -0.372      0.246 -1.514 1.30e-01
timeN23$tratEq2 -0.205      0.252 -0.813 4.16e-01
timeN23$tratEq6 -0.575      0.245 -2.348 1.89e-02
Log(scale)      -0.921      0.164 -5.620 1.91e-08

Scale= 0.398

Weibull distribution
Loglik(model)= -309.2   Loglik(intercept only)= -313.5
        Chisq= 8.6 on 3 degrees of freedom, p= 0.035
Number of Newton-Raphson Iterations: 6
n= 88
```

```
> alfaN23<-1/m1N23$scale
> alfaN23
```

```
[1] 2.512398
```

```
> muCtrlN23<-tapply(predict(m1N23),list(timeN23$trat),mean) [1]
> muCtrlN23
```

```
       C
5490.986
```

```
> trmCtrlN23<-muCtrlN23*gamma(1+(1/alfaN23))
> trmCtrlN23
```

```
       C
4872.553
```

```
> muCSN23<-tapply(predict(m1N23),list(timeN23$trat),mean) [2]
> muCSN23
```

```
      CS
3784.586
```

```
> trmCSN23<-muCSN23*gamma(1+(1/alfaN23))
> trmCSN23
```

```
     CS
3358.34
```

```
> muFGE2EqN23<-tapply(predict(m1N23),list(timeN23$trat),mean) [3]
> muFGE2EqN23
```

```
     Eq2
4474.411
```

```
> trmFGE2EqN23<-muFGE2EqN23*gamma(1+(1/alfaN23))
> trmFGE2EqN23
```

```
     Eq2
3970.472
```

```
> muFGE6EqN23<-tapply(predict(m1N23),list(timeN23$trat),mean) [4]
> muFGE6EqN23
```

```
     Eq6
3089.071
```

```
> trmFGE6EqN23<-muFGE6EqN23*gamma(1+(1/alfaN23))
> trmFGE6EqN23
```

```
     Eq6
2741.158
```

6.6 "N26"

```
> subset(dados, nest=="N26")->timeN26
> droplevels(timeN26)->timeN26
> summary(timeN26)
```

```
  nest     pDish       indiv         caste      trat         time
 N26:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   :  30
          P2:22   I10    : 4   workers :80   CS :22   1st Qu.:2520
          P3:22   I11    : 4                 Eq2:22   Median :2880
          P4:22   I12    : 4                 Eq6:22   Mean   :2400
                  I13    : 4                          3rd Qu.:2880
                  I14    : 4                          Max.   :2880
                  (Other):64
     censor
 Min.   :0.0000
 1st Qu.:0.0000
 Median :1.0000
 Mean   :0.7045
 3rd Qu.:1.0000
 Max.   :1.0000
```

```
> m0N26<-survreg(Surv(timeN26$time, timeN26$censor)~1)
> m1N26<-survreg(Surv(timeN26$time, timeN26$censor)~timeN26$trat)
```

```
> anova(m0N26,m1N26)
```

```
        Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1           1        86 1093.773      NA       NA        NA
2 timeN26$trat        83 1090.628    =  3 3.144943 0.3698131
```

```
> summary(m1N26)
```

```
Call:
survreg(formula = Surv(timeN26$time, timeN26$censor) ~ timeN26$trat)
                  Value Std. Error      z        p
(Intercept)      8.1090      0.111 72.947 0.00e+00
timeN26$tratCS  -0.0842      0.156 -0.541 5.88e-01
timeN26$tratEq2 -0.0832      0.158 -0.526 5.99e-01
timeN26$tratEq6 -0.2584      0.151 -1.713 8.66e-02
Log(scale)      -0.8547      0.118 -7.224 5.06e-13

Scale= 0.425

Weibull distribution
Loglik(model)= -545.3   Loglik(intercept only)= -546.9
        Chisq= 3.14 on 3 degrees of freedom, p= 0.37
Number of Newton-Raphson Iterations: 7
n= 88
```

```
> alfaN26<-1/m1N26$scale
> alfaN26
```

```
[1] 2.350756
```

```
> muCtrlN26<-tapply(predict(m1N26),list(timeN26$trat),mean) [1]
> muCtrlN26
```

```
       C
3324.183
```

```
> trmCtrlN26<-muCtrlN26*gamma(1+(1/alfaN26))
> trmCtrlN26
```

```
       C
2945.799
```

```
> muCSN26<-tapply(predict(m1N26),list(timeN26$trat),mean) [2]
> muCSN26
```

```
      CS
3055.785
```

```
> trmCSN26<-muCSN26*gamma(1+(1/alfaN26))
> trmCSN26
```

```
      CS
2707.952
```

```
> muFGE2EqN26<-tapply(predict(m1N26),list(timeN26$trat),mean) [3]
> muFGE2EqN26
```

```
     Eq2
3058.953
```

```
> trmFGE2EqN26<-muFGE2EqN26*gamma(1+(1/alfaN26))
> trmFGE2EqN26
```

```
     Eq2
2710.759
```

```
> muFGE6EqN26<-tapply(predict(m1N26),list(timeN26$trat),mean) [4]
> muFGE6EqN26
```

```
     Eq6
2567.345
```

```
> trmFGE6EqN26<-muFGE6EqN26*gamma(1+(1/alfaN26))
> trmFGE6EqN26
```

```
     Eq6
2275.111
```

6.7 "N28"

```
> subset(dados, nest=="N28")->timeN28
> droplevels(timeN28)->timeN28
> summary(timeN28)
```

```
  nest     pDish       indiv         caste     trat         time
 N28:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 480
          P2:22   I10    : 4   workers :80   CS :22   1st Qu.:2880
          P3:22   I11    : 4                 Eq2:22   Median :2880
          P4:22   I12    : 4                 Eq6:22   Mean   :2455
                  I13    : 4                          3rd Qu.:2880
                  I14    : 4                          Max.   :2880
                  (Other):64
     censor
 Min.   :0.0000
 1st Qu.:0.0000
 Median :1.0000
 Mean   :0.5568
 3rd Qu.:1.0000
 Max.   :1.0000
```

```
> m0N28<-survreg(Surv(timeN28$time, timeN28$censor)~1)
> m1N28<-survreg(Surv(timeN28$time, timeN28$censor)~timeN28$trat)
```

```
> anova(m0N28,m1N28)
```

```
         Terms Resid. Df    -2*LL Test Df Deviance Pr(>Chi)
1            1        86 890.9102      NA       NA       NA
2 timeN28$trat        83 888.5631    =  3 2.347131 0.503552
```

```
> summary(m1N28)
```

```
Call:
survreg(formula = Surv(timeN28$time, timeN28$censor) ~ timeN28$trat)
                  Value Std. Error      z        p
(Intercept)      8.1892      0.135 60.849 0.00e+00
timeN28$tratCS   0.0355      0.189  0.187 8.51e-01
timeN28$tratEq2 -0.2046      0.171 -1.193 2.33e-01
timeN28$tratEq6 -0.0785      0.181 -0.433 6.65e-01
Log(scale)      -0.8366      0.134 -6.235 4.53e-10

Scale= 0.433

Weibull distribution
Loglik(model)= -444.3   Loglik(intercept only)= -445.5
        Chisq= 2.35 on 3 degrees of freedom, p= 0.5
Number of Newton-Raphson Iterations: 5
n= 88
```

```
> alfaN28<-1/m1N28$scale
> alfaN28
```

```
[1] 2.308523
```

```
> muCtrlN28<-tapply(predict(m1N28),list(timeN28$trat),mean) [1]
> muCtrlN28
       C
3601.968
> trmCtrlN28<-muCtrlN28*gamma(1+(1/alfaN28))
> trmCtrlN28
       C
3191.175
> muCSN28<-tapply(predict(m1N28),list(timeN28$trat),mean) [2]
> muCSN28
      CS
3732.065
> trmCSN28<-muCSN28*gamma(1+(1/alfaN28))
> trmCSN28
      CS
3306.435
> muFGE2EqN28<-tapply(predict(m1N28),list(timeN28$trat),mean) [3]
> muFGE2EqN28
     Eq2
2935.471
> trmFGE2EqN28<-muFGE2EqN28*gamma(1+(1/alfaN28))
> trmFGE2EqN28
    Eq2
2600.69
> muFGE6EqN28<-tapply(predict(m1N28),list(timeN28$trat),mean) [4]
> muFGE6EqN28
     Eq6
3330.173
> trmFGE6EqN28<-muFGE6EqN28*gamma(1+(1/alfaN28))
> trmFGE6EqN28
     Eq6
2950.378
```

6.8 "N29"

```
> subset(dados, nest=="N29")->timeN29
> droplevels(timeN29)->timeN29
> summary(timeN29)
  nest     pDish       indiv         caste      trat         time
 N29:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 480
          P2:22   I10    : 4   workers :80   CS :22   1st Qu.:1440
          P3:22   I11    : 4                 Eq2:22   Median :2880
          P4:22   I12    : 4                 Eq6:22   Mean   :2231
                  I13    : 4                          3rd Qu.:2880
                  I14    : 4                          Max.   :2880
                  (Other):64
     censor
 Min.   :0.000
 1st Qu.:1.000
```

```
 Median :1.000
 Mean   :0.875
 3rd Qu.:1.000
 Max.   :1.000
> m0N29<-survreg(Surv(timeN29$time, timeN29$censor)~1)
> m1N29<-survreg(Surv(timeN29$time, timeN29$censor)~timeN29$trat)
> anova(m0N29,m1N29)
         Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1            1        86 1298.234      NA       NA        NA
2 timeN29$trat        83 1296.883    =  3 1.350989 0.7170624
> summary(m1N29)
Call:
survreg(formula = Surv(timeN29$time, timeN29$censor) ~ timeN29$trat)
                  Value Std. Error      z        p
(Intercept)      7.9360     0.0906 87.583 0.00e+00
timeN29$tratCS  -0.1402     0.1255 -1.117 2.64e-01
timeN29$tratEq2 -0.0949     0.1267 -0.749 4.54e-01
timeN29$tratEq6 -0.0603     0.1250 -0.482 6.30e-01
Log(scale)      -0.9560     0.1023 -9.346 9.06e-21

Scale= 0.384

Weibull distribution
Loglik(model)= -648.4   Loglik(intercept only)= -649.1
        Chisq= 1.35 on 3 degrees of freedom, p= 0.72
Number of Newton-Raphson Iterations: 7
n= 88
> alfaN29<-1/m1N29$scale
> alfaN29
[1] 2.601262
> muCtrlN29<-tapply(predict(m1N29),list(timeN29$trat),mean) [1]
> muCtrlN29
       C
2796.034
> trmCtrlN29<-muCtrlN29*gamma(1+(1/alfaN29))
> trmCtrlN29
       C
2483.503
> muCSN29<-tapply(predict(m1N29),list(timeN29$trat),mean) [2]
> muCSN29
    CS
2430.3
> trmCSN29<-muCSN29*gamma(1+(1/alfaN29))
> trmCSN29
      CS
2158.649
> muFGE2EqN29<-tapply(predict(m1N29),list(timeN29$trat),mean) [3]
> muFGE2EqN29
     Eq2
2542.803
```

```
> trmFGE2EqN29<-muFGE2EqN29*gamma(1+(1/alfaN29))
> trmFGE2EqN29
```

```
     Eq2
2258.577
```

```
> muFGE6EqN29<-tapply(predict(m1N29),list(timeN29$trat),mean) [4]
> muFGE6EqN29
```

```
     Eq6
2632.501
```

```
> trmFGE6EqN29<-muFGE6EqN29*gamma(1+(1/alfaN29))
> trmFGE6EqN29
```

```
     Eq6
2338.248
```

6.9 "N31"

```
> subset(dados, nest=="N31")->timeN31
> droplevels(timeN31)->timeN31
> summary(timeN31)
```

```
  nest     pDish       indiv         caste      trat         time
 N31:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 240.0
          P2:22   I10    : 4   workers :80   CS :22   1st Qu.: 480.0
          P3:22   I11    : 4                 Eq2:22   Median : 960.0
          P4:22   I12    : 4                 Eq6:22   Mean   : 992.7
                  I13    : 4                          3rd Qu.:1440.0
                  I14    : 4                          Max.   :2880.0
                  (Other):64
     censor
 Min.   :1
 1st Qu.:1
 Median :1
 Mean   :1
 3rd Qu.:1
 Max.   :1
```

```
> m0N31<-survreg(Surv(timeN31$time, timeN31$censor)~1)
> m1N31<-survreg(Surv(timeN31$time, timeN31$censor)~timeN31$trat)
```

```
> anova(m0N31,m1N31)
```

```
         Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1            1        86 1343.832      NA       NA        NA
2 timeN31$trat        83 1341.623    =  3 2.208225 0.5303301
```

```
> summary(m1N31)
```

```
Call:
survreg(formula = Surv(timeN31$time, timeN31$censor) ~ timeN31$trat)
                  Value Std. Error      z        p
(Intercept)      6.9520     0.1101 63.170 0.00e+00
timeN31$tratCS   0.1464     0.1561  0.938 3.48e-01
timeN31$tratEq2 -0.0228     0.1551 -0.147 8.83e-01
timeN31$tratEq6  0.1665     0.1563  1.066 2.87e-01
Log(scale)      -0.6648     0.0807 -8.234 1.82e-16

Scale= 0.514

Weibull distribution
```

```
Loglik(model)= -670.8   Loglik(intercept only)= -671.9
        Chisq= 2.21 on 3 degrees of freedom, p= 0.53
Number of Newton-Raphson Iterations: 5
n= 88
```

```
> alfaN31<-1/m1N31$scale
> alfaN31
```

```
[1] 1.944065
```

```
> muCtrlN31<-tapply(predict(m1N31),list(timeN31$trat),mean) [1]
> muCtrlN31
```

```
       C
1045.232
```

```
> trmCtrlN31<-muCtrlN31*gamma(1+(1/alfaN31))
> trmCtrlN31
```

```
       C
926.8888
```

```
> muCSN31<-tapply(predict(m1N31),list(timeN31$trat),mean) [2]
> muCSN31
```

```
      CS
1210.002
```

```
> trmCSN31<-muCSN31*gamma(1+(1/alfaN31))
> trmCSN31
```

```
      CS
1073.003
```

```
> muFGE2EqN31<-tapply(predict(m1N31),list(timeN31$trat),mean) [3]
> muFGE2EqN31
```

```
     Eq2
1021.683
```

```
> trmFGE2EqN31<-muFGE2EqN31*gamma(1+(1/alfaN31))
> trmFGE2EqN31
```

```
     Eq2
906.0055
```

```
> muFGE6EqN31<-tapply(predict(m1N31),list(timeN31$trat),mean) [4]
> muFGE6EqN31
```

```
    Eq6
1234.63
```

```
> trmFGE6EqN31<-muFGE6EqN31*gamma(1+(1/alfaN31))
> trmFGE6EqN31
```

```
     Eq6
1094.843
```

6.10 "N32"

```
> subset(dados, nest=="N32")->timeN32
> droplevels(timeN32)->timeN32
> summary(timeN32)
```

```
 nest    pDish       indiv         caste     trat         time
N32:88   P1:22   I1     : 4   soldiers: 8   C  :22   Min.   : 480
         P2:22   I10    : 4   workers :80   CS :22   1st Qu.:2880
         P3:22   I11    : 4                 Eq2:22   Median :2880
         P4:22   I12    : 4                 Eq6:22   Mean   :2569
                 I13    : 4                          3rd Qu.:2880
                 I14    : 4                          Max.   :2880
                 (Other):64
    censor
 Min.   :0.000
 1st Qu.:0.000
 Median :1.000
 Mean   :0.625
 3rd Qu.:1.000
 Max.   :1.000
```

```
> m0N32<-survreg(Surv(timeN32$time, timeN32$censor)~1)
> m1N32<-survreg(Surv(timeN32$time, timeN32$censor)~timeN32$trat)

> anova(m0N32,m1N32)

         Terms Resid. Df    -2*LL Test Df Deviance  Pr(>Chi)
1            1        86 967.5609      NA       NA        NA
2 timeN32$trat        83 963.5418    =  3 4.019112 0.2594078

> summary(m1N32)

Call:
survreg(formula = Surv(timeN32$time, timeN32$censor) ~ timeN32$trat)
                 Value Std. Error     z        p
(Intercept)      8.207      0.102 80.71 0.00e+00
timeN32$tratCS  -0.157      0.130 -1.21 2.26e-01
timeN32$tratEq2 -0.201      0.126 -1.60 1.09e-01
timeN32$tratEq6 -0.218      0.126 -1.73 8.42e-02
Log(scale)      -1.185      0.130 -9.08 1.06e-19

Scale= 0.306

Weibull distribution
Loglik(model)= -481.8   Loglik(intercept only)= -483.8
        Chisq= 4.02 on 3 degrees of freedom, p= 0.26
Number of Newton-Raphson Iterations: 7
n= 88

> alfaN32<-1/m1N32$scale
> alfaN32

[1] 3.270208

> muCtrlN32<-tapply(predict(m1N32),list(timeN32$trat),mean) [1]
> muCtrlN32

       C
3665.239

> trmCtrlN32<-muCtrlN32*gamma(1+(1/alfaN32))
> trmCtrlN32

       C
3286.285

> muCSN32<-tapply(predict(m1N32),list(timeN32$trat),mean) [2]
> muCSN32
```

```
     CS
3131.17
```

```
> trmCSN32<-muCSN32*gamma(1+(1/alfaN32))
> trmCSN32
```

```
      CS
2807.435
```

```
> muFGE2EqN32<-tapply(predict(m1N32),list(timeN32$trat),mean) [3]
> muFGE2EqN32
```

```
     Eq2
2996.379
```

```
> trmFGE2EqN32<-muFGE2EqN32*gamma(1+(1/alfaN32))
> trmFGE2EqN32
```

```
    Eq2
2686.58
```

```
> muFGE6EqN32<-tapply(predict(m1N32),list(timeN32$trat),mean) [4]
> muFGE6EqN32
```

```
     Eq6
2947.809
```

```
> trmFGE6EqN32<-muFGE6EqN32*gamma(1+(1/alfaN32))
> trmFGE6EqN32
```

```
     Eq6
2643.032
```

7 Análise para verificar se os tempos até a morte dos indiviíduos (considerando soldados e operários conjuntamente) foram influenciados pelos tratamentos

```
> treat<-c("C","C","C","C","C","C","C","C","C","C","CS","CS","CS","CS","CS","CS","CS","CS","CS","CS",
+ "FGE2Eq","FGE2Eq","FGE2Eq","FGE2Eq","FGE2Eq","FGE2Eq","FGE2Eq","FGE2Eq","FGE2Eq","FGE2Eq",
+ "FGE6Eq","FGE6Eq","FGE6Eq","FGE6Eq","FGE6Eq","FGE6Eq","FGE6Eq","FGE6Eq","FGE6Eq","FGE6Eq")
```

```
> nests<-c("N1","N3","N13","N18","N23","N26","N28","N29","N31","N32","N1","N3","N13","N18",
+ "N23","N26","N28","N29","N31","N32","N1","N3","N13","N18","N23","N26","N28","N29","N31",
+ "N32","N1","N3","N13","N18","N23","N26","N28","N29","N31","N32")
```

```
> TimeToDeath<-c(trmCtrlN1,trmCtrlN3,trmCtrlN13,trmCtrlN18,trmCtrlN23,trmCtrlN26,trmCtrlN28,
+ trmCtrlN29,trmCtrlN31,trmCtrlN32,trmCSN1,trmCSN3,trmCSN13,trmCSN18,trmCSN23,trmCSN26,
+ trmCSN28,trmCSN29,trmCSN31,trmCSN32,trmFGE2EqN1,trmFGE2EqN3,trmFGE2EqN13,trmFGE2EqN18,
+ trmFGE2EqN23,trmFGE2EqN26,trmFGE2EqN28,trmFGE2EqN29,trmFGE2EqN31,trmFGE2EqN32,trmFGE6EqN1,
+ trmFGE6EqN3,trmFGE6EqN13,trmFGE6EqN18,trmFGE6EqN23,trmFGE6EqN26,trmFGE6EqN28,trmFGE6EqN29,
+ trmFGE6EqN31,trmFGE6EqN32)
```

```
> TabTimeToDeath<-data.frame(treat,nests,TimeToDeath)
> TabTimeToDeath
```

```
   treat nests TimeToDeath
1      C    N1   2518.2291
2      C    N3   2548.0745
3      C   N13   2277.1716
4      C   N18   2941.9703
```

```
5       C   N23   4872.5531
6       C   N26   2945.7992
7       C   N28   3191.1750
8       C   N29   2483.5026
9       C   N31    926.8888
10      C   N32   3286.2853
11     CS    N1   2324.1387
12     CS    N3   1916.8668
13     CS   N13   2284.5895
14     CS   N18   3327.9235
15     CS   N23   3358.3402
16     CS   N26   2707.9524
17     CS   N28   3306.4354
18     CS   N29   2158.6491
19     CS   N31   1073.0029
20     CS   N32   2807.4349
21 FGE2Eq    N1   2624.9884
22 FGE2Eq    N3   2424.6945
23 FGE2Eq   N13   2064.2375
24 FGE2Eq   N18   3215.9459
25 FGE2Eq   N23   3970.4719
26 FGE2Eq   N26   2710.7592
27 FGE2Eq   N28   2600.6903
28 FGE2Eq   N29   2258.5767
29 FGE2Eq   N31    906.0055
30 FGE2Eq   N32   2686.5801
31 FGE6Eq    N1   2000.5391
32 FGE6Eq    N3   1986.2224
33 FGE6Eq   N13   2420.8420
34 FGE6Eq   N18   3590.8101
35 FGE6Eq   N23   2741.1585
36 FGE6Eq   N26   2275.1105
37 FGE6Eq   N28   2950.3780
38 FGE6Eq   N29   2338.2484
39 FGE6Eq   N31   1094.8427
40 FGE6Eq   N32   2643.0318
```

```
> summary(TabTimeToDeath)
```

```
    treat        nests     TimeToDeath
 C     :10   N1     : 4   Min.   : 906
 CS    :10   N13    : 4   1st Qu.:2271
 FGE2Eq:10   N18    : 4   Median :2574
 FGE6Eq:10   N23    : 4   Mean   :2569
             N26    : 4   3rd Qu.:2947
             N28    : 4   Max.   :4873
             (Other):16
```

7.1 Criação do modelo

```
> m1TimeToDeath<-glm(TabTimeToDeath$TimeToDeath~TabTimeToDeath$nests+TabTimeToDeath$treat)
```

7.2 Aplicação do teste "F"nos tempos até a morte dos inquilinos

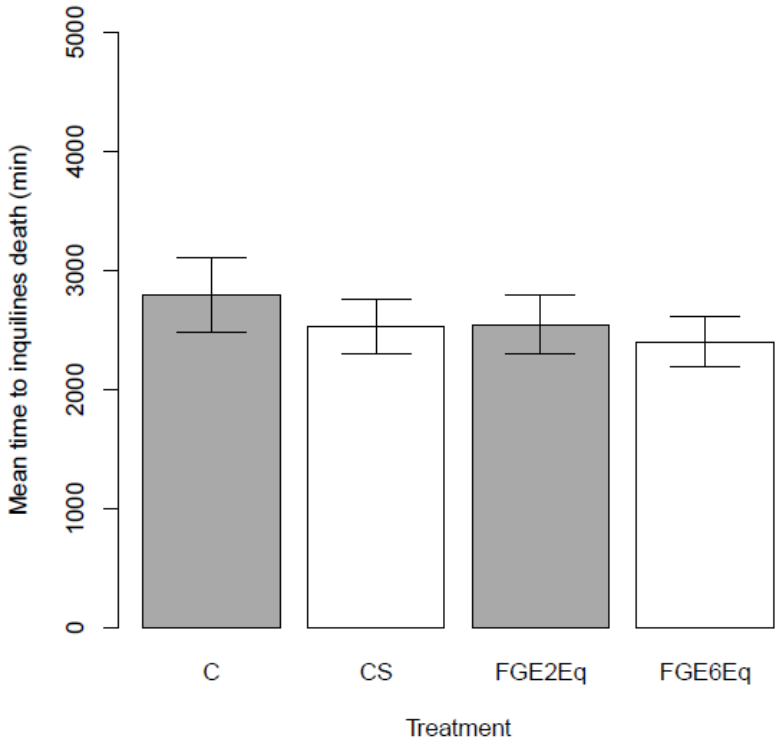
```
> anova(m1TimeToDeath,test="F")
Analysis of Deviance Table
```

```
Model: gaussian, link: identity

Response: TabTimeToDeath$TimeToDeath

Terms added sequentially (first to last)


                    Df Deviance Resid. Df Resid. Dev       F    Pr(>F)
NULL                                    39   23852966
TabTimeToDeath$nests  9 19697408        30    4155558 17.7414 3.683e-09 ***
TabTimeToDeath$treat  3   824808        27    3330750  2.2287    0.1078
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

O tempo até a morte dos inquilinos (considerando soldados e operários conjuntamente) não foi significativamente afetado pelos tratamentos (p= 0.1078).

8 Construção do gráfico de barras para os tratamentos não amalgamados

```
> library(gplots)
> media2<-tapply(predict(m1TimeToDeath),treat,mean)
> media2
       C       CS   FGE2Eq   FGE6Eq
2799.165 2526.533 2546.295 2404.118
> erro2<-tapply(TimeToDeath,treat,sd)/sqrt(tapply(TimeToDeath,treat,length))
> erro2
       C       CS   FGE2Eq   FGE6Eq
311.8280 230.3012 248.3969 209.5044
> barplot2(media2,plot.ci=TRUE,ci.u=media2+erro2,ci.l=media2-erro2,
+   ylim=c(0,5000),xlab="Treatment",ylab="Mean time to inquilines death (min)",
+   col=c("darkgray","white"))
```


Mean time to inquilines death (min)
0
1000
2000
3000
4000
5000
C
CS
FGE2Eq
FGE6Eq
Treatment

APÊNDICE B

Repelência de *Inquilinitermes microcerus* por exsudatos do seu hospedeiro *Constrictotermes cyphergaster*

15.07.2017

Resumo

O experimento objetivou verificar se inquilinos (*Inquilinitermes microcerus*) evitariam contato direto com os exsudatos do construtor (*Constrictotermes cyphergaster*). Para isso, inquilinos foram colocados numa placa de petri (20 op + 2 sold) forrada com papel filtro marcado diametralmente em duas metades. Em somente uma das metades da placa aplicou-se a substância sob teste. A outra metade serviu de controle local, pois nada foi aplicado. Após a morte de todos os inquilinos anotou-se quantos foram encontrados mortos em cada uma das metades da placa.

1 Preâmbulo

1.1 Tratamentos

Há quatro tratamentos:

1. somente papel (total)
2. somente solvente (hexano)
3. 2 individuos-equivalentes de exsudatos da glandula frontal
4. 6 individuos-equivalentes de exsudatos da glandula frontal

1.2 Hipótese biológica

Sabe-se que *Inquilinitermes microcerus* consegue reconhecer os feromônios de trilha de seus hospedeiros. É plausível supor, portanto, que reconheceriam também os feromônios de alarme e as substâncias de defesa que se encontram na glândula frontal, evitando ao máximo permanecer próximos da fonte de tais substâncias. Após suficiente lapso de tempo, os indivíduos **morrerão por inanição** neste lado da placa.

1.3 Hipótese estatística

Havendo repêlencia, os inquilinos tenderão a se agrupar na metade não tratada da placa. Após o final o experimento, mais indivíduos mortos estarão neste lado, quando comparado com o lado tratado. Isso deve ser mais evidente nas placas com maior quantidade de exsudatos (6 equivalentes) do que naquelas onde há menos exsudatos (solvente somente ou 2 equivalentes)

1.4 Estabelecimento do diretório de trabalho e do arquivo dos dados

```
> setwd("~/ISOP.DSC.AN.REPEL4")
> dados <- read.table("repelencia_tudo.csv", h=T)
> summary(dados)
```

```
      rep       n_individuos          metade     trat
 sn1    : 6   Min.   : 0    nao_tratada:30   eq2 :20
 sn13   : 6   1st Qu.: 4    tratada    :30   eq6 :20
 sn18   : 6   Median :11                     solv:20
 sn23   : 6   Mean   :11
 sn26   : 6   3rd Qu.:18
 sn28   : 6   Max.   :22
 (Other):24
```

```
> head(dados)
```

```
   rep n_individuos  metade trat
1  sn1            4 tratada  eq2
2  sn3           21 tratada  eq2
3 sn13            6 tratada  eq2
4 sn18            3 tratada  eq2
5 sn23           21 tratada  eq2
6 sn26           11 tratada  eq2
```

Neste conjunto de dados, cada linha é uma das metades de uma placa de petri onde se fez o teste. Os números se referem aos indivíduos mortos naquela metade.

Como todos as placas tinha sempre o mesmo número de indivíduos, o número de mortos no lado tratado da placa é *complementar* ao número de mortos no lado não-tratado. Assim, sabendo um desses números e o total de indivíduos (sempre 22), é suficiente para nossos cálculos. Por isso, vamos extrair dos dados somente as linhas referentes às metades não-tratadas da placa.

```
> sdados <- subset(dados,metade=="nao_tratada")
> sdados<-droplevels(sdados)
> summary(sdados)
```

```
      rep      n_individuos            metade     trat
 sn1    : 3   Min.   : 1.00   nao_tratada:30   eq2 :10
 sn13   : 3   1st Qu.: 8.00                    eq6 :10
 sn18   : 3   Median :13.50                    solv:10
 sn23   : 3   Mean   :12.93
 sn26   : 3   3rd Qu.:19.00
 sn28   : 3   Max.   :22.00
 (Other):12
```

```
> head(sdados)
```

```
    rep n_individuos      metade trat
11  sn1           18 nao_tratada  eq2
12  sn3            1 nao_tratada  eq2
13 sn13           16 nao_tratada  eq2
14 sn18           19 nao_tratada  eq2
15 sn23            1 nao_tratada  eq2
16 sn26           11 nao_tratada  eq2
```

```
> attach(sdados)
```

2 Análise

Caso nossa hipótese de trabalho esteja correta, os indivíduos tendem a se concentrar nas áreas não-tratadas nos tratamentos onde há maior quantidade de exsudatos. Logo, a percentagem de indíviduos encontrados nas áreas não tratadas deve aumentar no sentido trat: solv $<$ eq2 $<$ eq6. Como temos sempre 22 indivíduos, usar percentagens ou contagems é redundante. Usaremos contagens por ser mais simples. Por isso, a distribuição de erros é Poisson.

```
> m1 <- glm(n_individuos~trat, poisson)
> anova(m1,test="Chisq")

Analysis of Deviance Table

Model: poisson, link: log

Response: n_individuos

Terms added sequentially (first to last)


     Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                    29     120.03
trat  2   1.2596        27     118.77   0.5327
```

```
> summary(m1)
```

```
Call:
glm(formula = n_individuos ~ trat, family = poisson)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.4283  -1.5420   0.0127   1.4312   2.1482

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.595255   0.086387  30.042   <2e-16 ***
trateq6      0.007435   0.121943   0.061    0.951
tratsolv    -0.118716   0.125961  -0.942    0.346
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 120.03  on 29  degrees of freedom
Residual deviance: 118.77  on 27  degrees of freedom
AIC: 250.37

Number of Fisher Scoring iterations: 5
```

```
> 118.77/27

[1] 4.398889
```

Há sobredispersão. Corrigiremos usando Quasipoisson

```
> m2<-update(m1,family=quasipoisson)
> anova(m2,test="F")

Analysis of Deviance Table

Model: quasipoisson, link: log

Response: n_individuos

Terms added sequentially (first to last)


     Df Deviance Resid. Df Resid. Dev      F Pr(>F)
NULL                    29     120.03
trat  2   1.2596        27     118.77 0.1735 0.8417
```

Não há diferenças significativas entre os tratamentos solv, eq2 e eq6 ($p = 0.8417$). Logo, não parece ter havido qualquer repelência dos inquilinos pelos exsudatos dos construtores.

```
> summary(m2)

Call:
glm(formula = n_individuos ~ trat, family = quasipoisson)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.4283  -1.5420   0.0127   1.4312   2.1482

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  2.595255   0.164613  15.766 3.83e-15 ***
trateq6      0.007435   0.232366   0.032    0.975
tratsolv    -0.118716   0.240021  -0.495    0.625
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 3.631038)

    Null deviance: 120.03  on 29  degrees of freedom
Residual deviance: 118.77  on 27  degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 5

> library(gplots)

> media<-tapply(n_individuos,trat,mean)

> media

 eq2  eq6 solv
13.4 13.5 11.9

> erro<-tapply(n_individuos,trat,sd)/sqrt(tapply(n_individuos, trat,length))

> barplot2(media,plot.ci = TRUE, ci.u = media+erro, ci.l = media-erro,
+          ylim = c(0,25),ylab = "Mean number of dead inquiline individuals",
+          xlab = "Treatments",names.arg = c("CS","FGE-2Eq","FGE-6Eq"),
+          col = c("darkgray","white"))
```