LÍVIA FONSECA NUNES

TRIGGERS FOR BEHAVIOURAL COHESION IN SOCIAL GROUPS

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

Orientador: Og Francisco F. de Souza

Coorientadora: Gladys Julieth C. Quiroga

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Og Francisco Fonseca de Souza
Orientador

DEDICATÓRIA

Aos meus filhos, Helena e Jonas.
Tudo por vocês!

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EPÍGRAFE

"Only those who will risk going too far can possibly find out how far they can go".
(T.S. Eliot)

RESUMO

NUNES FONSECA, Lívia, D.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Triggers for behavioral cohesion in social groups**. Orientador: Og Francisco Fonseca de Souza. Coorientadora: Gladys Julieth Castiblanco Quiroga.

O comportamento coletivo é um fenômeno difundido no mundo animal. Para agir coletivamente, os indivíduos devem exibir comportamentos coesos que, por sua vez, implicam múltiplos indivíduos executando comportamentos semelhantes ou complementares. Apesar do grande progresso na compreensão dos mecanismos que regem o comportamento coletivo, pouco se sabe sobre os fatores que predispõem os indivíduos a convergir suas ações exibindo uma atitude comportamental coesa. Aqui mostramos que os estímulos externos podem ser um desses fatores. Para tanto, em laboratório, aplicamos diferentes tipos e intensidades de estímulos externos a grupos de cupins da espécie *Constrictotermes cyphergaster*. Nossos resultados demonstraram que em todos os casos, a coesão comportamental entre os indivíduos foi desencadeada pela inserção do estímulo. Argumentamos que a coesão comportamental foi alcançada pela resposta independente dos indivíduos à estimulação externa. A imagem que emerge de nossos resultados é que, embora frequentemente relacionada à motivação intrínseca dos indivíduos para permanecerem juntos, um traço onipresente em organismos sociais, a coesão comportamental também pode ser impulsionada por estímulos externos. Nossos resultados corroboram e acrescentam mais evidências sobre o papel dos fatores externos no comportamento coletivo em animais.

Palavras-chave: Comportamento coletivo. Comportamento coerente. Estímulo externo.

ABSTRACT

NUNES FONSECA, Lívia, D.Sc., Universidade Federal de Viçosa, February, 2023. **Triggers for behavioural cohesion in social groups**. Advisor: Og Francisco Fonseca de Souza. Co-advisor: Gladys Julieth Castiblanco Quiroga.

Collective behaviour is a widespread phenomenon in the animal world. In order to act collectively, individuals must exhibit cohesive behaviours which, in turn, imply multiple individuals performing similar or complementary behaviours. Despite the robust theory on mechanisms that govern collective behaviour, little is known about the factors that predispose individuals to converge their actions by exhibiting a cohesive behavioural attitude. Here we show that external stimuli can be one of these factors. To do so, we apply different types and intensities of external stimuli to laboratory groups of *Constrictotermes cyphergaster* termite individuals. Our results demonstrated that in all cases, behavioural cohesion among individuals was triggered by the stimulus insertion. We argue that behavioural cohesion was attained by the independent response of individuals to external stimulation. The picture that emerges from our results is that although frequently related to the intrinsic motivation of individuals to remain together, a ubiquitous trait in social organisms, behavioural cohesion can also be driven by external stimulation. Our results corroborate and add further evidence on the role of external factors in collective behaviour.

Keywords: Collective behaviour. Behavioural coherence. External stimulus.

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Thesis overview

The aim of the present work was to investigate the triggers for behavioural cohesion. More specifically, we tested whether external stimuli application would predispose individuals to converge their actions leading to an emergent behavioural cohesion that could, potentially, culminate in pattern formation at the group scale. Act cohesively seems to be an important trait, mainly in those organisms that need to perform complex collective tasks, such as social animals.

Chapter 1: Behavioural cohesion in social animals emerging from external stimuli

In chapter 1 we studied the collective behaviour in termite species *Constrictotermes cyphergaster*. More specific we investigated whether external stimuli would drive individuals to converge their actions to exhibit a cohesive behavioural response at group level. Using behavioural assays under laboratory conditions we showed that indeed the aplication of external stimulus predispose individuals to perform similar behaviours leading to the emergence of behavioural cohesion among group members. The results indicates that such cohesion was attained by the independend responses of the individuals to the stimulus rather than a byproduct of interindividual interaction. It suggest that instead of a internal motivation, a ubiquitous trait in social organisms, behavioural cohesion among individuals could also be guided by the external environment. Our results pointed out the importance of including external factors in the study of animal collective behaviour.

Chapter 2: Dataset on substrate-borne vibration of *Constrictotermes cyphergaster* (Blattodea: Isoptera) termites

The chapter 2 consisted of a published datapaper where we described the methods we used to collect the dataset used in chapter 1. By publishing the datapaper we aimed to encourage the accessibility and reuse of our dataset as also as to contribute to the principle of open science.

Chapter 1

Behavioural cohesion in social animals emerging from external stimuli

Lívia Nunes, Luana Rodrigues, Julieth Castiblanco & Og DeSouza

1.1 Introduction

Animal grouping is widespread in the world. Animals can group for a specific and temporary goal (*i.e.* to feed, to mate, to defend) or animals can live in permanent social groups. Forming a group demands behavioural coherence between group members in order to maintain group cohesion. By doing so, individuals gather the benefits of staying together, such as decreased predation risk (Lehtonen and Jaatinen, 2016), increased foraging efficiency (Gordon, 2013) or reduced energetic costs of locomotion (Marras et al., 2015). Behavioural coherence emerges when multiple individuals converge their actions to perform similar or complementary behaviours. Such behavioural dynamics is often referred to as collective behaviour. Collective behaviour is frequently linked to the emergence of a pattern at the group scale, as can be seem in a flock of birds (Storms et al., 2019), schoal of fish (Lopez et al., 2012), the trail formation in ants (Couzin and Franks, 2003), nest construction in termites (Mizumoto and Bourguignon, 2020; Heyde et al., 2021) or the marching bands in locusts (Buhl et al., 2006). However, not all collective behaviour will culminate in a neat, sheer geometric pattern formation at the group scale. Midges, for instance, get together in swarms which can be better described as chaotic than ordered (Attanasi et al., 2014; van der Vaart et al., 2020). But, by all means, their individual behaviours are complementary enough to keep them in a cohesive swarm, hence qualifying as "collective behaviour". That is to say, it seems more unbiased to associate collective behaviour to behavioural cohesion among individuals rather than to group pattern formation. It is in this sense that we will refer to collective behaviour throughout this paper.

The mechanisms governing collective behaviour have been the subject of several studies (Couzin et al., 2002; Sumpter, 2006, 2010; Camazine et al., 2020; Attanasi et al., 2014). It basically consists of a set of simple interaction rules between group members by which individuals are able to connect with each other through information exchange. Information exchange, in turn, mediates specific behavioral responses of individuals which, ultimately, impacts the collective dynamics of the group as a whole (Eftimie et al., 2007; Demartsev et al., 2022). However, despite the progress on how collective behaviour is created and how it persists, a pending question remains not entirelly solved: "which factors predispose individuals to get together and converge their actions to perform a cohesive behavioural

pattern?". That is, while most studies on collective behaviour aim to understand how behavioural cohesion gives rise to group pattern formation, here we take an important "step back" to understand the starting point of behavioural cohesion among individuals.

The starting point of behavioural cohesion relies on the propensity of individuals to get together and converge their actions towards a specific or limited set of behaviours. Individuals may converge their action by intrinsic or extrinsic motivation. Intrinsic motivation concerns to, for example, the individuals desire to move in certain direction as shown in migrating species (Buhl et al., 2006), or their natural behaviour to explore the environment, as shown by Jeanson et al. (2005) in juvenile cockroaches *Blattella germanica* or even the natural tendency of individuals to copy behaviours of nearby nestmates, an ubiquitous trait in social species such as termites (DeSouza et al., 2001), ants (Boulay et al., 1999), among others. On the other hand, extrinsic motivation consists of external stimuli driving independently the reaction of individuals to perform similar behaviours. Indeed, it was already suggested by Downes et al. (1955) that non-patterned swarming formation in midges is a mere outcome of independent interaction of each individual to an external landmark. Later on, it was demonstrated that individuals within such disordered swarm behave collectively (Attanasi et al., 2014; Mateo et al., 2017). Together the above results suggest that some landmark feature promoted the behavioural convergence among individuals by attracting them to fly within its vinicity which, in turn, allowed the individuals to enter a state of a cohesive behavioural pattern. It is then, plausible to assert that an external stimuli (the landmark feature) was the starting point that triggered behavioural cohesion in midges.

Here, then, we tested such a hypothesis, namely that, behavioural cohesion can be triggered by external stimuli. It is important to note that behavioural cohesion here means a group of individuals performing similar or complementary behaviours. Whether or not this will culminate in pattern formation at the group scale is beyond the scope of this paper.

To test this hypothesis we exposed groups of *Constrictotermes cyphergaster* (Silvestri, 1901) termite individuals, to external stimuli that varied in quality (stimulus type) and intensity (stimulus volume). The experiment aimed to inspect whether stimuli would drive individuals to converge their actions to perform similar behaviours culminating in

a cohesive behavioural attitude. Termites provide a good model for this purpose. As eusocial organisms they perform a variety of collective behaviours such as nest construction (Mizumoto and Bourguignon, 2020; Heyde et al., 2021) and social coordination against desease (Davis et al., 2018; Rosengaus et al., 1999), to name a few. In addition, termites are known to respond to external stimuli by adjusting their behaviours regarding the stimuli type and intensity (Cristaldo et al., 2015; Oberst et al., 2017; Evans et al., 2009), giving us a range of possible stimulus to test. Finally, their manipulation in laboratory conditions is relatively easy.

1.2 Methods

Full details about the experimental procedures and setup development can be acessed in Chapter 3 wich consists of the datapaper *"Dataset on Substrate-Borne Vibrations of Constrictotermes cyphergaster (Blattodea: Isoptera) Termites"* written by Nunes et al. (2019), published in DATA journal.

1.2.1 Ethics Statment

All necessary permits were obtained for the described study, which complied with all relevant regulations of Brazil. This includes collecting and transportation permit from IBAMA (The Brazilian Institute for the Environment and Renewable Natural Resources), as well as tacit approval from the Brazilian Federal Government implied by granting the authors the post of Scientific Researcher. Species collected for the present study are neither endangered nor protected ones and, thus, no specific permits were required for laboratory experiments.

1.2.2 Study site and collection

Assays were conducting using 15 wild nests of *C. cyphergaster*, collected in April 2017 in the Brazilizan "Cerrado", in the municipality of Sete Lagoas, Minas Gerais, Brazil (S 27°19′ , W 14°44′; altitute 800-900m above the sea level). The colonies were transported to the campus of the Federal University of Viçosa, in the municipality of Viçosa, Minas Gerais, Brazil, where they were kept in the laboratory under laboratory conditions. Water and food was offered, *ad libitum* during the whole period of the assays.

1.2.3 Experimental setup

The experimental arena, here called of tympanic arena, consisted of a frame covered by tracing paper as if forming a drum. On the tympanic arena, we placed a lid of a pastic Petri dish ($\varnothing$ = 53 mm) to hold the termite individuals to be assayed. The tympanic arena (frame plus Petri dish) was placed inside a wooden box lined with a thick layer of glass wool and styrofoam to prevent termites from external perturbations. Inside the wooden box, the Petri dish was connected by a hose to the injection chamber placed outside the wooden box. In the injection chamber, we placed each stimulus tested. Each stimulation was carried to the termites by gently injecting, into the Petri dish, a known volume of air carring the stimulus odours.

Termites were submitted to the following treatments: (i) empty chamber, thence only air (AR), (ii) air + paper (AP), (iii) air + paper + hexane (APH) and (iv) air + paper + soldiers' head extract (APE). For the stimulus AR, the injection chamber was empty whereas for the other stimuli, the chamber received a piece of filter paper (7x 3 mm). For stimuli APH and APE this piece of paper was loaded with known volume of hexane or soldier's head extract, respectively. The stimuli APH and APE also varied in volume to provide different intensities.

1.2.4 Termite soldier's head extract preparation

To prepare the soldiers' head extracts (APE) stimulus, we picked up 20 soldiers from their respective nests, anesthetized them on ice until their movements slowed down, and then severe their heads from the rest of their body using a micro scissors and a tweezers. Then, we exposed the contents of the frontal gland (the gland where the alarm pheromone is produced and stored) by slicing their heads in halves. The heads were then immersed in hexane (10 µL per head) and placed in a freezer at 2.4 °C for 24 hours. The resulting extract was then collected with the help of a microsyringe and stored again in the freezer until its use in the assays.

From each nest, we prepared only one extract, totaling 15 extracts. Because termites' heads vary in size and, consequently, in the amount of hexane they absorb, the volume of extract varied between nests. As a consequence, the volume of extract applied at the termites' group could be different but the proportional equivalence of their heads was

maintained. An overview of the stimuli with the respective volume of hexane (APH) or frontal gland extract (APE), as well as the corresponding equivalence of the number of head used in the assays, can be found at Table 2.1.

Table 1.1: Overview of tested stimuli. Column readings: NestID = the identity of the nest where the stimuli were prepared and applied; Treatment = the type of stimulus used in the assays; Chemical stimulus volume = the volume of the chemical stimulus (hexane or extract) used in the assays; Head equivalence = the number of soldier heads used to prepare the soldiers' head extract (APE).

NestID	Treatment	Chemical stimulus volume (µL)	Head equivalence
N01YCC2017	AR	0.00	0
	AP	0.00	0
	APH	3.26	0
	APE	3.26	1
N02YCC2017	AR	0.00	0
	AP	0.00	0
	APH	6.69	0
	APE	6.69	3
N03YCC2017	AR	0.00	0
	AP	0.00	0
	APH	13.5	0
	APE	13.5	5
N04YCC2017	AR	0.00	0
	AP	0.00	0
	APH	24.68	0
	APE	24.68	7
N05YCC2017	AR	0.00	0
	AP	–	–
	APH	47.57	0
	APE	47.57	9
N06YCC2017	AR	0.00	0
	AP	0.00	0
	APH	4.61	0
	APE	4.61	1
	AR	0.00	0

Table 1.1 continued from previous page

NestID	Treatment	Chemical stimulus volume (µL)	Head equivalence
N07YCC2017	AP	0.00	0
	APH	11.98	0
	APE	11.98	3
N08YCC2017	AR	0.00	0
	AP	0.00	0
	APH	28.9	0
	APE	28.9	5
N09YCC2017	AR	0.00	0
	AP	0.00	0
	APH	25.2	0
	APE	25.2	7
N10YCC2017	AR	0.00	0
	AP	–	–
	APH	49.32	0
	APE	49.32	9
N11YCC2017	AR	0.00	0
	AP	0.00	0
	APH	1.6	0
	APE	1.6	1
N12YCC2017	AR	0.00	0
	AP	0.00	0
	APH	15.54	0
	APE	15.54	3
N13YCC2017	AR	0.00	0
	AP	0.00	0
	APH	24.5	0
	APE	24.5	5
	AR	0.00	0
	AP	0.00	0
	APH	20.79	0

Table 1.1 continued from previous page

NestID	Treatment	Chemical stimulus volume (µL)	Head equivalence
N14YCC2017	APE	20.79	7
N15YCC2017	AR	0.00	0
	AP	0.00	0
	APH	45.36	0
	APE	45.36	9

1.2.5 Behavioural assay

From each termite nest we collected four different groups of termites. Each of these groups was subjected to a certain stimulus (described below), only once. The assayed groups consisted of 15 individuals (12 workers and 3 soldiers) which were collected from their colonies and placed, one by one, inside the tympanic arena. Termites were left acclimatizing for two hours prior to the begining of the experiment. The group size and caste ratio were chosen so that to conform to the natural proportion found in the field nests (4.5 workers:1 soldier) (Cunha et al., 2003) and to the density known to improve interindividual interactions and survival (DeSouza et al., 2001). A total of 60 termite groups (15 nests * 4 treatments) and 900 individuals (60 termites groups * 15 individuals) were assayed.

1.2.6 Behavioural video recordings

The behavioral responses were video recorded with a digital video camera (SONY HDR-CX405 TM), set to record 30 frames per second at full HD (resolution: 1920x1080 pixels). Each assay was recorded for a total of seven minutes: two minutes before stimulus injection and five minutes after stimulus injection. This setting enabled the assessment of usual group activities (i.e. before stimulus excitement) and the activities performed by the group after appying the stimulus. In doing so, a total of 24,360 seconds [(7 minutes * 60 seconds * 4 stimuli * 15 assays) - (2 missing assays * 420 seconds)] were video recorded for the whole experiment.

1.2.7 Behavioural analysis

We collected the behavioural data from the video recordings using the Boris software (Friard and Gamba, 2016). For that, at the beginning of each video (time = 0), a printscreen of the video frame was taken and the focal termite was marked with a colored paint in *Microsoft Paint* software in order to prevent duplicate identities. A total of 900 individuals, including soldiers and workers, were visually singled out (15 individuals *per* termite groups *per* treatment). To avoid distinct interpretations of the behaviours performed by termites, only one observer was allowed to analyse the videos. We cut fragments of the video recordings such that the segments before and after contained the same amount of frames. The fragments at the moment "after" stimulus application consisted of the first minutes after stimulus insertation. A total 120 seconds before and 120 seconds after stimulus application were analysed *per* assay, totaling aprox. 7200 (240 seconds * 30 frames/sec) frames.

The information acquired at the Boris software was used to construct an ethogram. In the context of this work, the concept of behaviour was defined according to Tinbergen (2020): *"The total movements made by the intact animal"*. This broad definition includes all behavioural changes executed by the individuals, including a change in motion to nonmotion. The definition of the each behaviour observed here (discribed in Table 2.2) followed Hugo et al. (2020) description. Individuals who died during the acclimatization period were excluded from the analysis. Ethograms were then used to assess the propensity of individuals to converge their actions in response to stimulus received.

Table 1.2: List of behaviours performed by individuals observed in Boris sofware.

Behaviour	**Definition**
Antennation Plate	To antennate the plate (lateral or above)
Antennation Termite	To antennate another termite
Attack	To attack another termite (aggressive behaviour)
Bypass	To pass by another termite and continue walking/running in the same direction
Eat	To eat the filter paper
Explore walk	To walk around the arena
Explore run	To run around the arena
Grooming mate	To get or to give groom to a nestmate
Grooming plate	To groom the plate
Grooming self	To groom itself
Poop	To defecate
Reverse	To pass by another termite and change the direction of the walk/run
Rest	To rest, be without movement for a while
Tropholaxy bucal	To exchange substances via oral
Tropholaxy proctodeal	To exchange substances via anus
Vibrate	To vibrate the body (jerk or drum)

1.2.8 Statistical analysis

To obtain the number of times behaviours were executed *per* individual for each moment (before and after) we divided the total number of times each behaviour was registered by the total number of individuals within group. In order to inspect whether the stimuli application (*x-var*) would affect the number of times behaviours were executed within group (*y-var*), we divided the total number of times behaviours were executed *per* individual within the group by the total number of termite groups. We, then, perfomed a Generalized Linear Model (GLM) with Gaussian error distribution and selected the best model using the second-order Akaike Information Criterion (AICc). Because each nest was assayed before and after stimulus insertation the nest indentity entered in the model as blocking factor. Models with $\Delta \leq 2$ were considered significantly supported.

To obtain the Delta of the number of times each behaviour was executed *per* individual we

first calculated the "Delta value" of the number of times each behaviour were registered for each treatment for each termite group. The delta value consisted of the difference between the number of times a given behaviour was executed after and before stimulus aplication. In doing so, we obtained the number of times a given behaviour was executed given the stimuli application. Each of these values were, then, divided by the number of individuals within termite groups. To investigated whether the delta number of times each behaviour was performed within termite group *(y-var)* is affected by the type of stimulus (x-var), we divided the total number of times each behaviour were executed *per* individual within the group by the total number of termite groups. Then, we perfomed a Generalized Linear Model (GLM) with Gaussian error distribution and selected the best model using the second-order Akaike Information Criterion (AICc). **This was done for each stimulus separately**. We performed a contrast analysis in order to verify whether the number of times each behaviour type was executed *per* individual differed from each other according to the stimulus type. Because several behaviours were recorded in the same nest, the nest indentity entered in the model as blocking factor. Models with $\Delta \leq 2$ were considered significantly supported.

Finally, to investigated whether the delta number of times each behaviour was performed within termite group *(y-var)* is affected by the stimulus volume (x-var), we divided the total number of times each behaviour were executed *per* individual within the group by the total number of termite groups. Then, we perfomed a Generalized Linear Model (GLM) with Gaussian error distribution for each treatment and selected the best model using the second-order Akaike Information Criterion (AICc). Because several behaviours were recorded in the same nest, the nest indentity entered in the model as blocking factor. Models with $\Delta \leq 2$ were considered significantly supported.

All the analyses were performed in (R Core Team, 2020) using the MuMIn package (Barton and Barton, 2012). The residual a analysis of the models was done in order to check the suitable of the error distribution and model fitting.

1.3 Results

1.3.1 Stimuli application triggered behavioural responses within termite groups

All stimuli tested were effective in trigering behavioural responses within termite groups (Fig. 2.1, Table 2.3). That is, the average number of times termites performed acts was higher after stimulus application to all kind of stimuli (Table 2.3).

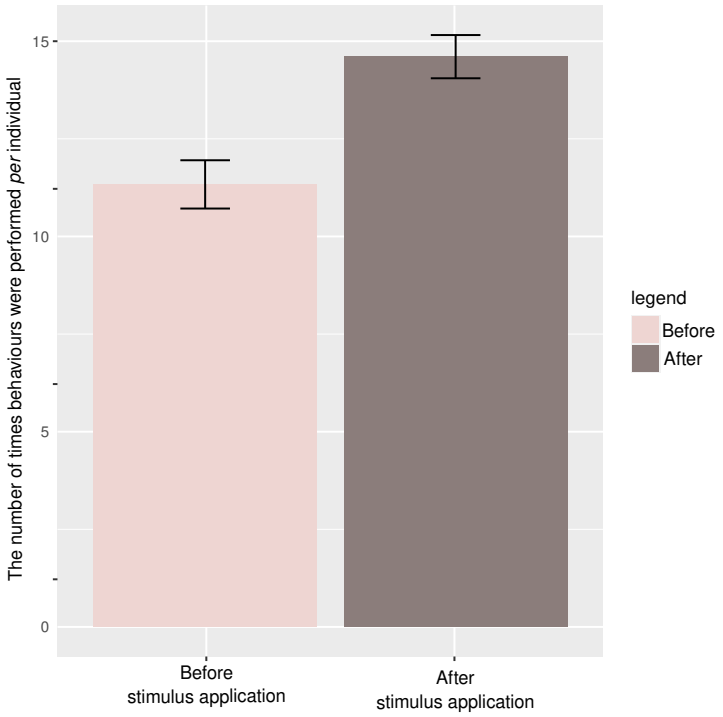

Figure 1.1: Barplot of the number of times behaviours were performed within termite groups according to the moment of stimulus application (before vs after). The number of times termites performed some behaviour increased after stimulus application. See Tables 2.3 and 2.4 for statistical details.

Table 1.3: Models with substantial empirical evidence ($\Delta \leq 2$) predicting the effect of the moment of stimulus application (before and after) on the the number of times behaviours were performed within termite groups. Explanatory variables include (1) the moment of stimulus application (before x after) *(Moment)*; (2) the nest indentity *(NestID)* from which individuals were extracted. columns readings: Model = possible models describing the relationship between the moment of stimulus application, the nest identity and the number of times behaviours were perfomed *per individual*; (Intercept) = model intercept, Moment = the moment of stimulus application (before vs after), NestID = id of the nest from which individuals were extracted, df = degree of freedmon, AICc = second-order Akaike information criterion, Δ = AICc difference between the model in concern and the best model, weight = Akaike weight. Global model: $y \sim$ *Moment + NestID*.

Model	**adjR²**	**df**	**Loglik**	**AICc**	**Δ**	**weight**
Moment	0.3597	3	-66.163	139.2	0.00	0.995
Moment + NestID	0.8913	17	165.9	26.62	26.62	0.005

Table 1.4: The coefficients of the model predicting the effect of the moment of stimulus insertion (before x after) on the number of times behaviours were performed *per* individual.

	Estimate	**Std. Error**	**t value**	**Pr(>—t—)**
(Intercept)	14.6044	0.5868	24.888	<2e-16 ***
Before	-3.2711	0.8299	-3.942	0.000491 ***

1.3.2 The stimuli identity modulated the number of times each behaviour was executed within termite groups

Additionally to eliciting more behavioural actions as a whole, the application of some of the stimuli elicited more behavioural actions than the application of others. That is, the total number of times behaviours were performed under AR and AP is lower that the number of times behaviours were performed under APH and APE. This can be easily spotted observing that bars in Figs. 2.2**a** and 2.2**b** (AR and AP respectivelly) barely reach the 0.25 boundary in y-axis and no bar ever crosses the 0.5 boundary. Conversely, the bars in Figs. 2.2**c** and 2.2**d** (APH and APE) easily overcome this value, with some bars easily exceeding the 0.5 value. Behaviours were never enacted more than 0.5 times after application of AR and AP (Figs. 2.2**a** and 2.2**b**). This same number exceeded 0.5 times for at least one behaviour ("explore run"), after the application of APH and APE (Figs. 2.2**c** and 2.2**d**). In all cases, the stimuli application drove individuals to perform, mainly, the "explore run" (ER) behaviour.

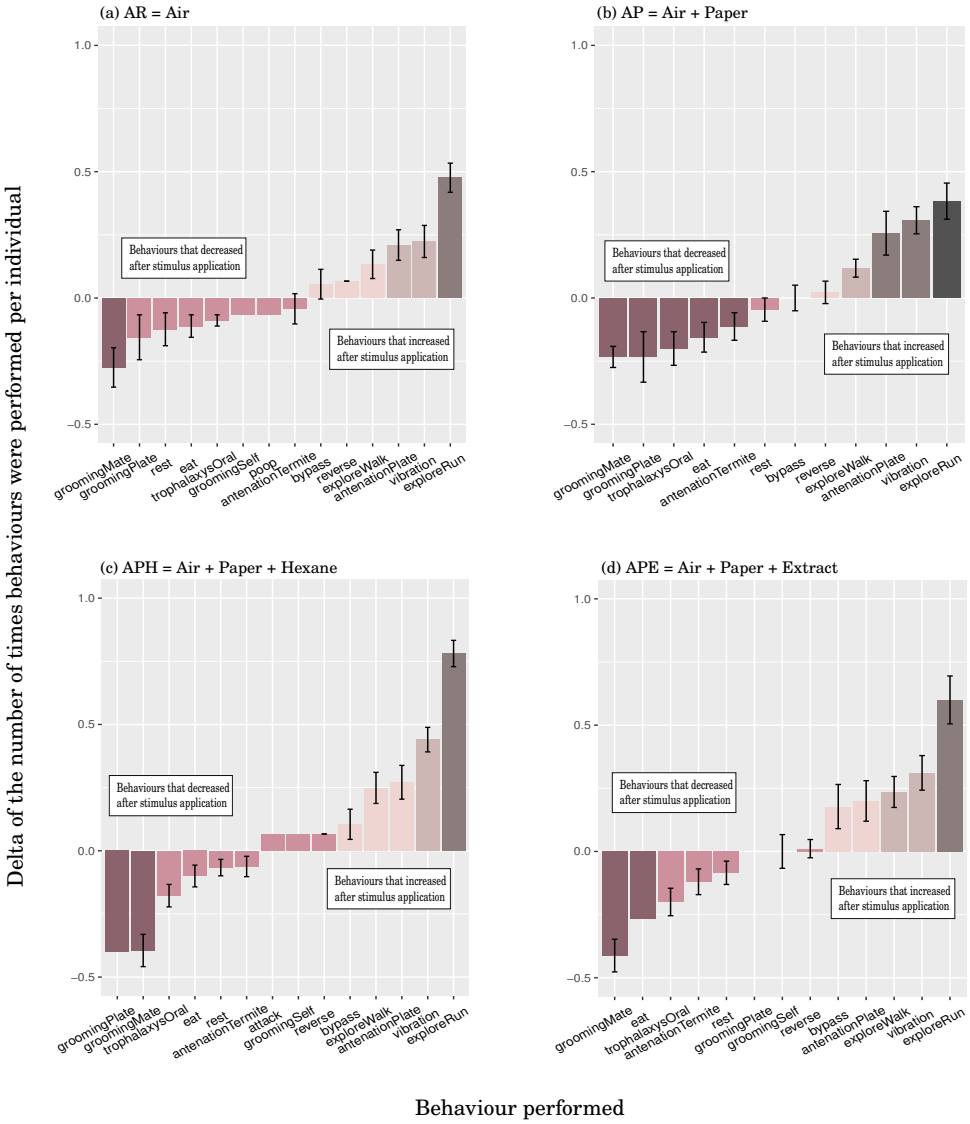

Figure 1.2: Barplot of the number of times each behaviour was performed within termite groups according to the type of stimulus. The x-axis represents the set of behaviours triggered by each stimulus. The y-axis represents the delta (after - before application) of the number of times behaviours were executed *per* individual. Each bar represents a specific behaviour. Negative values on y-axis means that termites performed such a behaviour more times before stimulus application than after. Positive values means that termites performed such a behaviour more times after stimulus application than before. See Tables 2.5, 2.6, 2.7, 2.8 and 2.9 for statistical details.

Table 1.5: Models with substantial empirical evidence ($\Delta \leq 2$) predicting the effect of the identity of the stimulus on the delta number of times each behaviour was performed within termite groups. Explanatory variables include (1) the type of behaviour *(BehaviourType)* were perfomed; (2) the nest indentity *(NestID)* from which individuals were extracted. columns readings: Model = possible models describing the relationship between the type of behaviour, the nest identity and the delta number of times behaviours were perfomed per individual; (Intercept) = model intercept; df = degree of freedmon; AICc = second-order Akaike information criterion; Δ = AICc difference between the model in concern and the bestmodel; weight = Akaike weight.

Stimulus AR

Model	df	Loglik	AiCc	Δ	weight
BehaviourType	15	20.235	-5.7	0.00	0.839
BehaviourType + NestID	28	20.235	-2.4	3.30	1.161

Stimulus AP

Model	df	Loglik	AiCc	Δ	weight
BehaviourType	13	26.897	-23.8	0.00	0.956
BehaviourType + NestID	25	42.053	-17.7	6.14	0.044

Stimulus APH

Model	df	Loglik	AiCc	Δ	weight
BehaviourType	15	33.794	-33.1	0.00	1
BehaviourType + NestID	29	46.801	-16.7	16.37	0

Stimulus APE

Model	df	Loglik	AiCc	Δ	weight
BehaviourType + NestID	28	23.183	26.7	0.00	0.76
BehaviourType	14	1.408	29	2.31	0.24

Table 1.6: The coefficients of the model predicting the effect of the stimulus "AR" insertion on the delta number of times behaviours were performed within termite groups.

Stimulus AR

	Estimate	Std. Error	t value	Pr(>—t—)
(Intercept)	0.2946099	0.0896350	3.287	0.001451 **
Antenation Termite	-0.2523810	0.0749100	3.369	0.001116 **
Bypass	-0.1479838	0.0805768	-1.837	0.069614 .
Eat	-0.2920467	0.1310846	-2.228	0.028406 *
Explore Run	0.2666667	0.0749100	3.560	0.000598 ***
Explore Walk	-0.0761905	0.0749100	-1.017	0.311866
Grooming Mate	-0.5383368	0.0894796	-6.016	3.89e-08 ***
Grooming Plate	-0.3732658	0.1312401	-2.844	0.005524 **
Grooming Self	-0.0523810	0.2194664	-0.239	0.811907
Poop	-0.0523810	0.2194664	-0.239	0.811907
Rest	-0.3333333	0.0749100	-4.450	2.48e-05 ***
Reverse	-0.1138991	0.1569050	-0.726	0.469798
Typetrophalaxys Oral	-0.3099332	0.1309962	-2.366	0.020153 *
Vibration	0.0142857	0.0749100	0.191	0.849191
Ninho02	-0.3088956	0.1106484	-2.792	0.006417 **
Ninho03	-0.0775686	0.1067952	-0.726	0.469542
Ninho04	-0.1523810	0.1059388	-1.438	0.153832
Ninho05	-0.1225888	0.1042518	-1.176	0.242774
Ninho06	-0.0612008	0.1030023	-0.594	0.553906
Ninho08	-0.2253204	0.1036931	-2.173	0.032440 *
Ninho09	-0.1143513	0.0996287	-1.148	0.254137
Ninho10	0.2081457	0.1067952	1.949	0.054443 .
Ninho11	-0.0013514	0.0996085	-0.014	0.989206
Ninho12	-0.0975130	0.1011913	-0.964	0.337832
Ninho13	-0.0008463	0.1012141	-0.008	0.993347
Ninho14	-0.1054994	0.1000167	-1.055	0.294364
Ninho15	-0.1318347	0.1036962	-1.271	0.206915

Table 1.7: The coefficients of the model predicting the effect of the stimulus "AP" insertion on the delta number of times behaviours were performed within termite groups.

Stimulus AP

	Estimate	**Std. Error**	**t value**	**Pr(>—t—)**
(Intercept)	2.480e-01	8.123e-02	3.053	0.003064 **
Antenation Termite	-3.692e-01	7.240e-02	-5.100	2.19e-06 ***
Bypass	-2.279e-01	7.833e-02	-2.910	0.004664 **
Eat	-3.526e-01	1.232e-01	-2.861	0.005377 **
Explore Run	1.260e-01	7.411e-02	1.700	0.092881
Explore Walk	-1.385e-01	7.240e-02	-1.913	0.059342
Grooming Mate	-5.232e-01	8.431e-02	-6.206	2.19e-08 ***
Grooming Plate	-5.558e-01	1.465e-01	-3.793	0.000286 ***
Rest	-3.026e-01	7.240e-02	-4.179	7.34e-05 ***
Reverse	-2.657e-01	1.232e-01	-2.156	0.034036 *
Typetrophalaxys Oral	-5.361e-01	1.465e-01	-3.659	0.000450 ***
Vibration	5.128e-02	7.240e-02	0.708	0.480764
Nest 02	-1.726e-01	9.593e-02	-1.800	0.075620
Nest 03	7.185e-02	9.419e-02	0.763	0.447780
Nest 04	1.781e-02	9.375e-02	0.190	0.849803
Nest 06	-2.402e-03	9.755e-02	-0.025	0.980418
Nest 07	-1.130e-01	9.375e-02	-1.205	0.231600
Nest 08	-7.500e-02	9.229e-02	-0.813	0.418789
Nest 09	-8.468e-02	9.058e-02	-0.935	0.352626
Nest 11	4.465e-02	9.573e-02	0.466	0.642135
Nest 12	1.250e-01	9.229e-02	1.354	0.179359
Nest 13	-3.731e-16	9.229e-02	0.000	1.000000
Nest 14	1.043e-01	8.844e-02	1.179	0.241901
Nest 15	1.933e-01	9.585e-02	2.017	0.046992 *

Table 1.8: The coefficients of the model predicting the effect of the stimulus "APH" insertion on the delta number of times behaviours were performed within termite groups.

Stimulus APH

	Estimate	**Std. Error**	**t value**	**Pr(>—t—)**
(Intercept)	0.223907	0.088576	2.528	0.01314 *
Antenation Termite	-0.333333	0.068588	-4.860	4.69e-06 ***
Attack	-0.267404	0.203936	-1.311	0.19298
Bypass	-0.151016	0.088205	-1.712	0.09017
Eat	-0.360268	0.109723	-3.283	0.00144 **
Explore Run	0.514338	0.069946	7.353	7.08e-11 ***
Explore Walk	-0.022222	0.068588	-0.324	0.74666
Grooming Mate	-0.651878	0.071600	-9.104	1.47e-14 ***
Grooming Plate	-0.493326	0.203556	-2.424	0.01728 *
Grooming Self	-0.239118	0.203670	-1.174	0.24334
Rest	-0.337778	0.068588	-4.925	3.61e-06 ***
Reverse	-0.205733	0.123733	-1.663	0.09970
Typetrophalaxys Oral	-0.452358	0.123678	-3.658	0.00042 ***
Vibration	0.168889	0.068588	2.462	0.01562 *
Nest 02	0.023043	0.104811	0.220	0.82646
Nest 03	0.081879	0.100592	0.814	0.41772
Nest 04	0.110164	0.101624	1.084	0.28112
Nest 05	0.013520	0.104811	0.129	0.89764
Nest 06	0.120542	0.102532	1.176	0.24270
Nest 07	0.013520	0.104811	0.129	0.89764
Nest 08	-0.003464	0.099191	-0.035	0.97221
Nest 09	0.045323	0.099041	0.458	0.64828
Nest 10	0.277778	0.108446	2.561	0.01202 *
Nest 11	-0.024576	0.104811	-0.234	0.81513
Nest 12	-0.130580	0.100376	-1.301	0.19647
Nest 13	0.067064	0.100592	0.667	0.50660
Nest 14	0.061139	0.104811	0.583	0.56107
Nest 15	0.052718	0.102205	0.516	0.60720

Table 1.9: The coefficients of the model predicting the effect of the stimulus "APE" insertion on the delta number of times behaviours were performed within termite groups.

Stimulus APE

	Estimate	**Std. Error**	**t value**	**Pr(>—t—)**
(Intercept)	0.289739	0.099271	2.919	0.004370 **
Antenation Termite	-0.320000	0.082863	-3.862	0.000203 ***
Bypass	0.007054	0.097287	0.073	0.942346
Eat	-0.447134	0.246977	-1.810	0.073325
Explore Run	0.400000	0.082863	4.827	5.16e-06 ***
Explore Walk	0.035556	0.082863	0.429	0.668811
Grooming Mate	-0.659681	0.091025	-7.247	1.03e-10 ***
Grooming Plate	-0.229863	0.247252	-0.930	0.354849
Grooming Self	-0.067117	0.179339	-0.374	0.709037
Rest	-0.284444	0.082863	-3.433	0.000880 ***
Reverse	-0.082620	0.112627	-0.734	0.464980
Typetrophalaxys Oral	-0.454780	0.132510	-3.432	0.000882 ***
Vibration	0.111111	0.082863	1.341	0.183086
Nest 02	-0.092016	0.120216	-0.765	0.445877
Nest 03	-0.445243	0.111526	-3.992	0.000127 ***
Nest 04	-0.214737	0.116787	-1.839	0.069017
Nest 05	-0.214729	0.116034	-1.851	0.067276
Nest 06	0.123458	0.116440	1.060	0.291655
Nest 07	-0.130111	0.120216	-1.082	0.281796
Nest 08	-0.098458	0.113633	-0.866	0.388378
Nest 09	-0.109271	0.115845	0.347896	-0.943
Nest 10	0.032395	0.115845	0.280	0.780348
Nest 11	-0.059875	0.116440	-0.514	0.608270
Nest 12	-0.097921	0.113144	-0.865	0.388926
Nest 13	-0.104958	0.111142	-0.944	0.347331
Nest 14	0.090729	0.115845	0.783	0.435426
Nest 15	-0.025340	0.119380	-0.212	0.832348

1.3.3 The stimulus volume modulated the number of times behaviours were perfomed within termite groups

The stimulus volume modulated the average number of times termites performed behaviours (see Table 2.10; Fig. 2.3). With the increments in stimulus volume termites performed behaviours more times on average (Fig. 2.3), mainly when exposed to stimulus APH. Termites performed more times "explore run" than the other behaviours despite the type of stimulus and volume (see Table 2.10; Fig. 2.4).

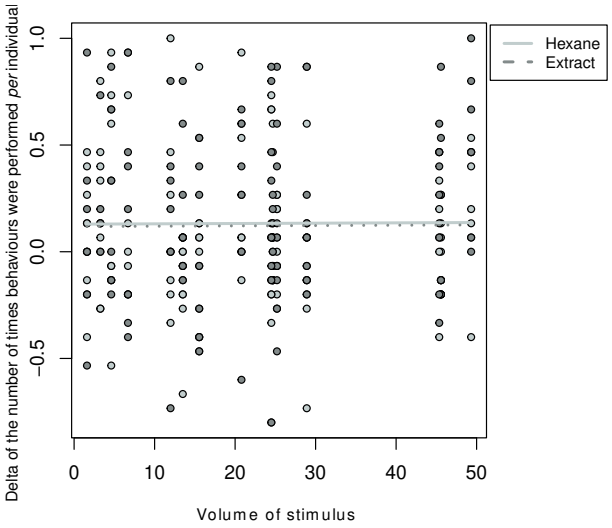

Figure 1.3: The effect of stimulus volume on the delta of the average number of times behaviours were performed within termite groups.The y-axis represents the difference between the number of times behaviours were performed before vs after the stimulus application. The x-axis represents the volume of stimulus tested. Each dot represents the termite group observed. Solid lines = hexane stimulus (APH), dotted lines = extract stimulus (APE). Light gray = termites individuals exposed to hexane, dark gray = individuals exposed to soldiers' head extract. See Tables 2.10 and 2.11 for statistical details.

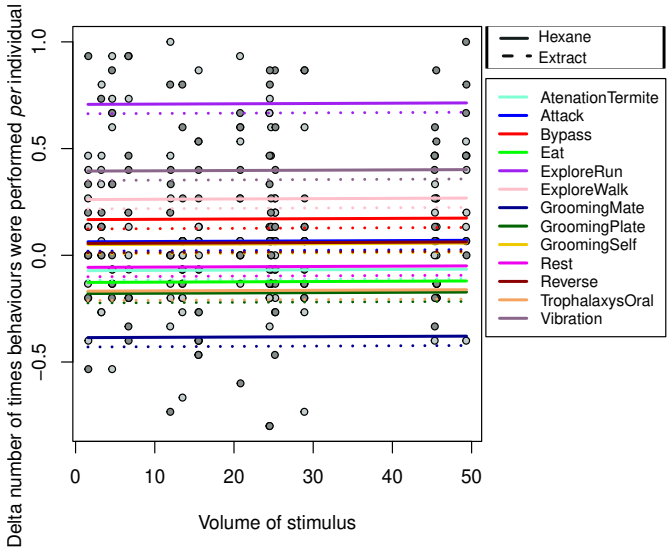

Figure 1.4: The effect of stimulus volume on the delta of the number of times each behaviour was performed within termite groups.The y-axis represents the difference between the number of times behaviours were performed before vs after the stimulus application. The x-axis represents the voulume of stimulus tested. Each dot represents the termite group. The lines correspond to the number of times each behaviour type were performed *per* individual. Lines with different colors represents different behaviours types. Solid lines = hexane stimulus (APH), dotted lines = extract stimulus (APE). See Tables 2.10 and 2.11 for statistical details.

Table 1.10: Models with substantial empirical evidence ($\Delta \leq 2$) predicting the effect of the insertion of different stimulus volume (dosis) on the delta number of times behaviours were performed within termite groups. Explanatory variables include (1) the type of behaviour *(BehaviourType)* were perfomed; (2) the nest indentity *(NestID)* from which individuals were extracted; (3) the volume of chemical *(dosis)* applied; (4) the type of stimulus *(stimulus)* was used (APH x APE). Columns readings: Model = possible models describing the relationship between the stimulus volume (dosis), the stimulus type, the nest identity, the behaviour type and the delta number of times behaviours were perfomed per individual; (Intercept) = model intercept; df = degree of freedmon; AICc = second-order Akaike information criterion; Δ = AICc difference between the model in concern and the bestmodel; weight = Akaike weight. Global Model: *y* $\sim$ *BehaviourType* + *dosis* + *NestID* + *stimulus* .

Model	df	Loglik	AICc	Δ	weight
BehaviourType + nestID + stimulus	30	46.186	-23.7	0.00	0.393
BehaviourType + dosis + nestID + stimulus	30	46.186	-23.7	0.00	0.393
BehaviourType + nestID	29	43.025	-20.0	3.73	0.061

Table 1.11: Full average for the models predicting the effect of the insertion of different stimulus volume (dosis) on the delta number of times behaviours were performed within termite groups. Only models presenting ($\Delta \leq 2$) in the model selection procedure were included (Table 2.10). The minimal adequate model was obtained by averaging all candidate models presenting ($\Delta \leq 2$). The coefficients of the average model were used to plot curves in Figures 2.3 and 2.4.

	Estimate	Std. Error	Adjusted SE	z value	Pr(>—z—)
(Intercept)	2.361e-01	7.104e-02	7.144e-02	3.305	0.00095 ***
Antenation Termite	-3.267e-01	5.513e-02	5.544e-02	5.892	<2e-16 ***
Attack	-1.736e-01	2.233e-01	2.245e-01	0.773	0.43934
Bypass	-6.107e-02	6.690e-02	6.727e-02	0.908	0.36402
Eat	-3.846e-01	1.063e-01	1.069e-01	3.599	0.00032 ***
Explore Run	4.520e-01	5.564e-02	5.595e-02	8.078	<2e-16 ***
Explore Walk	6.667e-03	5.513e-02	5.544e-02	0.120	0.90429
Grooming Mate	-6.419e-01	5.865e-02	5.898e-02	10.884	<2e-16 ***
Grooming Plate	-3.803e-01	1.598e-01	1.607e-01	2.367	0.01794 *
Grooming Self	-1.083e-01	1.347e-01	1.355e-01	0.799	0.42408
Rest	-3.111e-01	5.513e-02	5.544e-02	5.611	<2e-16 ***
Reverse	-1.263e-01	8.339e-02	8.386e-02	1.506	0.13195
Trophalaxis Oral	-4.216e-01	9.150e-02	9.202e-02	4.581	4.6e-06 ***
Vibration	1.400e-01	5.513e-02	5.544e-02	2.525	0.01157 *
Nest 02	-4.110e-02	7.977e-02	8.022e-02	0.512	0.60839
Nest 03	-1.899e-01	7.211e-02	7.252e-02	2.619	0.00881 **
Nest 04	-5.934e-02	7.270e-02	7.311e-02	0.812	0.41696
Nest 05	-1.103e-01	7.933e-02	7.978e-02	1.383	0.16664
Nest 06	1.087e-01	7.853e-02	7.897e-02	1.376	0.16877
Nest 07	-6.447e-02	7.783e-02	7.827e-02	0.824	0.41012
Nest 08	-7.247e-02	7.111e-02	7.151e-02	1.013	0.31085
Nest 09	-4.702e-02	7.100e-02	7.140e-02	0.659	0.51019
Nest 10	1.433e-01	8.242e-02	8.288e-02	1.729	0.08374
Nest 11	-4.191e-02	8.039e-02	8.084e-02	0.518	0.60415
Nest 12	-1.338e-01	7.208e-02	7.248e-02	1.846	0.06496
Nest 13	-3.573e-02	7.064e-02	7.103e-02	0.503	0.61498
Nest 14	7.435e-02	7.457e-02	7.498e-02	0.992	0.32140

Table 1.11: Full average for the models predicting the effect of the insertion of different stimulus volume (dosis) on the delta number of times behaviours were performed within termite groups. Only models presenting ($\Delta \leq 2$) in the model selection procedure were included (Table 2.10). The minimal adequate model was obtained by averaging all candidate models presenting ($\Delta \leq 2$). The coefficients of the average model were used to plot curves in Figures 2.3 and 2.4.

	Estimate	Std. Error	Adjusted SE	z value	Pr(>—z—)
Nest 15	-3.520e-03	5.672e-02	5.704e-02	0.062	0.95079
APH	6.557e-02	2.759e-02	2.774e-02	2.363	0.01810 *
Dosis	8.361e-05	1.347e-03	1.355e-03	0.062	0.95079

1.4 Discussion

Despite the knowledge on the mechanisms governing collective behaviour in animals, little is known about the factors that will predispose individuals to converge their behavioural actions leading to a cohesive behaviour attitude. Here we showed that external stimuli can be one of these factors. We found that externally applied stimuli induced individuals to increase their behavioural actions (Fig. 2.1). Such results demonstrated the efficacy of the stimuli used in the assays. Individuals are know alter their own action in order to adapt to external stimuli (Wong and Candolin, 2014; Schaerf et al., 2017; Cristaldo et al., 2015). Accordingly we found that all types of stimuli favoured individuals to perform some behaviours at expense of others (Figs. 2.2**a**, 2.2**b**, 2.2**c** and 2.2**d**). Such a result suggests that the application of stimuli imposed some behavioural restrictions on termite individuals. The behavioural restriction, in turn, caused individuals to respond similarly, leading to the emergence of behavioral cohesion mainly into the "explore run" behaviour. This effect is particularly noticeable for APH and APE stimuli (Figs. 2.2**c** and 2.2**d**). Further, our results also demonstrated that the behavioural cohesion among individuals is modulated by the stimuli intensity. The higher the intensity of stimli (*i.e.* by increasing stimuli volume), the higher the propensity of individuals to converge their behavioural actions toward the "explore run" behaviour (Figs. 2.3 and 2.4). These results indicate that although all types of external stimuli used here have the potential to drive individuals to come together in a cohesive behavioural attitude, the level of such cohesion may also be modulated by the intensity of the stimuli. The picture that emerges from our results is that although frequently related to intrinsic motivations, behavioural cohesion can also be driven by external stimuli.

That is to say, the behavioural cohesion could emerge from: (i) the intrinsic motivation of the individuals to follow the behaviours of their nestmates or (ii) it could emerge from the independent response of the individuals to external stimuli. Although we can not fully eliminate some degree of internal motivation, our results strongly point out that the external stimuli was the primary cause for the emergence of behavioural cohesion among individuals. The most classical example of external stimulus driving individuals to get together in a cohesive attitude is the formation of insect swarms (Downes et al., 1955). Individuals are independently attracted to fly toward the vicinity of a landmark. Thus behavioural cohesion among individuals is established (all individuals flying around the landmark). Behavioural cohesion, in turn, allows individuals to interact. By doing so, individuals maintain group cohesion and function (Downes et al., 1955; Attanasi et al., 2014; van der Vaart et al., 2020).

In termites, behavioural cohesion among individuals has already been correlated with external stimuli but, to our best knowledge, this was never directly tested before. For example, Myles (2002) demonstrated that groups of *Reticulitermes flavipes* termite individuals in contact with fungus exposed termite, tend to converge their behavioural actions toward vibration behaviour. The authors demonstrated that at the peak of the infection (about 15 minutes after group exposure to the infected termite) 50% of the unexposed termites were engaged in vibration behaviour. This seems to be in line with our findings. However, a crucial difference must be pointed out here between what has been done by Myles (2002) and what we did. The external stimulus used by the authors can be considered locally applied as it consisted of a single individual, infected with fungus conidia, exposed to groups composed of 40 non-infected termite individuals. Therefore, it can be difficult to know which termites are responding directly to the external stimulus or which are responding to their neighbors. Indeed, the authors pointed out that direct contact between the infected and non-infected termites was not always clear observed prior the execution of the vibratory movement. This suggest that such reaction could be dependent on interindividual contact. That is, the emergence of behavioural cohesion among non-infected individuals could be related to a secondary contact between individuals rather than with the exposition to the external stimuli, *per se*. In our work, on the other hand, because it was important to discriminate direct and indirect responses to the applied stimuli, we took care to apply the stimuli in a manner that reached all individuals at the same

time. As a result, individuals instantaneously reacted to the stimuli application (L.F. Nunes personal observation). So, it is plausible to infer that, the behavioural cohesion, in our case, was triggered by individuals independently responding to the external applied stimuli with similar behaviours rather than by means of the interindividual contact.

Interesting, although very often (if not always) behavioural cohesion have been implicitly linked to the intrinsic motivation of individuals to stay close to their nestmates, a ubiquitous trait of social life, here we argue that individuals' inclination to get together in a cohesive way cannot be fully understood without considering external motivation. Our results corroborate and add further evidence of the importance to include external factors in the study of animal collective behaviour (van der Vaart et al., 2020; King et al., 2018; Reynolds, 2018; Van Gestel and Tarnita, 2017).

1.5 Conclusion

Despite the implicit thought that behavioral cohesion is an emergent phenomenon related to the intrinsic motivation of social individuals to stay together, here we provide evidence that external factors cannot be neglected. Our results demonstrated that external applied stimuli drove individuals to respond with similar behaviour, giving rise to a cohesive behavioural attitude. We argue that such cohesion was attained through the independent response of individuals to the external stimuli application rather than as a function of interindividual interaction.

1.6 Acknowledgements

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1.7 Author contribution

Conceived and designed the experiments: LFN, JC, ODS. Performed the experiments: LFN. Collected the data: LFN, LC. Statistical modelling and analyses: LFN, JC, ODS. Biological interpretation of the models: LFN, JC, ODS. Contributed reagents/materials/analysis tools: ODS, LFN. Wrote the paper: LFN, JC, ODS.

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Chapter 2

Dataset on Substrate-Borne Vibrations of *Constrictotermes cyphergaster* (Blattodea: Isoptera) Termites

data

MDPI

Data Descriptor

Dataset on Substrate-Borne Vibrations of *Constrictotermes cyphergaster* (Blattodea: Isoptera) Termites

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Abstract: Here we present data on distinct stimuli as elicitors of substrate-borne vibrations performed by groups of termites belonging to the species *Constrictotermes cyphergaster* (Blattodea: Isoptera: Termitidae: Nasutitermitinae). The study consisted of assays where termite workers and soldiers were exposed to different airborne stimuli and the vibrations thereby elicited were captured by an accelerometer attached under the floor of the arena in which the termites were confined. A video camera was also used as a visual complement. The data provided here contribute to fill a gap currently existing in published datasets on termite communication.

Dataset: 10.5281/zenodo.2790686.

Keywords: termite vibrational reaction; stimuli; soldier's head extract

1. Summary

Vibration signaling is widespread in insects [1]. Such a communication channel can be used in a range of situations, e.g., to detect predators [2], prey [3], mates [4], and to recruit nestmates [5]. Furthermore, vibration can also be used as an important component to establish communication with another individual or a group of individuals.

Termites are social insects that are well known to use vibrations to convey information to their nestmates or to gather contextual information about the environment. For instance, by combining substrate-borne vibrations with chemical scents, termites communicate alarm [6]. They are also able to use the resonant frequency of a block of wood to assess its size, thereby choosing one food item over another [7]. Termites are also sensitive to heterospecific vibration, using that information for their own benefit: as recently demonstrated [8], termites can escape danger by eavesdropping the vibrational cues emitted by predatory ants. In spite of such an importance, vibratory communication in termites is relatively underexplored, and published datasets on the subject are definitely very rare.

To cover such a gap, here we present a large dataset (c.a. 13 million lines) containing the intensity of the substrate-borne vibration of termite groups confined in arenas specially designed to amplify

such vibrations (see [9]). Vibrations were measured using an accelerometer and were triggered by airborne stimuli.

2. Data Descriptor

Here we provide the raw data describing the intensity of substrate-borne vibrations produced by groups of termites confined in arenas bearing a flexible floor. Such arenas (Figure 1; so-called "tympanic" in allusion to their flexible floor) were designed to amplify the feeble vibrations produced by the termites and are fully described and tested by Nunes et al. [9]. Vibrations were measured by an accelerometer whose sensor was attached to the outer surface of the arenas' floor.

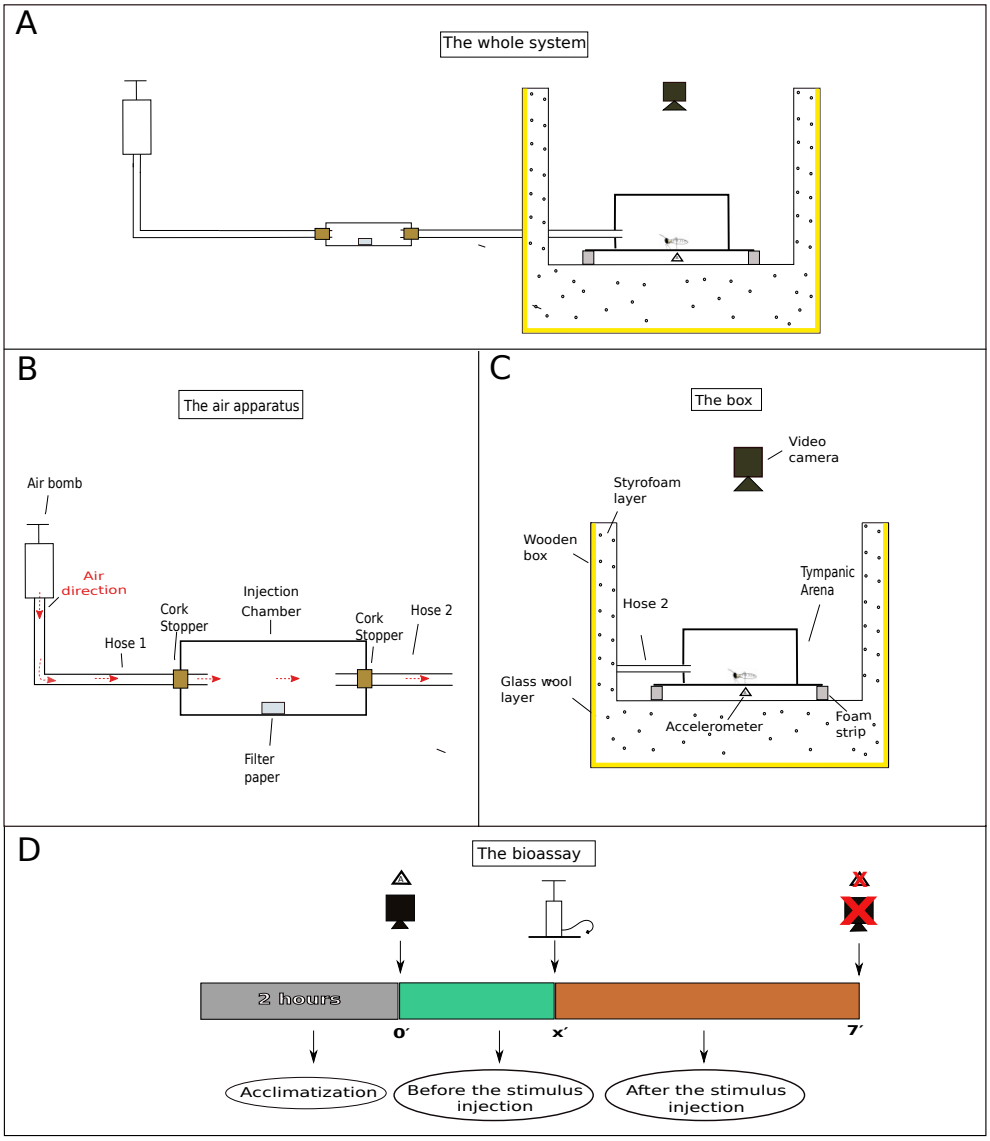

Figure 1. Schematic depiction of the setup designed to measure termite vibrational responses when subjected to different stimuli. (**A**) Global view of the setup, showing an air pump connected by hoses through a injection chamber and to a tympanic arena housed in a wooden box. (**B**) Detailed view of the air pumping system, showing the injection chamber used to house the sources of stimuli. (**C**) Detailed view of the wooden box lined with styrofoam and glass wool to minimize external noise disturbance. (**D**) The chronology of each assay: (i) each termite group was allowed at least 2 h of acclimatization before the beginning of the assays; (ii) video and accelerometer recordings start about 2 min before the stimulus injection; (iii) after injecting the stimulus, recordings proceed for another 5 min, totaling 7 min of readings. "x" is the exactly time at which the stimulus was injected, which is given in Table 1.

Each termite group was composed of 12 workers and 3 soldiers from a given nest. Vibrations were triggered by subjecting these termites to distinct airborne stimuli gently pumped into the arena after termites were allowed to acclimatize for 2 h.

Readings thereby produced are presented here in a series of comma-separated values (.csv) files. Each file corresponds to a full assay on a single termite group from a single nest and with a single stimulus. Each line in the the file presents the readings of termite vibrations captured by the accelerometer at the "x" (horizontal), "y" (horizontal), and "z" (vertical) axes (Figure 2) at a given time. The accelerometer was configured at high gain. Readings are expressed in "counts" units. There are 13,108 counts per *g* (the acceleration due to gravity) in high gain mode. Each set of 512 lines corresponds to 1 s (more details are given in Section 3.5.

The columns in the datafiles are:

time: the moment, from the beginning of the recording session, when vibration was recorded

Ax: counts read by the accelerometer at the horizontal x axis

Ay: counts read by the accelerometer at the horizontal y axis

Az: counts read by the accelerometer at the vertical z axis

NestID: the field identification of the nest from which the termite group was collected. Codes within `NestID` column are built as `nnncccyyyy`, where `nnn` = the nest sequential number used as a field label; `ccc` = the initials of the collector (name and surname); yyyy = the year in which the nest was taken from the field to the lab.

TypeStimulus: the type of stimulus that was deposited within the injection chamber to be carried by the air injected into the arena to trigger termite vibrations. Codes within `TypeStimulus` are: `air` = only air; `air_paper` = air plus a piece of filter paper was deposited into the injection chamber and the air therein was injected into the arena; `air_paper_hexane` = a piece of filter paper onto which hexane was applied was deposited into the injection chamber and the air therein was injected into the arena; `air_paper_extract` = a piece of filter paper onto which a hexane extract from soldiers' heads was applied was deposited into the injection chamber and the air therein was injected into the arena.

TermiteGroup: the identification of the nest from which the termite group was collected and the stimulus they were exposed to. Codes within the `TermiteGroup` column are built as `gnns`, where: `g` = "group"; `nn` = the nest sequential number at which the termite group was collected; `s` = the stimulus that the termite group was exposed to.

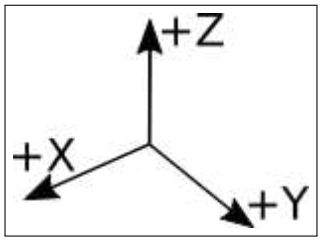

Figure 2. The orientation of the X, Y, and Z axes from which vibrations were captured by the accelerometer.

3. Methods

3.1. Ethical Statement

The current study is in compliance with the relevant regulations of Brazil, including collection and transportation permits from The Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA), and permission from The Brazilian Enterprise for Agricultural Research (EMBRAPA, CNPMS) to conduct the study on their site. O. DeSouza holds a permanent collecting and transporting permit (# 10014-1) from IBAMA. Tacit approval from the Brazilian Government is implied by the authors being hired as scientific researchers. The species collected for the present

study are neither endangered nor protected, and thus no specific permits were required for laboratory experiments. No genetic information was accessed.

3.2. Termite Material

Assays were conducted using termites from 15 wild colonies of *C. cyphergaster* (Silvestri, 1901) collected in April 2017 in the Brazilian "cerrado", near the town of Sete Lagoas (27°19′ S, 14°44′ W; altitude 800–900 m above sea level), Minas Gerais State, Southeastern Brazil. The colonies were transported to Viçosa (Minas Gerais, Brazil), where they were kept in laboratory in room-level conditions of humidity, temperature, and light. As food, the bark of trumpet trees (*Tabebuia aurea*, Bignoniaceae) was offered ad libitum to all nests. Water was also offered ad libitum through a piece of cotton attached to the opening of a test tube full of water.

3.3. Experimental Procedures

The assays were conducted from April to mid-July 2017. They were designed to measure the vibrational reaction exhibited by workers and soldiers of *C. cyphergaster* when they are subjected to different stimuli.

To perform the assays, we designed an experimental setup to minimize noise and vibrations from human traffic and other activities in nearby labs (Figure 1A). This setup consisted of a wooden box lined with (approx.) a 5-cm thick layer of glass wool plus an 8-cm thick styrofoam layer.

In order to amplify the feeble vibrations exhibited by the termites, we used an arena bearing a flexible floor (so as to mimic a tympanum) as described and tested by Nunes et al. [9]. The tympanic arena was placed inside the wooden box described above, over a pair of egg crate foam strips lying on a hollowed, cubic styrofoam structure (Figure 1C). Groups of 15 termite individuals (12 workers + 3 soldiers) were taken from their colonies and placed inside the arena (one group at a time). At least 2 h were allowed for termites to acclimatize before the beginning of the assays. The number of termites and caste ratio of the groups were chosen according to natural proportions found in field nests (4.5 workers: 1 soldier) [10] and within the range of densities known to improve interindividual interactions and survival [11].

Stimuli were offered to termites by gently pumping air through a hose to an injection chamber and from there through another hose to the arena (Figure 1A). This injection chamber (internal space: ⌀ 18 mm × 80 mm long) was used to house any source of stimulus in addition to air (Figure 1B). Both ends of the injection chamber were sealed with cork stoppers. A small hole was made in the cork stoppers to connect the ends of the two hoses (⌀ = 4 mm). The other end of the first hose (Figure 1B, hose 1; ≈1190 mm long) was coupled to an air pump and the end of the other hose (Figure 1B, hose 2; ≈650 mm long), which flowed directly into the tympanic arena. A light touch was given to the air pump lever, which descended by weight and gravity, blowing the stimulus from the injection chamber to the arena where the individuals were confined (Figure 1C). A total of c.a. 230 cm^3 of air was injected into the system by the air pump.

The stimuli consisted of injecting into the arena:

1. the air present in the injection chamber;
2. the air present in the injection chamber after it had contact with a piece of filter paper (7 × 2 cm) previously deposited therein;
3. the air present in the injection chamber after it had contact with a known amount of hexane that was loaded onto a piece of filter paper deposited in the injection chamber;
4. the air present in the injection chamber after it had contact with hexane extracts of termite soldier heads. These extracts were loaded onto a piece of filter paper deposited in the injection chamber.

Each assay was recorded on video and registered by the accelerometer for a total of 7 min divided into "time before the stimulus injection" and "time after the stimulus injection" (Figure 1D). This was

done to differentiate the activity of the groups before the stimulus injection from the activity after the stimulus injection, in order to obtain only the real effect of each stimulus on the termites' behavior.

Due to operational reasons, both the time of the stimulus injection and the volume of stimulus applied onto the filter paper varied among assays. These are specified in Table 1. Each nest provided four termite groups to be independently assayed with a given stimulus. Each group was assayed only once.

Table 1. Overview of stimuli at which termites were exposed to. Nest—the identification of the nest from which the termite group was collected. The nest identity code followed the sequence "nnncccyyyy", where *nnn* = nest sequential number used as field label; *ccc* = initials of collector (name and surname); *yyyy* = year in which the nest was taken from the field to the lab. Stimulus—the kind of stimulus applied to each termite group, where "A" is air; "AP" is air + filter paper; "APH" is air + paper + hexane, and "APE" is air + paper + extract. Injection time—the time (s) at which the stimulus was injected. Aliquot applied—the proportional volume of hexane or extract applied at each assay. Head equivalence—how many heads of soldiers the aliquot corresponds to. Missing values are indicated by a dash.

Nest	Stimulus	Injection Time (s)	Aliquot Applied (μL)	Head Equivalence
N01YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	120	3.26	0
	APE	120	3.26	1
N02YCC2017	A	122	0.00	0
	AP	120	0.00	0
	APH	120	6.69	0
	APE	122	6.69	3
N03YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	120	13.5	0
	APE	140	13.5	5
N04YCC2017	A	129	0.00	0
	AP	120	0.00	0
	APH	125	24.68	0
	APE	123	24.68	7
N05YCC2017	A	123	0.00	0
	AP	–	–	–
	APH	120	47.57	0
	APE	123	47.57	9
N06YCC2017	A	122	0.00	0
	AP	120	0.00	0
	APH	120	4.61	0
	APE	120	4.61	1
N07YCC2017	A	122	0.00	0
	AP	144	0.00	0
	APH	120	11.98	0
	APE	120	11.98	3
N08YCC2017	A	122	0.00	0
	AP	120	0.00	0
	APH	120	28.9	0
	APE	180	28.9	5
N09YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	120	25.2	0
	APE	123	25.2	7

Table 1. *Cont.*

Nest	Stimulus	Injection Time (s)	Aliquot Applied (µL)	Head Equivalence
N10YCC2017	A	120	0.00	0
	AP	–	–	–
	APH	126	49.32	0
	APE	122	49.32	9
N11YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	120	1.6	0
	APE	120	1.6	1
N12YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	122	15.54	0
	APE	120	15.54	3
N13YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	120	24.5	0
	APE	120	24.5	5
N14YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	113	20.79	0
	APE	141	20.79	7
N15YCC2017	A	120	0.00	0
	AP	120	0.00	0
	APH	141	45.36	0
	APE	120	45.36	9

3.4. Extract Preparation

The soldiers of philogenetic advanced termites species have a secretory epithelium located inside a large sac in their head known as a frontal gland. This gland is responsible for the production of a blend of chemicals that is related to alarm situations [12,13]. In *C. cyphergaster*, such chemicals are composed of (1S)-α-pinene, myrcene, and (E)-β-ocimene [6], which are known to be soluble in hexane. To provide assayed termites with this type of stimulus, we prepared hexane extracts from soldiers' heads, following Cristaldo et al., 2015 [6], as described below and depicted in Figure 3.

A total of 20 soldiers were taken from their respective nest and anesthetized on ice to have their heads severed and cut so as to expose the frontal gland. The heads were then immersed in hexane (10 µL per head) and left for 24 h in a freezer at ≈2.4 °C, after which the resulting extract was separated from the termite heads with the help of a microsyringe and again stored in the freezer at ≈2.4 °C until used in the assays. From each termite nest, we prepared only one extract.

Because termite heads vary in the amount of hexane they absorb, each termite group from a given nest produced a distinct initial volume of extract. For each nest, a given aliquot of extract would thus represent a particular amount of termite heads. Due to this, we kept track of the precise volume of extract offered to termites in a given assay so that we could know how many "head equivalents" this volume represented. For comparability, this same volume was used in the assays using only hexane. The volumes used in each assay, as well as their head equivalences, are listed in Table 1.

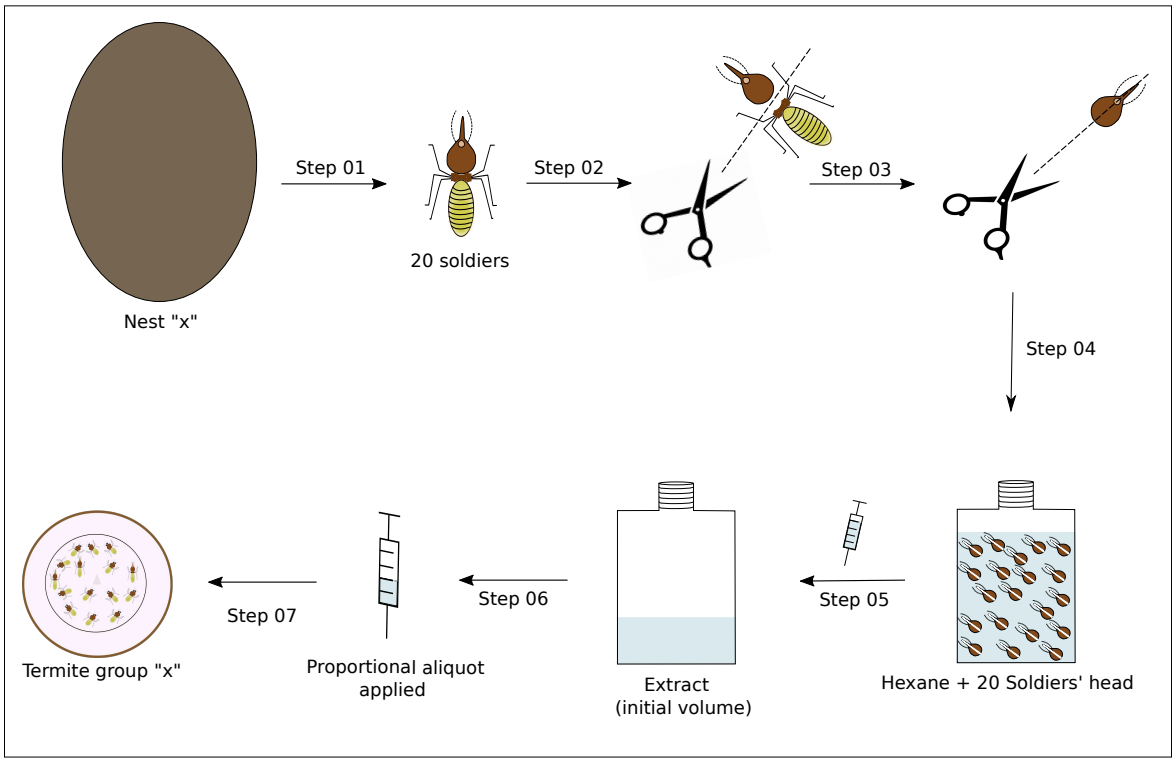

Figure 3. Overview of steps used to prepare the soldiers' head extracts. *Step 01*: 20 termites soldiers were taken from their respective colonies; *Step 02*: the soldiers were anesthetized on ice and dissected into the head and rest of body; *Step 03*: the soldiers' heads were cut from the base of the neck to the nasus; *Step 04*: the heads were placed into hexane (10 µL per head) for 24 h at ≈2.4 °C; *Step 05*: the resulting extract was separated from the termite heads with the help of a microsyringe; *Step 06*: a known aliquot of this extract was collected from its initial volume, the aliquot being proportional to the amount of soldier heads we wanted to apply; *Step 07*: the aliquot was offered to a given termite group in a given assay. Assays are detailed in Table 1.

3.5. Behavioral Response and Parameters Measured

Substrate-borne vibrations produced by the assayed termites were recorded using a USB accelerometer (Gulf Coast Data Concepts, LLC™ model X2-2 logger) equipped with a Kionix KXRB5-2050™ sensor at 2.5 volts, which results in a sensitivity factor of 500 mv/g. These electrical stimuli are recorded by the accelerometer independently in three axes (x, y, and z, Figure 2) as "counts". The number of counts is recorded 512 times in each second. Therefore, each line in the files produced by the accelerometer (henceforth referred to as a "reading") contains the number of counts read in $^1/_{512}$ s. These files form the basis on which we have built the datafile here presented (Section 2). To convert these counts into *g* (gravity acceleration), it suffices to divide the number of counts by 13,108 because this is a correcting factor corresponding to the high gain mode in which we operated the accelerometer (Section 2). To facilitate the assay, the sensor was removed from the accelerometer's case while keeping it connected to the recording unit by electric wires. In doing so, we could attach this sensor directly to the external bottom surface of the arenas. This setup allowed us to record a series of 215,040 readings (512 readings × 60 s × 7 min) per assayed termite group, totaling about 12,840,000 readings = (215,040 readings × 4 stimuli × 15 assays) – (2 missing assays × 215,040 readings) for the whole experiment. As a visual complement, we also recorded the termite group's activity in each assay using a SONY HDR-CX405™ digital video camera set to record 30 frames per second at Full HD (1920 × 1080 60p). The camera positioning and the chronology of this footage are explained in detail in Figure 1.

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