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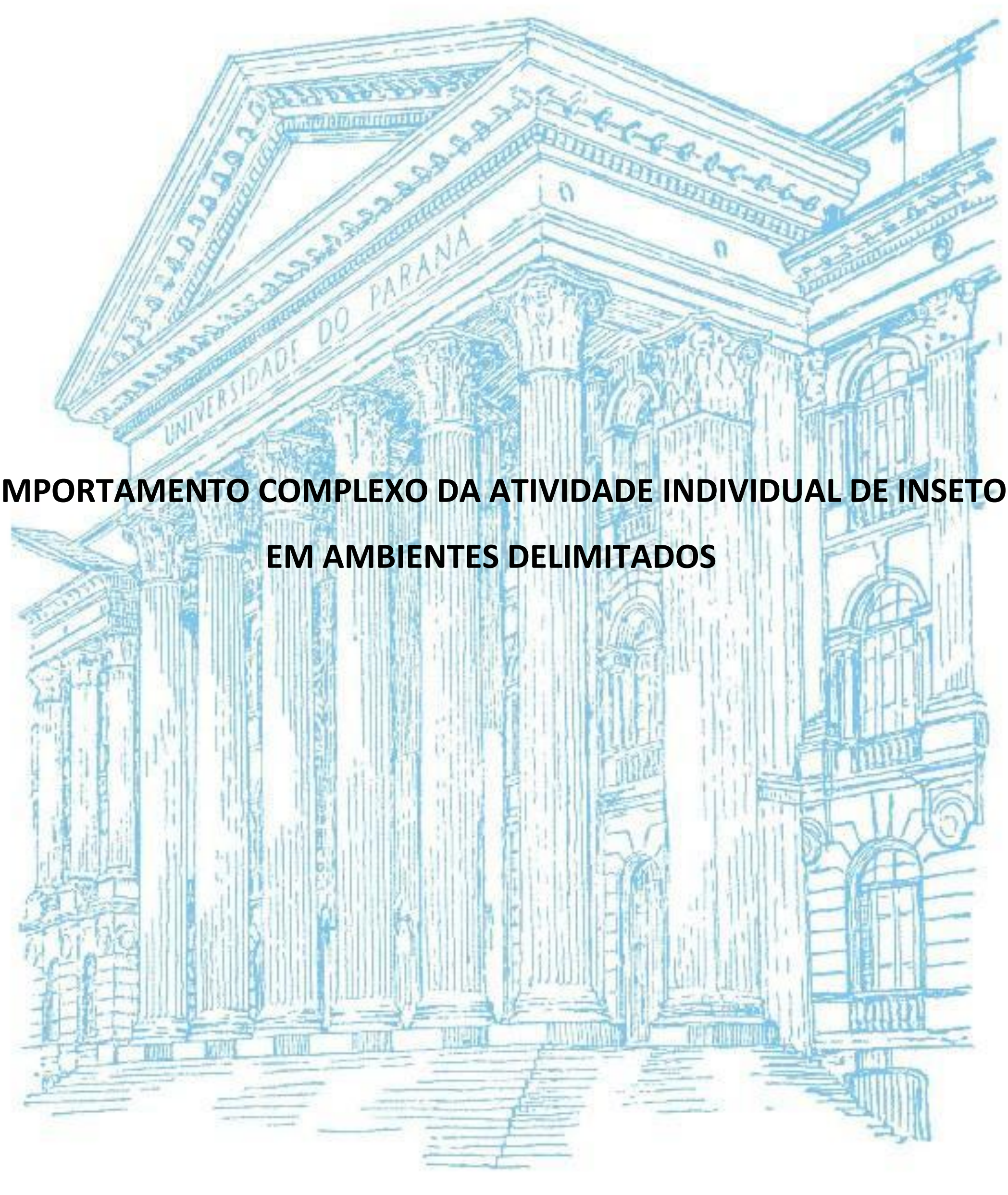

COMPORTAMENTO COMPLEXO DA ATIVIDADE INDIVIDUAL DE INSETOS EM AMBIENTES DELIMITADOS

CURITIBA

2016

FELIPE MARCEL NEVES

COMPORTAMENTO COMPLEXO DA ATIVIDADE INDIVIDUAL DE INSETOS EM AMBIENTES DELIMITADOS

Tese apresentada como requisito parcial à obtenção do título de Doutor em Ciências Biológicas, pelo Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Entomologia, da Universidade Federal do Paraná. Orientador: Prof. Dr. Marcio Roberto Pie e Coorientador: Prof. Dr. Ricardo Luiz Viana

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Tese aprovada como requisito parcial para obtenção do grau de "Doutor em Ciências", no Programa de Pós-graduação em Ciências Biológicas, Área de Concentração em Entomologia, da Universidade Federal do Paraná, pela Comissão formada pelos professores:

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Curitiba, 30 de junho de 2016.

Dedico esta tese a memória da minha amada avó, Tereza (In memoriam), aos meus pais Glaceir e Mauricio e ao meu irmão Lucas, por sempre me ajudarem em todos os aspectos.

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RESUMO

Como podemos mensurar a complexidade do comportamento animal? Nesta tese utilizo análises de recorrência (i.e. Plots de recorrência e RQA – Recurrence quantification analysis), descrição de comportamentos de movimento, incluindo o uso de modelagem matemática, para a compreensão do comportamento complexo de atividade individual de insetos em ambiente delimitados. Embora o estudo de insetos como sistemas complexos, em especial insetos com algum nível de organização social (colônias, agregações), seja promissor, a influência do comportamento individual na emergência de padrões auto organizados é pouco estudada. Estruturei a tese em três capítulos distintos. O primeiro capítulo desta tese é sobre o uso de análises de recorrência no estudo comportamental da atividade de insetos, sua aplicação e significância na interpretação de sinais estocásticos e determinísticos no comportamento animal. Comparei o padrão de atividade de três espécies de formigas, assim como de uma espécie solitária/gregária (*Tenebrio molitor*) para explorar a influência de níveis diferentes de complexidade social. Esta foi a primeira aplicação de análises de recorrência no estudo do comportamento de atividade, e uma das primeiras aplicações do método para fenômenos biológicos. Nossos resultados demonstram o potencial de análises de recorrência na análise de padrões de comportamento complexo. O segundo capítulo introduz a pouco conhecida espécie de vespa *Perreyia flavipes*, onde descrevemos pela primeira vez na literatura sobre o seu comportamento. *Perreyia flavipes* apresenta comportamentos interessantes em um intervalo curto de vida (36 hrs), tal como tanatose e cuidado maternal primitivo. Atos comportamentais são descritos e comparados entre os sexos, e suas possíveis funções são discutidos. O terceiro capítulo enfoca no comportamento das larvas de *Perreyia flavipes*, que apresentam comportamento gregário, se movimentando em grupos e de modo aparentemente coordenado. Neste capítulo abordo a influência de espaços delimitados no comportamento individual de *P. flavipes* através de dados experimentais e modelagem matemática. Espero que o presente estudo auxilie nos avanços dentro da área de mensuramento de testes empíricos de sistemas complexos em contextos biológicos.

Palavras-chave: Sistemas complexos, tracking de movimento, comportamento de insetos, dinâmica de movimentos

ABSTRACT

How could we measure the complexity of animal behavior? In this thesis I use recurrence analysis (i.e. recurrence plots and RQA - Recurrence quantification analysis), motion behavior description, including mathematical modelling to understand the complex behavior of individual activity of insects in boundaries spaces. Although the study of insects as complex systems, specially, insects with some degree of social organization (e.g. eusocial colonies, aggregations) is promising, the influence of individual behavior in the emergence of self-organized patterns is not well studied. I structured the thesis in three distinct chapters. The first chapter of this thesis is about the use of analysis of recurrences in the behavioral study of insect activity, its application and significance in the interpretation of stochastic and deterministic signals in animal behavior. I compared the activity patterns of three ant species, as well as a solitary/gregarious species (Flour beetle), to explore varying levels of social complexity. This was the first application of recurrence analysis to the study of animal activity, and one of the first applications to biological phenomena. In particular, our results underscore the potential of recurrence analyses in the analysis of complex behavioral patterns. The second chapter introduces the not well studied species of sawfly *Perreyia flavipes*, where we describe for the first time in the literature its behavior. *Perreyia flavipes* presents interesting behaviors in a short life span (36 hours), such as thanatosis and primitive maternal care. Individual behavioral acts are described and compared among sexes, and their potential functions are discussed. The third chapter focuses on the behavior of *Perreyia flavipes* larvae, which have gregarious behavior, moving in groups and apparently coordinated mode. In this chapter, I study the influence of boundaries spaces in the movement behavior of individual larvae by experimental data and statistical modelling. I hope that the present study helps in the advances in the area of behavioral measurement of empirical experiments of complex systems.

Keywords: Complex systems, movement tracking, behavior of insects, movement dynamics.

LISTA DE FIGURAS

CAPÍTULO I - Recurrence analysis of ant activity patterns

CAPÍTULO II - On the behavior of the sawfly *Perreyia flavipes* Konow, 1899 (Hymenoptera: Pergidae)

CAPÍTULO III - Wall following behavior in larvae of the sawfly *Perreyia flavipes* (Hymenoptera: Pergidae)

Appendix A - Wall following behavior in larvae of the sawfly *Perreyia flavipes* (Hymenoptera: Pergidae)

ANEXO I - Métodos para a extração de dados de atividade em insetos através de sistemas de tracking de movimento

LISTA DE TABELAS

CAPÍTULO I - Recurrence analysis of ant activity patterns

CAPÍTULO II - On the behavior of the sawfly *Perreyia flavipes* Konow, 1899 (Hymenoptera: Pergidae)

CAPÍTULO III - Wall following behavior in larvae of the sawfly *Perreyia flavipes* (Hymenoptera: Pergidae)

SUMÁRIO

CAPÍTULO III - Wall following behavior in larvae of the sawfly *Perreyia flavipes* (Hymenoptera: Pergidae)

ANEXO I - Métodos para a extração de dados de atividade em insetos através de sistemas de *tracking* de movimento

INTRODUÇÃO

> REDUCTIONISM is the most natural thing in the world to grasp. It's simply the belief that "a whole can be understood completely if you understand its parts, and the nature of their 'sum."
>
> —Douglas Hofstadter, Gödel, Escher, Bach: an Eternal Golden Braid

Qual é a definição de complexidade? Na literatura em geral, não existe senso comum sobre o seu significado (Mitchell 2009). Porém, alguns conceitos básicos do que entendemos como complexidade podem ser definidos, especialmente se nos focarmos em sistemas biológicos. Complexidade em sistemas biológicos se refere a: comportamento coletivo em algum nível, seja em células, organismos multicelulares e grupos de indivíduos, tais como colônias de formigas (1); Processamento de sinais do ambiente, de um modo interno ou externo (2); Adaptação, para aumentar suas chances de sobrevivência e sucesso reprodutivo, através de aprendizagem, em um curto prazo, ou evolutivamente, através de processos determinísticos de larga escala (3) (Mitchell 2009). O comportamento biológico complexo, portanto, pode ser interpretado de um ponto de vista hierárquico, através de um nível crescente de complexidade, de células individuais, grupos multicelulares, até grupos gigantescos de animais auto organizados (Novikoff 1945). Dentre inúmeros táxons de animais, insetos, em especial, possuem uma diversidade gigantesca, além de uma grande importância econômica, o que os tornam interessantes sob vários aspectos para estes estudos (Finlay et al. 2006; Wilson 1985). Não obstante, insetos em sua maioria possuem incrível adaptação a estudos experimentais. Seu tamanho relativamente diminuto e fácil realocação a laboratório são grandes vantagens para estudos comportamentais empíricos com condições delimitadas. Insetos também possuem diversos níveis de complexidade social, e podem ser comparados em relação a seu nível organizacional (Wilson 1971; Costa 1996).

Historicamente, o estudo de comportamento de insetos pode ser artificialmente dividido em três fases distintas que são complementares uma as outras. Cada uma destas fases está relacionada ao desenvolvimento de novos métodos e análises e estudo: história natural, fisiologia de indivíduos e sociedades e estudos de interações entre indivíduos. Na primeira fase de história natural, as espécies foram descobertas, catalogadas e descritas biologicamente (e.g. Lubbock 1881). A segunda fase cria pontes cada vez mais estruturadas entre comportamento e fisiologia, nas quais ocorreram os primeiros estudos experimentais em colônias de insetos sociais (e.g. formigas, abelhas e vespas). Um dos seus precursores pode ser considerado Karl V. R. Frisch, (um dos ganhadores do prêmio Nobel de Medicina em 1973, juntamente com Konrad Lorenz e Nickolaas Tinbergen) pelos seus estudos em comportamento animal. As pesquisas de Frisch em abelhas possuem princípios ainda aplicáveis em várias áreas, como as determinações de castas (e.g. Frisch 1953). Outro exemplo é a linha de pesquisa de Edward O. Wilson e colaboradores na comunicação e a descoberta da função dos feromônios na organização social em formigas (Wilson, 1962). A terceira fase é mais recente e interdisciplinar, através do uso de ferramentas oriundas de outras áreas, tais com os modelos matemáticos, novos equipamentos e análises. Baseiam-se em experimentação e postulações teóricas, tal como o início do estudo de sociedades de insetos como sistemas complexos, em outras palavras, o estudo do comportamento coletivo. Aliás, formigas foram um dos primeiros animais a serem estudados sob esta ótica (Deneubourg et al. 1985, 1986). Motivada por uma maior possibilidade de extração de dados, a biologia em geral está indo ao encontro de uma ciência mais quantitativa.

Embora exista um início promissor do estudo de insetos como sistemas complexos, em especial em insetos com algum nível de organização social (colônias, agregações), a influência do comportamento individual na emergência de padrões auto organizados é pouco estudada. Em geral, não se sabe ao certo a influência da complexidade individual na emergência de sistemas complexos auto organizados, menos ainda o seu grau próprio de complexidade. Isto se deve a uma série de fatores, primeiramente temos o problema de coleta de dados. Apesar das inúmeras facilidades metodológicas providas por insetos, como discutido anteriormente, o mensuramento do quão complexo tal comportamento pode ser é limitado pela dificuldade na extração de dados comportamentais em grande quantidade e de maneira confiável. Para estudarmos o comportamento complexo de uma forma abrangente e o mais próximo da realidade, é indispensável o acúmulo de uma grande quantidade de dados de maneira ininterrupta. Se o comportamento tem um componente estocástico substancial, este pode ser limitado em uma média dentro de um intervalo de tempo relativamente grande para discernir alguma ordem. Contudo, para um comportamento que varia temporalmente, este procedimento obscurece qualquer padrão que ocorra em um tempo curto. Cole (1991), apesar de limitações metodológicas de seu tempo, foi capaz de mostrar atividade caótica em formigas individuais. Nos últimos anos, somente mais um trabalho demonstrou atividade caótica no comportamento de forma empírica, no caso de vigilância em grupos de aves (Ferriere et al. 1996). Encontrar e classificar sinais determinísticos e estocásticos no comportamento animal pode trazer profundas consequências na interpretação de evolução comportamental. Por exemplo, se uma fração do comportamento em uma escala tempo for descoberta como ilusória, como tendo pouca informação prática para a formação do fenótipo comportamental, então teremos uma perspectiva diferente da forma de atuação e poder da seleção natural (Cole 1994). Apesar destes esforços iniciais, nenhuma metodologia nova foi proposta para o mensuramento de padrões determinísticos no comportamento animal, e embora exista análises de dinâmicas comportamentais, elas são limitadas (Webber et al. 2009).

Extrair dados que façam sentido, construir novos modelos, fazer as perguntas corretas e o desenho experimental necessário para responder estas questões é cada vez mais importante para se obter uma visão mais holística do comportamento animal. Desta maneira, nosso principal objetivo é utilizar novas ferramentas e métodos para a descrição do comportamento complexo de indivíduos de insetos. Deste modo temos acesso a dados de comportamento em grande escala e com o menor ruído instrumental possível. Devido à abrangência do tema, nosso escopo foi direcionado a espécies com características distintas e uma problemática comportamental para a compreensão de seus padrões de comportamento para a formação de grupos (sejam através de colônias eusociais ou de agregações). Partimos do pressuposto lógico de que para entender os macros padrões formados por comportamentos sociais em insetos, primeiramente é necessário um estudo minucioso da sua atividade de comportamento individual. Utilizamos duas variáveis importantes como nossos aliados metodológicos. Primeiramente, escolhemos estudar sob essa ótica apenas o nível de atividade de insetos. A atividade de movimento é um dos comportamentos que mais intrinsicamente estão ligados aos demais comportamentos exibidos por animais, além de poder ser interpretada como uma série temporal. Na literatura, existem fortes evidências sobre como o nível de atividade está ligado à agressividade, exploração, forrageio entre outros comportamentos básicos exibidos por animais (Korb & Heinze 2004; Brodin 2009; Biro et al. 2010; Realé et al. 2010; Chapman et al 2013; Sweeney et al. 2013). Também focamos no estudo de comportamento em ambientes limitados. Teoricamente, a influência de barreiras no comportamento de atividade em animais é universal (Creed & Miller 1990). Em insetos, principalmente terrestres, sua compreensão e influência pode ser mensurável e controlada de uma maneira mais eficaz.

Deste modo, a tese é dividida em três capítulos. O primeiro capítulo discorre sobre o uso das análises de recorrência no estudo comportamental de atividade de insetos, sua aplicação e significância na interpretação de sinais estocásticos e determinísticos no comportamento animal. Análises de recorrência são métodos de análise de dinâmica não lineares ainda poucos explorados na biologia. Sua capacidade quantitativa pode trazer informações importantes sobre a dinâmica de movimentos e como classificar padrões complexos. O segundo capítulo introduz a pouco conhecida espécie de vespa *Perreyia flavipes* Konow, 1899, na qual descrevemos pela primeira vez seu comportamento. O terceiro capítulo enfoca no comportamento das larvas de *Perreya flavipes*, que apresentam comportamento gregário, se movimentando em grupos e de modo aparentemente coordenado. Neste capítulo, tentamos compreender a influência de espaços delimitados no comportamento individual de *P flavipes*, e de que modo a influência de barreiras naturais podem influenciar a formação de grupos nestes animais e em espécies com comportamento semelhante. Esperamos que desta maneira, contribuíramos para uma compreensão maior sobre a dinâmica de movimento em insetos, principalmente no mensuramento e consideração de comportamento individuais complexos na emergência de sistemas auto organizados.

Referências

Biro PA, Beckmann C, Stamps JA. 2010 Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proc. R. Soc. Lond. B*. 277(1678), 71e77 (doi:10.1098/rspb.2009.1346)

Brodin T. 2009 Behavioral syndrome over the boundaries of life-carryovers from larvae to adult damselfly. *Behav. Ecol.* 20, 30e37 (doi: 10.1093/beheco/arn111)

Chapman BB, Hegg A, Ljungberg P. 2013 Sex and the syndrome: individual and population consistency in behaviour in rock pool prawn, *Palaemon elegans*. *PLoS One*, 8(3), e59437. (doi: 10.1371/journal.pone.0059437)

Cole BJ. 1991 Is animal behaviour chaotic? Evidence from the activity of ants. *Proc. Roy. Soc. Lond. B. Bio.* 244, 253–259. (doi*:*10.1098/rspb.1991.00)

Cole, B. J. 1994. Chaos and behavior: the perspective of Nonlinear Dynamics In: *Behavioral Mechanisms in Evolutionary Ecology* (Ed. by L. Real), pp. 423-443. Chicago: University of Chicago Press.

Creed Jr, R. P., & Miller, J. R. 1990 Interpreting animal wall-following behavior. *Experientia*, *46*(7), 758-761.

Deneubourg, J.-L., Pasteels, J. M. & Verhaeghe, J. C. 1983 Probabilistic behaviour in ants: a strategy of errors*? J. Theor. Biol.* 105, 259{271.79)

Finlay BJ., et al. 2006 Self-similar patterns of nature: insect diversity at local to global scales. *Proc. R. Soc. Lond. B*. 273, 1596: 1935-1941.

Frisch KV, *The Dancing Bees: An Account of the Life and Senses of the Honey Bee*, Harvest Books New York (1953)

Korb J, Heinze J. 2004 Multilevel selection and social evolution of insect societies. *Naturwissenschaften* 91, 291–304. (doi:10.1007/s00114-004-0529-5)

Lubbock, J. 1881 Observations on ants, bees, and wasps. IX. Color of flowers as an attraction to bees: Experiments and considerations thereon. *J. Linn. Soc. Lond. (Zool.)* 16: 110–112.

Mitchell, M. 2009 *Complexity: A guided tour*. Oxford University Press.

Webber Jr. CL, Marwan N, Facchini A, Giuliani A. 2009 Simpler methods do it better: Success of Recurrence Quantification Analysis as a general purpose data analysis tool. *Phys. Lett. A.* 373, 3753 (doi: 10.1016/j.physleta.2009.08.052.)

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO. 2010 Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos. T. R. Soc. B.* 365(1560), 4051e4063. (doi:10.1098/rstb.2010.0208)

Sweeney K, Cusack B, Armagost F, O'Brien T, Keiser CN, Pruitt JN. 2013 Predator and prey activity levels jointly influence the outcome of long-term foraging bouts. *Behav. Ecol.* 24, 1205e1210. (doi:10.1093/beheco/art052)

CAPÍTULO I

Recurrence analysis of ant activity patterns

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Recurrence analysis of ant activity patterns

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Abstract

Measuring complexity in biological systems is an inherently difficult endeavour, particularly because of their intrinsic nonlinear and noisy nature. In this study, we compare activity patterns of three ant species, as well as a solitary/gregarious species, to explore varying levels of social complexity. We used two methods of nonlinear analysis: recurrence quantification analysis (RQA) and recurrence plots (RPs). RQA and RPs quantifies the number and duration of recurrences of a dynamical system, resulting in a detailed description of its behaviour, including the quantification of signals that could be stochastic, deterministic, or both. The implications of our study are threefold. First, we found differences between species according to their level of social organization, from the gregarious beetles to the highly complex social ant species. Second, workers from different ant species varied with respect to their dynamics, varying from stochastic to deterministic behaviour. Finally, considerable differences were found among minor and major caste of the same (dimorphic) ant species. This study provided the first application of recurrence analysis to the study of animal activity, and one of the first applications to biological phenomena. In particular, our results underscore the potential of recurrence analyses in the analysis of complex behavioural patterns.

Keywords: Biological Systems, Chaotic Behaviour, Time Series, Animal Activity, Ants, Nonlinear Data Analysis.

Introduction

Animal behaviour is characterized by broad differences in hierarchical organization and variability, from individual organisms to societies. The variability often detected in animal behaviour seems unpredictable at first sight, resulting from genetic, developmental, neural and physiological processes, as well as environmental effects (Cole 1991a). However, this apparent intrinsic variability could hide nonlinear and unstable deterministic signals. In a seminal work, Cole (1991a) observed that single, isolated individuals of the ant *Temnothorax* (=*Leptothorax*) *allardycei* had movement activity characteristic of low-dimensional chaos. The existence of chaos in ant activity has been suggested as a necessary dynamic for foraging and exploration (Cole 1991b; Solé et al. 1993). However, empirical studies about the dynamics of activity (movement) behaviour are still in their infancy (e.g. Cole 1991a, b, 1992, 1995; Miramontes 1992; Boi et al. 1999; Lone and Sharma 2011; Christensen et al. 2014). Ants, in particular, display several advantages as a model organism for these kinds of study, given that they show considerable variation in social, morphological, ecological and behavioural traits at the colony-level (Wilson 1985; Grimaldi and Agosti 2000). Furthermore, ants are easy to manipulate and rear under laboratory conditions, and to observe in 2D experimental setups (Wilson 1985).

Activity could be considered one of the most vital biological feature of animals, being correlated with a wide spectrum of behavioural syndromes, such as aggressiveness and exploration (Korb and Heinze 2004; Brodin 2009; Biro et al. 2010; Réale et al. 2010; Chapman et al. 2013; Sweeney et al. 2013). A highly informative way to collect and investigate movement activity probably could be provided by the investigation of spatio-temporal data of several species. However, the study of time series patterns imposes some difficulties, not the least of which being data collection. A large amount of instrumental noise (as often occurs in real data, e.g. from EEG analysis, cardiology or geology) reduces the ability to detect deterministic signals. Fortunately, the possibility of computational tracking of individuals in the recent years offers better ways to obtain qualitative reliable data (Dell et al. 2014). Characterizing irregular behaviour of deterministic or stochastic processes is not a straightforward task to perform either. Several analysis methods have been proposed to investigate the presence of determinism in time series (e.g. Lyapunov exponents, Fourier analysis, Power spectral analysis), however, usually with very limited applications (Webber et al. 2009).

In this study, we introduce the use of recurrence analyses for the study of animal behaviour. Recurrence analyses are a new reliable and robust method of nonlinear data analysis that could be used for an improved understanding of biological time series. It is composed of visual diagnostics known as Recurrence Plots (RPs) and measures of complexity, such as Recurrence Quantification Analysis (RQA) (Eckmann et al. 1987). Using these tools, one can distinguish regimes of recurrence behaviour, which may be characteristic of different processes, such as white noise, chaotic maps, and (quasi-) periodic processes (Marwan et al. 2007). RQA has several advantages when compared to other time series analysis, such as its mathematical simplicity, non-restrictive modelling assumptions, and the capacity to deal with inherent noise (Webber et al. 2009). RQA has been used to interpret and correlate complex patterns in dynamic systems, such as in physics (Guimarães-Filho et al. 2008), physiology (Webber and Zbilut 1994), meteorology (Marwan et al. 2003), economics (Holyst et al. 2001), geophysics (Marwan et al. 2002) and cardiology (Marwan et al. 2009). The use of the RQA measures could give a more detailed and qualitative approach to time series analysis of complex dynamics. Here, we examine the complex temporal pattern of movement activity in individuals of species with varying levels of social complexity and behavioural specialization.

Methods

Three ant species with different degrees of social organization were used in our study: *Gnamptogenys striatula* (Ectatomminae), *Linepithema micans* (Dolichoderinae), and *Pheidole rudigenis* (Myrmicinae). Workers from three colonies of each chosen species were collected in the campus of the Universidade Federal do Paraná in Curitiba, state of Paraná, Brazil. *Gnamptogenys striatula* is typically found in open habitats and rainforests, showing a suite of primitive behavioural and morphological traits (Lattke 1990). Colonies of *G. striatula* are small (150-200 individuals) and have either one or several queens and gamergates (i.e. workers with reproductive capacity) (Giraud et al. 2000; Lommelen et al. 2006). *Linepithema micans* belongs to a widespread genus that includes an important invasive species [i.e. *Linepithema humile*, (Wild 2009), which could be an indicative of its own potential as an invasive species. Colony size in *L. micans* might exceed 1000 individuals, leading to a fairly complex social organization (Wild 2007). Finally, *P. rudigenis*, as is the norm for its genus, is characterized by a dimorphic sterile worker caste, with regular workers (minors) carrying out quotidian colony tasks, whereas larger, big-headed workers (majors) are specialized in specific tasks, such as colony defence or seed milling (Wilson 2003). Furthermore, for comparison with non-social insects, we used the beetle *Tenebrio molitor*, a cosmopolitan pest of stored grains with gregarious behaviour.

Foraging ants were collected manually by attracting them outside their nests using sugar water or tuna baits between 10:30 am and 5:00 pm (*n*=30 workers for each species, except for *P. rudigenis*, in which 30 minor and major workers were tested separately). Assays lasting for two hours (*n*=7200 seconds) typically started 45 min after the collection, whereas *T. molitor* were reared in laboratory in three acrylic boxes, stored in a well-ventilated, dark place, at ambient humidity and temperature with food (i.e. wheat bran) *ad libitum*. Trials were carried with different combinations of colonies and species per day, for a total of 150 experiments (*n*=300 hours).

Experimental setup

The experimental apparatus for the trials consisted of an environmental chamber made with cardboard (51 x 26 cm) with a tracking Petri dish arena inside (92 mm in diameter) (Figure 1). The arena was brightly lit (≈880 lux) by two fluorescent light spots (Taschibra ® TKT15 15W 120 VAC 60 Hz 370 mA 6.400 *K*) positioned at opposite corners. Individuals were placed inside the arena under controlled environmental conditions (21 ± 2 °C and 65 % ± 10 relative humidity), between a glass cover plate and the substrate, which was shallow enough to constraint movement into only two dimensions. The colour of the floor of the arena was opaque white for better image contrast, consisting of odourless white rubber silicon RTV (i.e. Room Temperature Vulcanizing silicon CS1000). After each trial, both the arena and the substrate were cleaned, washed in bleach (80 %), dried and not used for at least five hours before a new experiment.

We recorded the movements of the individuals with a camera mounted 20 cm above the experimental setup (Everio GZ-MG435BUB). Trials were recorded at 20 frames *per* second. The extracted raw movie (MPEG-2; 720x480 pixels) was transformed into uncompressed AVI video files by the program Virtual Dub software by Avery Lee, version 1.5.10. The Cartesian displacement between the consecutively coordinates (x, y) of each frame, was considered the measure of activity (at an interval of three seconds; *n*= 2400 frames *per* trial). Thus, a high level of activity produced large number of pixel differences. Our definition of the activity measurement is similar to that of Cole (1991a) in a study on the behaviour of the ant *Temnothorax albipennis*.

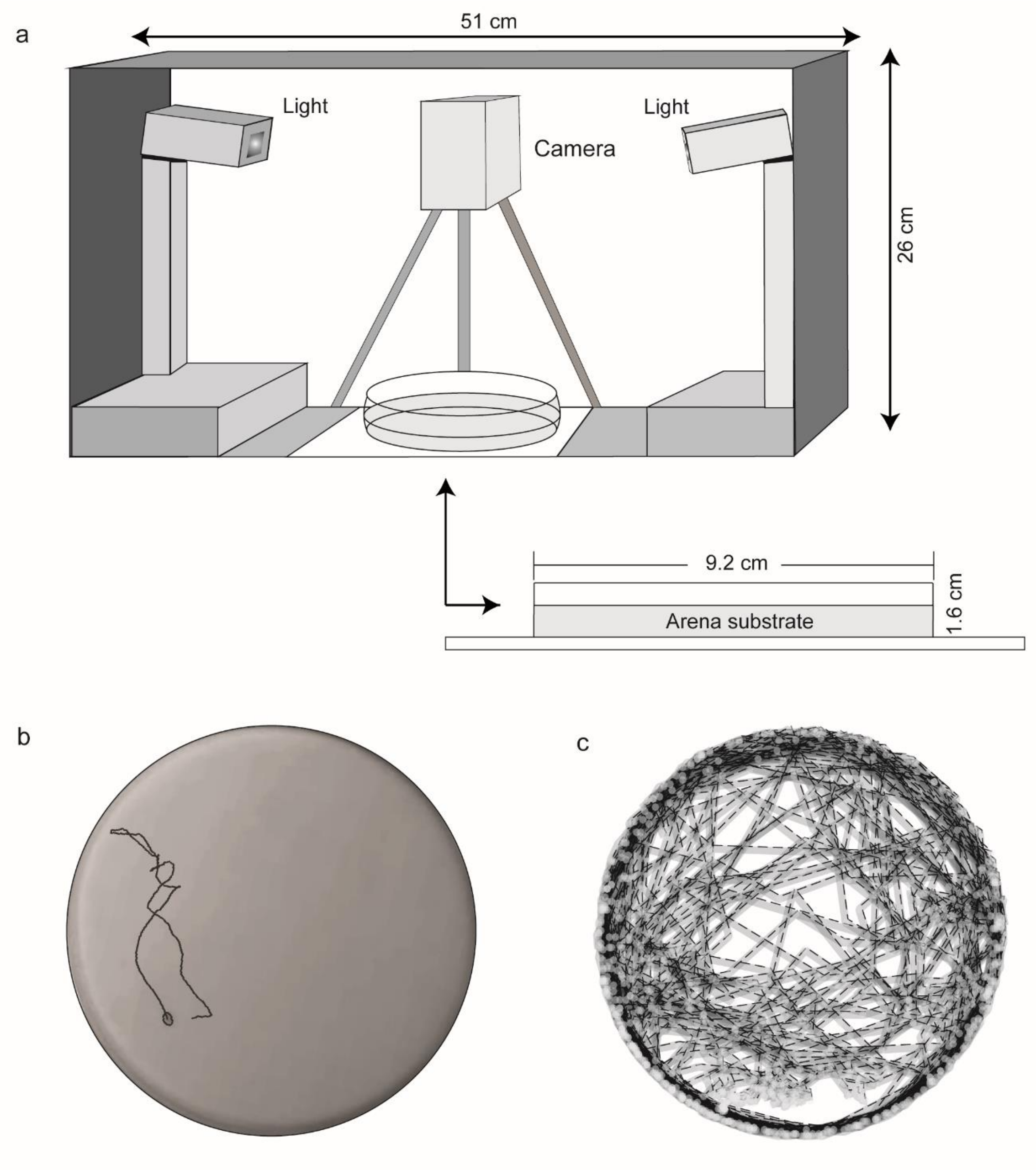

Figure 1. The experimental setup. Measurements, disposition view of the light spots, camera and the tracking arena (a). Video frame of an ant (*Linepithema micans*) in the arena during the tracking, the black line corresponds to the movement of the ant (b).

Final tracking coordinates (x,y) of 2.400 frames extracted from a time series (*Linepithema micans*), the grey dashed lines are the coordinates obtained (c).

Tracking system

The x-y coordinates of the individual positions were extracted using the Ctrax software (Caltech Multiple Fly Tracker; version 0.3.12) and the associated FixErrors toolbox forMATLAB© (v. 7.10.0 2010; MathWorks, Inc., Natick, MA, USA) (Branson et al. 2009; MATLAB v. 7.10.0). Ctrax was primarily made for tracking fruit flies, but is able to track individuals and large groups of animals (e.g. cockroaches, fishes, ants) while maintaining their individual identities (Branson et al. 2009; Bender et al. 2011; Reid et al. 2011). Furthermore, Ctrax has high accuracy in movement tracking and requires simple recording equipment (Branson et al. 2009). During preliminary experiments, we observed false positives errors (type I) and false negative errors (type II). Type I errors (mismatches of object identity) were corrected manually with the help of the FixErrors toolbox available for the Ctrax software, whereas type II errors (loss of tracking) were accepted only when they accounted for less than 5% of the time series.

Recurrence Plots

We investigated the obtained time series using the Recurrence Plot (RP), which is a technique of nonlinear data analysis first proposed by Eckmann et al. (Eckmann et al. 1987). RPs consists of a visualization (or a graph) of a square matrix in which the matrix elements correspond to those times at which a state in a dynamical system recurs, i.e. approaches itself after some period of time (Eckmann et al. 1987; Marwan et al. 2007). Consequently, the RP reveals all the times when the phase space trajectory of the dynamical system visits roughly the same region in the phase space (Marwan et al. 2007). We constructed recurrence matrices by comparing embedding vectors with each other at different times, drawing pixels when the distance between vectors falls within an ε-neighborhood (Marwan and Kurths 2005; Marwan et al. 2007; Marwan 2011). Such an RP can be mathematically expressed as

$$\mathbf{R}_{i,j} = H\left(\varepsilon - \left\| x_i - x_j \right\|\right),\ (i, j, = 1,2 \ldots N),$$

where ε is a threshold, $H(.)$ is the Heaviside unit step function $\|\ldots\|$ stands for some norm (e.g., the Euclidean norm), i =1, 2,…N is represented in the horizontal axis, and j with the same range in the vertical axis. The RP is thus obtained by assigning a black (white) dot to the points for which $\mathbf{R}_{i,j} = 1(0)$. By construction, the recurrence matrix is always symmetric ($\mathbf{R}_{i,j} = \mathbf{R}_{j,i}$), and a point is always recurrent to itself, i.e., $\mathbf{R}_{i,j} = 1$ forming the main diagonal line of the RP (Marwan et al. 2007).

Graphic representations of recurrence points permit to observe and interpret the general overview pattern of each individual time series. Eckmann et al. (1987) made distinctions on how to visually read some of the plots. Marwan et al. (2007) classifies recurrence plot structures into large-scale and small-scale patterns. Homogeneous patterns, characterizing white noise; Periodic patterns, when recurrence plots present diagonal lines and/or checkerboard structures; Drift patterns, when there is fading in the corners, for systems which are non-stationary; Disrupted patterns, for extreme (and rare) events, when white bands are present, indicating transitions. Furthermore, small-scale patterns can also be characterized, as in the case of isolated points for rare states. Diagonal and vertical lines are important structural elements of RPs, creating the basis for its quantification. Diagonal lines occur when the system visits the same region of the phase space at different moments, whereas vertical or horizontal lines occur when the system either does not change or it changes slowly (Marwan and Kurths 2005). Moreover, Zbilut and Webber (Zbilut 1992; Webber and Zbilut 1994) introduced measures based on the information extracted from the RPs to quantify dynamical features of the data (Webber and Zbilut 1994). Recurrence quantification analyses (RQA) provide a characterization of the type of dynamics present in the system (e.g. periodic, chaotic) (Marwan et al. 2007). RPs and RQA were obtained through the CRP toolbox (v. 5.17) developed by Norbert Marwan for MATLAB© (MATLAB 7.10.0). The toolbox can be found at http://www.agnld.uni-potsdam.de/˜marwan/toolbox/.

Recurrence Quantification Analysis

RQA comprises many quantitative diagnostics of the distribution of dots (actually pixels) in a recurrence plot to provide quantification of important aspects revealed through the

RPs in detail. We choose five measures to examine our data: Recurrence rate (RR), Determinism (DET), Entropy (ENT), Laminarity (LAM) and Trapping Time (TT). The *recurrence rate* (RR) is the probability of finding a black recurrence point (for which $\boldsymbol{R}_{i,j}$=1), or

$$\mathrm{RR} = \frac{1}{N(N-1)} \sum_{i,j=1;i\neq j}^{N} \mathbf{R}_{i\,,j},$$

where N^2 is the total number of pixels (black or white) in a RP [21]. Recurrence rate is nearly the same as the definition of correlation sum, which does not include the main diagonal line, it is related with the probability that a specific state will recur. Higher RR (for the same value of the ε parameter) would indicate that there only few overall changes in the dynamics of the responses over time and that performance are confined to few different states.

Determinism (DET) measures the percentage points in an RP belonging to diagonal lines, indicating deterministic components in the recurrence plot (Marwan et al. 2007). The DET measure is calculated by,

$$\mathrm{DET} = \frac{\sum_{l=l_{min}}^{l_{max}} lP(l)}{\sum_{i,j=1;\, i\neq j}^{N} \mathbf{R}_{i,j}},$$

the l_{min} is the minimum and the l_{max} is the maximum length allowed for a diagonal line. $P(l) = \{l_i\,; i = 1,2, \dots, N_l\}$ is the frequency distribution of the lengths l_i of diagonal lines, and N_l is the absolute number of diagonal lines, except for the main diagonal line (Marwan et al. 2007). The higher the DET value, more it reflects the predictability of the system over time. We can also compute estimates for the *Shannon entropy* (ENT),

$$\mathrm{ENT} = -\sum_{l=l_{min}}^{l_{max}} p(l) 1\mathrm{n}\, p(l)\,,$$

where

$$p(l) = \frac{P(l)}{\sum_{l=l_{min}}^{l_{max}} P(l)},$$

is the probability distribution of the diagonal line lengths. The ENT reflects the complexity of the deterministic structure of the system (Marwan et al. 2007). Higher entropy would indicate more inherent complexity of the correspondent time series, e.g. for uncorrelated noise the value of ENT would be rather small, indicating its low complexity. Laminarity (LAM) is the percentage of RP points forming vertical lines, or of these laminar phases,

$$\mathrm{LAM} = \frac{\sum_{v=v_{min}}^{v_{max}} vP(v)}{\sum_{i,j=1;\, i\neq j}^{N} \mathbf{R}_{i,j}},$$

where v_{min} is the minimum lengths of a vertical line and v_{max} is the maximum vertical length. Analogously to diagonal lines, we can obtain the frequency distribution of the lengths v_i of vertical lines $P(v) = \{v_i\,; i = 1,2, \dots, N_v\}$, where N_v is the absolute number of vertical lines. LAM represents the occurrence of laminar states in the system without describing the length (Marwan et. al 2007). Moreover, we calculated the trapping time (TT) that is the average length of a vertical line, it's given by

$$\mathrm{TT} = \frac{\sum_{v=v_{min}}^{v_{max}} vP(v)}{\sum_{v=v_{min}}^{v_{max}} P(v)},$$

TT estimates the mean time that the system will remain at a specific state or how long the state will be trapped (Marwan et. al 2007).

There are different ways into how to apply RQA, mostly based in order of magnitude (large and small scale) and data format (e.g. networks, time series). Here, we applied a global time series approach for each individual replicate. The global time series approach focuses in a large scale encompassing the entire time series with the five chosen RQA measures been extracted from it.

Recurrence parameters

The recurrence plots and the correspondent recurrence analyses used in the experiments might be affected by the chosen parameters from each time series and the embedding parameters affecting the quality of the phase space reconstruction, namely, time delay τ, embedding dimension m and the threshold value ε. The time delay τ determines the predictability of the components in the reconstructed vectors of the system state (Marwan et. al 2007). It should be chosen in a way such that the elements in the embedding vectors are no longer correlated. We estimate the time delay as the one where average mutual information reaches its first minimum (Zbilut 1992). The embedding dimension m determines the number of the components in the reconstructed vector of the system state. It should be large enough to unfold the system trajectories from self-overlaps, but not too large as the noise will be amplified. We employ the false nearest neighbour (FNN) method as suggested by Kennel et al. (1992) to determine a good value for our system. The threshold value $\mathcal{E}$ was defined accordingly to each time series recurrence plot, it was chosen using the value that corresponds to 10% of the maximum or mean phase space diameter of the data (Eckmann et al. 1987). Through the methods exposed here, the recurrence measures were stipulated in such a way that the embedding dimension and the time delay for all the time series were defined with the value of three and the threshold value was defined individually according to each time series.

Surrogate data and Statistical tests

We compared each time series original data with a shuffled surrogate, which is a common approach used for validation of results in time-series analysis (Theiler et al. 1992). In the context of RQA, the comparison between the data and its surrogates could be indicative of a lack of strong determinism or a spurious element in the determinist structure present by a system (Riley et al. 1999; Marwan et al. 2007). The shuffling preserves the statistical distribution of the data (e.g. mean, variance) but destroys the phasic time-correlated information in the dynamics. We shuffled the data $x(n)$ randomly choosing a pair of points from the data chain and randomly exchanged the positions of such points for each trial. This procedure has been repeated N times, where N is the number of all the data points. Given that normality was not met for our data based on a preliminary Lilliefors test (Lilliefors 1967), we used the two tailed Mann-Whitney non-parametric rank sum test for independence (Kruskal 1952) to compare the recurrence measures between the original data for each species and it is correspondent shuffled data (with $p \leq 0.05$). The comparison between species was made by the Kruskal-Wallis test (Mann and Whitney 1947); it is also a non-parametric one-way analysis of variance by ranks for testing equality of three or more population medians. Statistical analyses were performed by the R software v. 3.2.3 (R Core Team 2015).

Results

We obtained a series of RQA global measures (*n*=750) and individual RPs (*n*=75) from 150 time-series. The results highlight similarities and disparities between the activity dynamics of each species. Due to the topological nature of recurrence plots, we can also infer by visual inspection of RPs some of the features presented by all species and some particularities of them (Figure 2). All RPs showed several white bands, characterizing non-stationarity due to transitions. RPs showed diagonal and horizontal structures for almost all the species supporting the hypothesis of a deterministic content present in the data. Furthermore, the RPs did not show homogeneous topologies, clearly rejecting the idea of a random process for most of the species. However*, P. rudigenis* had relatively short lines and isolated dots, mainly in the major subcaste, indicating heavy fluctuation in the process. This can imply the existence of uncorrelated random motions or even anti-correlated process. RPs of *T. molitor* also had some peculiarities, such as irregular periodicities in the process, the presence of long lines with different distances to each other suggests a quasi-periodic dynamic (several frequencies in the system, whose ratios are irrational).

We compared the global RQA measures of the original data matching the shuffled ones. In general, the surrogate data possess lower significant values of the RQA measures compared to the original data, with the exception of REC in both *P. rudigenis* castes (Table 1). Differences between normal and surrogate data were usually higher in *G. striatula* and *L. micans,* with a typical percentage change between 70% and 74%. Differently, *P. rudigenis* and *T. molitor* had a percentage change of only 26% to 29%. The largest the impact of the shuffling, most deterministic the process.

RQA measures were also compared between species (Table 2). *Tenebrio molitor* presented higher RQA measures than the ants, however, these results must not be interpreted as a display of higher complexity. The RPs of *T. molitor* (Figure 2) have substantial phases of inactivity (black clusters) with a few sparse bouts of activity (white clusters), endorsing the role of a deterministic quasi-periodic pattern. The ant species

G. striatula and *L. micans* had higher significant RQA measures when compared with *P. rudigenis,* whereas both *P. rudigenis* worker castes presented the lower results among all of the species (Table 2).

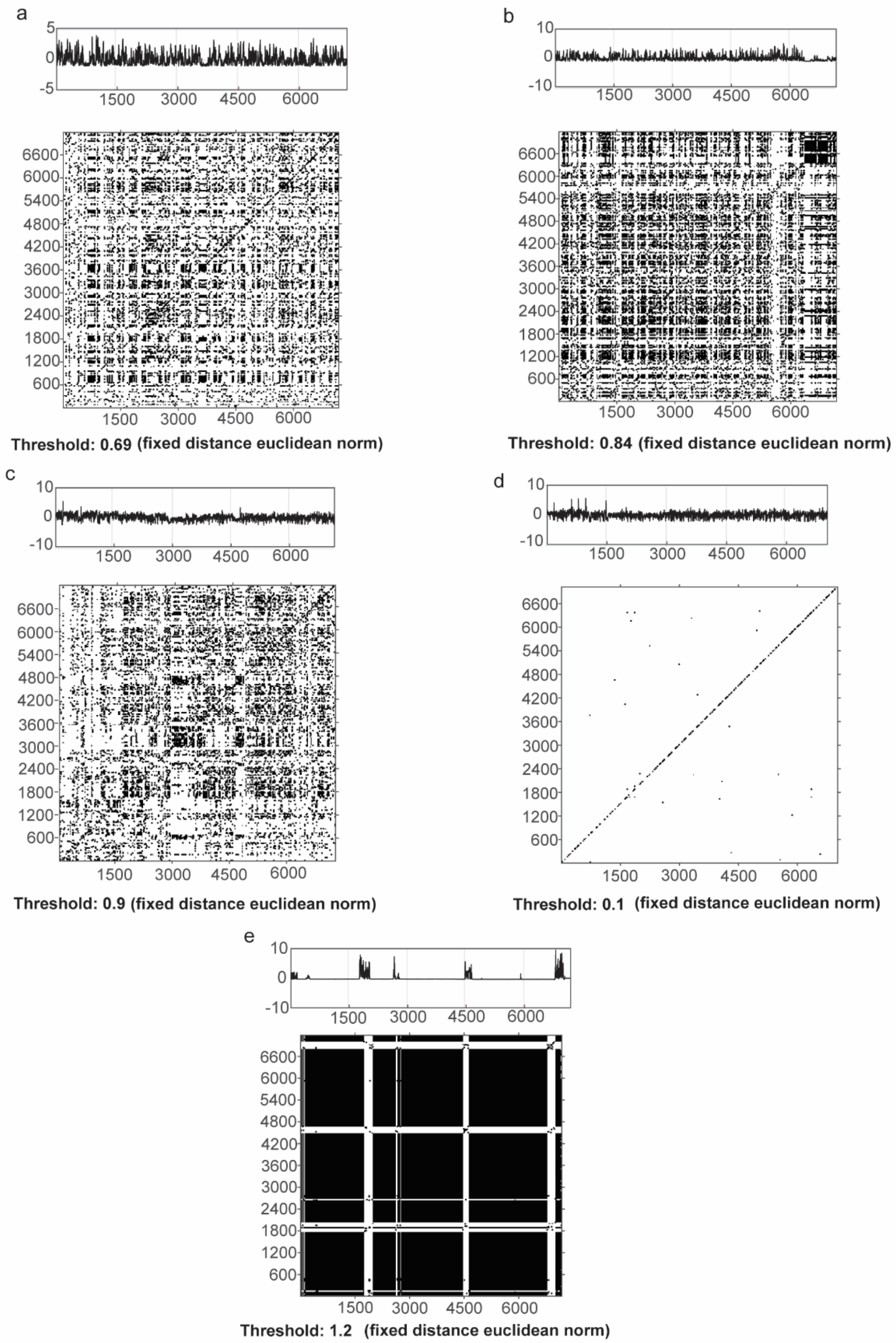

Figure 2. Recurrence plots (RP's) of the time series. Each species had a characteristic recurrence plot pattern, here represented by a time series with measures near the

median RQA values from the replicates. The species are *Gnamptogenys striatula* (a), *Linepithema micans* (b), *Pheidole rudigenis* minor (c) and major subcaste (d), and *Tenebrio molitor* (e). In the RP's, white dot are maximum distance and black dots are minimum. Estimated parameters: Dimension = 3, Time delay = 3 and the Threshold values were variable with each time series and are signaled in each RP.

Table 1. RQA percentage measures of the species (columns) compared with each respective surrogate data series (surr). Recurrence rate (REC), determinism (DET), entropy (ENT), laminarity (TT) and trapping time (TT) of the original and surrogate data (surr).

DATA	*Gnamptogenys striatula*		*Linepithema micans*		*Pheidole rudigenis*$^{\text{min}}$		*Pheidole rudigenis*$^{\text{maj}}$		*Tenebrio molitor*	
RR	**0.07**	$p<0.001$	**0.11**	$p<0.001$	0.07	ns	0.04	ns	**0.69**	$p<0.001$
RR$_{\text{surr}}$	0.03	(58%)	0.04	(64%)	0.06	(15%)	0.04	(0%)	0.41	(41%)
DET	**0.51**	$p<0.001$	**0.45**	$p<0.001$	**0.20**	$p<0.03$	**0.15**	$p<0.001$	**0.97**	$p<0.001$
DET$_{\text{surr}}$	0.05	(90%)	0.08	(83%)	0.13	(45%)	0.09	(40%)	0.65	(33%)
ENT	**1.43**	$p<0.001$	**1.06**	$p<0.001$	**0.40**	$p<0.004$	**0.32**	$p<0.001$	**2.57**	$p<0.001$
ENT$_{\text{surr}}$	0.13	(91%)	0.16	(85%)	0.23	(43%)	0.17	(47%)	0.87	(11%)
LAM	**0.61**	$p<0.001$	**0.71**	$p<0.001$	**0.37**	$p<0.04$	**0.21**	$p<0.001$	**0.96**	$p<0.001$
LAM$_{\text{surr}}$	0.16	(74%)	0.21	(71%)	0.20	(46%)	0.10	(53%)	0.86	(11%)
TT	**5.15**	$p<0.001$	**3.92**	$p<0.001$	**2.31**	$p<0.003$	**2.19**	$p<0.001$	**35.7**	$p<0.001$
TT$_{\text{surr}}$	2.11	(60%)	2.18	(45%)	2.14	(8%)	2.06	(6%)	4.41	(88%)

The p value is based on the Mann-Whitney rank sum test for independent samples, it was considered significant when it was less than $p<0.05$. Significant statistics are indicated in bold followed by the p value, non-significant (ns) results are also indicated. Moreover, results are followed by the percentage (%) difference between the normal and shuffled data. The subscribed acronyms $_{\text{min}}$ and $_{\text{maj}}$ mean the words "minors" and "majors", respectively.

Table 2. Comparison of the RQA measures: Recurrence rate (RR), determinism (DET), entropy (ENT), laminarity (LAM) and trapping time (TT) between the species.

DATA	RR	DET	ENT	LAM	TT
Gnamptogenys striatula	**0.07**+-0.13	**0.51**+-0.25	**1.43**+-0.65	**0.61**+-0.21	**5.15**+-12
Linepithema micans	**0.11**+-0.07	**0.45**+-0.25	**1.06**+-0.73	**0.71**+-6.40	**3.92**+-17.7
Pheidole rudigenis ^{min}	**0.07**+-0.14	0.20+-0.36	0.40+-1.05	**0.37**+-0.02	**2.31**+-42
Pheidole rudigenis ^{maj}	0.04+-0.03	0.15+-0.12	0.32+-0.44	0.21+-0.15	2.19+-1.45
Tenebrio molitor	**0.69**+-0.17	**0.97**+-0.03	**2.57**+-1.10	**0.96**+-0.02	**35.7**+-42

The *p* value is based on the Kruskal-Wallis rank sum test and it was considered significant when less than *p*>0.05. The results were indicated with each correspondent standard deviation (median ± sd), significant statistics results are indicate in bold. The subscribed acronyms $_{min}$ and $_{maj}$ mean the words “minors” and “majors”, respectively.

Discussion

To the best of our knowledge, the present study provided the first application of recurrence analysis to the study of animal activity, as well as one of the first applications to biological phenomena outside the study of physiological and cardiac rhythms. The implications of our study are threefold. First, we found intriguing differences between species according to their level of social organization, from the gregarious beetles to the highly complex social ant species. Second, workers from different ant species varied with respect to their dynamics, varying from stochastic to deterministic behaviour. Finally, considerable differences were found among minor and major caste of the same (dimorphic) ant species. Each of these issues will be discussed in turn.

The activity pattern observed in the flour beetle *T. molitor* was characterized by the presence of periodic short bursts of activity, interspersed with longer periods of quiescence, as indicated by the extremely high values of vertical lines measures (LAM and TT) that indicate the presence and consistency of wide laminar states of inactivity (i.e. black clusters within the RPs). The underlying causes of this behavior are still poorly known, but one possibility is that it is an involuntary response to the non-optimal condition. In general, *T. molitor* is more inactive during daylight (Fondacaro and Butz 1970), normally evading natural light in search of darker areas (Cotton 1927).

Differently, the time series of ants were more dynamic, reflecting RQA measures with deterministic signals but mixed with stochasticity. Contrary to solitary animals, ants and other social insect must rely on other workers to carry out the necessary tasks for the survival of their colony and to ensure its ergonomic efficiency (Hölldobler and Wilson 1990). As a consequence, ants need to respond not only to their own individual stimuli, but also to the cues provided by nestmates in a variety of contexts, from the recruitment of nestmates during foraging to colony-level alarm behavior (Hölldobler and Wilson 1990; Nicolis and Deneubourg 1999). Therefore, it is not surprising that ant activity patterns are more complex than those of solitary or even gregarious species, and the use of recurrence analyses might provide a valuable framework to investigate these differences in a quantitative framework. There were interesting differences in activity patterns within the worker caste of different ant species. For instance, both *L. micans* and *G. striatula*, which are monomorphic species, had strong deterministic features (i.e. higher REC and DET values). In monomorphic species, all colony tasks are performed by workers of equivalent morphology, with specialization only being possible through behavioral or age differences among workers (Öster and Wilson 1978). On the other hand, in species with a dimorphic worker caste, some workers are morphologically adapted to specific tasks (e.g. colony defense, seed milling) whereas other workers can focus on more quotidian tasks, such as nest maintenance and brood care (Hölldobler and Wilson 1990; Detrain and Pasteels 1991; Detrain and Pasteels 1992; Pie and Traniello 2007). Such differences are probably reflected in their intrinsic propensity to respond to specific cues in a way that is different from a colony with monomorphic workers. Indeed, in *P. rudigenis*, a species with polymorphic workers, both castes showed lower RQA measures, with major caste presenting particularly higher stochastic traits. Given that major workers in *P. rudigenis* probably play an important role in colony defense, the types of cues that they should respond to are more stochastic in nature (e.g. encountering a forager from a competing colony or a predator) than the more predictable tasks of foraging and brood care, so that an activity behavior with a strongly random dynamic could be more adaptive given that randomness is an efficient response to environmental unpredictability, as explored in mathematical models based of ant behavior (Deneubourg et al. 1986; Gordon et al. 1992; Solé et al. 1993). Alternatively, the lower complexity (i.e. lower RQA values) in a minor worker caste could reflect the counterpart aspects of the intrinsic division of labor in a dimorphic species, even though minor workers maintained an intrinsic determinism that was similar to those of monomorphic species when compared with their majors counterparts.

The search and interpretation of complex patterns in biological systems is an ambitious task and must be made with caution. Through our approach using recurrence analysis, we propose the use of several RQA measures in conjunction with RPs, for a more consistent and comprehensive interpretation of the results. The possible use of recurrence analyses in the study of animal behaviour are vast, from the interpretation of transitions within time series to the detection of synchronization and network complexity, we expect it to be an important tool in new empirical studies. Furthermore, the present study is among the first studies measuring individual complexity with comparison between species. The data generated by this kind of analysis could be interesting for behavioural ecologists as to physicists and correlated fields interested in the modelling and theoretical investigations of biological complex systems. The recurrence analysis permits further investigations to understand deeply patterns within time series. For instance, preliminary results indicate a progress to a more deterministic behaviour with increasing densities, however by very different processes (Neves et al. 2012). Our first study using recurrence plots and recurrence quantification analysis in animal behaviour suggests that the activity dynamics of ants are composed by a plethora of complex patterns that ranges from random to deterministic signals.

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Authors' contributions

FMN collected field data, carried out the lab work, carried out the analysis, participated in the design of the study and drafted the manuscript; RV conceived the analysis, helped interpret the results, helped coordinated the study and helped draft the manuscript; MRP conceived the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

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Conflict of Interest

The authors declare that they have no interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Bender JA, Simpson EM, Tietz BR, Daltorio KA, Quinn RD, Ritzmann RE (2011) Kinematic and behavioral evidence for a distinction between trotting and ambling gaits in the cockroach, *Blaberus discoidalis.* J Exp Biol 214:2057-2064. doi: 10.1242/jeb.056481

Biro PA, Beckmann C, Stamps JA (2010) Small within-day increases in temperature affects boldness and alters personality in coral reef fish. Proc R Soc Lond B. 277(1678):71e77 doi: 10.1098/rspb.2009.1346

Boi S, Couzin ID, Del Buono N, Franks NR, Britton NF (1999) Coupled oscillators and activity waves in ant colonies. Proc Royal Soc B Bio Sci 266:371-378. doi: 10.1098/rspb.1999.0647

Branson K, Robie AA, Bender J, Perona P, Dickinson MH (2009) High-throughput ethomics in large groups of *Drosophila*. Nat methods 6:451-457. doi*:* 10.1038/nmeth.1328

Brodin T (2009) Behavioral syndrome over the boundaries of life-carryovers from larvae to adult damselfly. Behav Ecol 20:30-37. doi: 10.1093/beheco/arn111

Chapman BB, Hegg A, Ljungberg P (2013) Sex and the syndrome: individual and population consistency in behaviour in rock pool prawn, *Palaemon elegans*. PLoS One 8(3):e59437. doi: 10.1371/journal.pone.0059437

Christensen K, Papavassiliou D, de Figueiredo A, Franks NR, Sendova-Franks AB (2014) Universality in ant behaviour. J R Soc Interface 12:20140985. doi: 10.1098/rsif.2014.0985

Cole BJ (1991a) Is animal behaviour chaotic? Evidence from the activity of ants. Proc Roy Soc Lond B Bio 244:253-259. doi*:* 10.1098/rspb.1991.0079

Cole BJ (1991b) Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. J Insect Behav 4:129-137. doi: 10.1007/BF01054607

Cole BJ (1992) Short-term activity cycles in ants: age-related changes in tempo and colony synchrony. Behav Ecol Sociobiol 31:181-188. doi: 10.1007/BF00168645

Cole BJ (1995) Fractal time in animal behaviour: the movement activity of *Drosophila*. Anim Behav 50:1317-1324. doi*:* 10.1016/0003-3472(95)80047-6

Cotton RT (1927) Notes on the biology of the meal worms, *Tenebrio molitor* Linne and *T. obscurus* Fab. Ann Entomol Soc Am 20:81-86. doi: 10.1093/aesa/20.1.81

Dell AI, Bender JA, Branson K, Couzin ID, Polavieja de GG, Noldus LPJJ, Pérez-Escudero A, Perona P, Straw AD, Wikelski M, Brose U (2014) Automated image-based tracking and its application in ecology, Trends Ecol Evol 29:417-428. doi: 10.1016/j.tree.2014.05.004

Deneubourg JL, Aron S, Goss SAPJM, Pasteels JM, Duerinck G (1986) Random behaviour, amplification processes and number of participants: how they contribute to the foraging properties of ants. Physica D 22(1):176-186

Detrain C, Pasteels JM (1991) Caste differences in behavioral thresholds as a basis for polyethism during food recruitment in the ant, *Pheidole pallidula*. J Insect Behav 4(2):157-176. doi: 10.1007/BF01054609

Detrain C, Pasteels JM (1992) Caste polyethism and collective defense in the ant *Pheidole pallidula*: the outcome of quantitative differences in recruitment. Behav Ecol Sociobiol 29:405-412. doi: 10.1007/BF00170170

Eckmann JP, Kamphorst SO, Ruelle D (1987) Recurrence plots of dynamical systems. Europhys Lett 5:973-977. doi: 10.1209/0295-5075/4/9/004

Fondacaro JD, Butz A (1970) Circadian rhythm of locomotor activity and susceptibility to methyl parathion of adult *Tenebrio molitor* (Coleoptera: Tenebrionidae). Ann Entomol Soc Am 63(4):952-955. doi: 10.1093/aesa/63.4.952

Gordon DM, Goodwin BC, Trainor LE (1992) A parallel distributed model of the behaviour of ant colonies. J Theor Biol 156(3):293-307

Giraud T, Blatrix R, Poteaux C, Solignac M, Jaisson P (2000) Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. Mol Ecol 9:1835-1841. doi: 10.1046/j.1365-294x.2000.01085.x

Grimaldi D, Agosti D (2000) A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants. P Natl Acad Sci USA 97:13678-13683. doi: 10.1073/pnas.240452097

Guimarães-Filho ZO, Caldas IL, Viana RL, Kurths J, Nascimento IC, Kuznetsov YK (2008) Recurrence quantification analysis of electrostatic fluctuations in fusion plasmas. Phys Lett A 372:1088-1095. doi: 10.1016/j.physleta.2007.07.088

Hölldobler B, Wilson EO (1990) The ants. Harvard, MA: The Belknap Press of Harvard University Press.

Holyst JA, Zebrowska M, Urbanowicz K (2001) Observation of deterministic chaos in financial time series by Recurrence Plots, can one control chaotic economy? Eur Phys J B 20:531-535. doi: 10.1007/PL00011109

Lattke JE (1990) Revisión del género *Gnamptogenys* Roger en Venezuela (Hymenoptera: Formicidae). Acta Terramaris 2:1-47

Lilliefors H (1967) On the Kolmogorov–Smirnov test for normality with mean and variance unknown. J Am Stat Assoc 62:399-402. doi: 10.1080/01621459.1967.10482916

Lommelen E, Johnson CA, Drijfhout FP, Billen J, Wenseleers T, Gobin B (2006) Cuticular hydrocarbons provide reliable cues of fertility in the ant *Gnamptogenys striatula*. J Chem Ecol 32:2023-2034. doi: 10.1007/s10886-006-9126-8

Lone SR, Sharma VK (2011) Timekeeping through social contacts: Social synchronization of circadian locomotor activity rhythm in the carpenter ant *Camponotus paria*. Chronobiol Int 28:862-872. doi*:* 10.3109/07420528.2011.622676

Kennel MB, Brown R, Abarbanel HDI (1992) Determining embedding dimension for phase-space reconstruction using a geometrical construction. Phys Rev A 45:3403 (1992). doi: 10.1103/PhysRevA.45.3403

Korb J, Heinze J (2004) Multilevel selection and social evolution of insect societies. Naturwissenschaften 91:291-304. doi: 10.1007/s00114-004-0529-5

Kruskal WH (1952) Use of ranks in one-criterion variance analysis. J Am Stat Assoc 47:583-621. doi: 10.1080/01621459.1952.10483441

Marwan N, Thiel M, Nowaczyk NR (2002) Cross Recurrence Plot Based Synchronization of Time Series. Nonlinear Proc Geoph 9:325-331. doi: 10.5194/npg-9-325-2002

Marwan N, Trauth MH, Vuille M, Kurths J (2003) Comparing modern and Pleistocene ENSO-like influences in NW Argentina using nonlinear time series analysis methods. Clim Dynam 21:317-326. doi: 10.1007/s00382-003-0335-3

Marwan N, Kurths J (2005) Line structures in recurrence plots. Phys Lett A 336:349-357. doi: 10.1016/j.physleta.2004.12.056

Marwan N**,** Romano MC, Thiel M, Kurths J (2007) Recurrence Plots for the Analysis of Complex Systems. Phys Rep 438:237-329. doi: 10.1016/j.physrep.2006.11.001

Marwan N, Donges JF, Zou Y, Donner RV, Kurths J (2009) Complex network approach for recurrence analysis of time series. Phys Lett A 373:4246-4254. doi: 10.1016/j.physleta.2009.09.042

Marwan N (2011) How to avoid potential pitfalls in recurrence plot based data analysis. Int J Bifurcat Chaos 21:1003-1017. doi: 10.1142/s0218127411029008

Mann HB, Whitney DR (1947) On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. Ann Math Stat 18:50-60. doi: 10.1214/aoms/1177730491

MATLAB version 7.10.0 (2010) The MathWorks Inc, Natick, Massachusetts

Miramontes O (1992) Complexity and Behaviour in Leptothorax Ants. Master Thesis, Open University, UK

Neves FM, Pie MR, Viana RL (2012) Self-organization in the movement activity of social insects (Hymenoptera: Formicidae), AIP Conf Proc 1479:658-661. doi: 10.1063/1.4756220

Nicolis SC, Deneubourg JL (1999) Emerging patterns and food recruitment in ants: an analytical study. J Theor Biol 198:575-592. doi: 10.1006/jtbi.1999.0934

Öster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton.

Pie MR, Traniello JFA (2007) Morphological evolution in a hyperdiverse clade: the ant genus *Pheidole*. J Zool 271(1):99-109 doi: 10.1111/j.1469-7998.2006.00239.x

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (url:http://www.R-project.org/)

Riley MA, Balasubramaniam R, Turvey MT (1999) Recurrence quantification analysis of postural fluctuations. Gait Posture 9:65-78. doi: 10.1007/s10439-013-0790-x

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos T R Soc B 365(1560):4051-4063. doi: 10.1098/rstb.2010.0208

Reid CR, Sumpter DJT, Beekman M (2011) Optimisation in a natural system: Argentine ants solve the Towers of Hanoi. J Exp Biol 214:50-58. doi: 10.1242/jeb.048173

Solé RV, Miramontes O, Goodwin BC (1993) Oscillations and chaos in ant societies. J Theor Biol 161: 343-357. doi: 10.1006/jtbi.1993.1060

Sweeney K, Cusack B, Armagost F, O'Brien T, Keiser CN, Pruitt JN (2013) Predator and prey activity levels jointly influence the outcome of long-term foraging bouts. Behav Ecol 24:1205-1210. doi: 10.1093/beheco/art052

Theiler J, Eubank S, Longtin A, Galdrikian B, Farmer JD (1992) Testing for nonlinearity in time series: the method of surrogate data. Physica D 58(1-4):77-94. doi: 10.1016/0167-2789(92)90102-S

Webber Jr. CL, Zbilut JP (1994) Dynamical assessment of physiological systems and states using recurrence plot strategies. J Appl Physiol 76:965-973

Webber Jr. CL, Marwan N, Facchini A, Giuliani A (2009) Simpler methods do it better: Success of Recurrence Quantification Analysis as a general purpose data analysis tool. Phys Lett A 3753:3756. doi: 10.1016/j.physleta.2009.08.052

Wild AL (2007) Taxonomic revision of the ant genus *Linepithema* (Hymenoptera: Formicidae). Univ of California Press, 126

Wild AL (2009) Evolution of the Neotropical ant genus *Linepithema*. Syst Entomol 34:49-62. doi: 10.1111/j.1365-3113.2008.00435

Wilson EO (1985) The sociogenesis of insect colonies. Science 228:1489-1495. doi: 10.1126/science.228.4707.1489

Wilson EO (2003) *Pheidole* in the New World: a dominant, hyperdiverse ant genus. Harvard University Press, Cambridge, MA

Zbilut JP (1992) Embeddings and delays as derived from quantification of recurrence plots. Phys Lett A 171:199-203. doi: 10.1016/0375-9601(92)90426-M

CAPÍTULO II

On the behavior of the sawfly *Perreyia flavipes* Konow, 1899 (Hymenoptera: Pergidae)

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On the behavior of the sawfly *Perreyia flavipes* Konow, 1899 (Hymenoptera: Pergidae)

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Abstract *Perreyia flavipes* Konow, 1899 (Pergidae: Perreyinae) is a sawfly species with gregarious larvae commonly found in open areas in Southern Brazil through Uruguay and northern Argentina. The combination of highly gregarious larvae with the production of a variety of toxins in *P. flavipes* has led to severe cases of intoxication in a variety of livestock species. Along the years, considerable information was discovered on the larval natural history of *P. flavipes*, however, virtually nothing is known about the adult behavior, particularly because of its short lifespan. In this study, we report on the first extensive study on the adult behavior of *P. flavipes*, including movement, mating behavior, oviposition and thanatosis. Our results show some unusual behavioral adaptations presented by *P. flavipes*, such as irregular activity behavior (i.e. deficient gait pattern), thanatosis behavior-like display and primitive maternal care. Individual behavioral acts are described and compared among sexes, and their potential functions are discussed.

Keywords: behavioral repertoire, natural history, sawflies, thanatosis.

Introduction

The family Pergidae comprises about 500 sawfly species in 14 subfamilies and 57 genera (Smith 1993), with the majority of these being restricted to South and Central America (Smith 1990). *Perreyia flavipes* Konow, 1899 has been reported to occur in Argentina, Brazil and Uruguay (Smith 1990; Dutra et al. 1997). The larvae of *P. flavipes* are gregarious and strongly poisonous, with common reports of livestock death due to ingestion during larval outbreaks, affecting cattle (Dadswell et al. 1985; Dutra et al. 1997; Soares et al. 2008; Raymundo et al. 2009), sheep (Dutra et al. 1997; Raymundo et al. 2008) and pigs (Riet-Correa et al. 1998; Soares et al. 2001; Jonck et al. 2010). At least 40 outbreaks occurred in Uruguay from 1993 to 1995, with cattle losses in 1995 exceeding 1000 head (Dutra et al. 1997). Mortality rates following ingestion vary from 0.8 % to 33% (e.g. Dutra et al. 1997; Soares et al. 2008; Jonck et al. 2010). Recent studies on *P. flavipes* demonstrated that their main toxin is pergidin, a heptapectide containing a phosphoseryl residue, although small amounts of lophyrotomin are also present (Oelrichs et al. 1999; Boevé et al. 2014). It has been hypothesized that the function of these peptides is defense against natural enemies, with the poisoning of livestock being an epiphenomenon. Poisoning of pigs is so common in southern Brazil that the larvae of *P. flavipes* (and also probably *Perreyia lepida*) are commonly dubbed “pig killers” (“mata-porcos” in Portuguese) (Costa 1941). They have also been named “bicho-da-chuva” (rain grub) (Camargo 1955; Soares et al. 2008).

During the winter season, from June to September, aggregates of *P. flavipes* larvae are commonly found in open grasslands, forming an orderly moving column of approximately 100 individuals (varying from six individuals to nearly 200), particularly after rainfalls and on cloudy days (Soares et al. 2001). In particular, these larvae form small closely packed masses on the ground and display group living, as in the case of other pergid species such as *Lophyrotoma interrupta* and *Perreyia lepida* from Australia and South America, respectively (Dadswell et al. 1985; Costa 2006). Larvae are seen feeding both on young leaves and senescent vegetation from different grass species and may also be fungivorous, as in *Perreyia tropica* (Flores et al. 2000; Soares et al. 2001). Pupation occurs in the soil, 3-10 cm below the surface, between late August and September (Soares et al. 2001; Dutra 2003). Cocoons are constructed from the surrounding soil, forming a compact, black round shell. Cocoons are oblong in shape with a diameter of approximately 1 cm in length by 0.6 cm wide (Dutra 2003).

Adults of *P. flavipes* are very odd wasps with a short lifespan (< 72 h), emerging in February and March (Soares et al. 2001; Dutra 2003). They are easily differentiated from other *Perreyia* species due to a suite of morphological characteristics: brightly orange leg parts and 13-15 antennal articles, which are serrate in the female and pectinate in the male (Smith 1990). These adults do not feed. Females have little activity and fly only short distances, usually staying on the top of grasses or trying to reach a position above the ground level. Males are less robust and fly longer distances than the females. Eggs are deposited in the soil and laid in clusters of 100-700 eggs, and each egg is oblong and yellowish-white, although inseminated ones develop a blackish yellow color (Riet-Correa et al. 1998; Dutra 2003). The incubation period is limited to 4-8 weeks and larvae start to emerge in March or April and develop through the autumn and winter (Soares et al. 2001). Despite considerable information on the larval natural history of *P. flavipes*, virtually nothing is known about their adult behavior. In this study we report on a series of observations on the behavior of adults of *P. flavipes*, including movement, oviposition, parental care and thanatosis.

Material and methods

Four groups of *P. flavipes* larvae (42 - 150 individuals) were collected in the campus of the Universidade Federal do Paraná, Curitiba, Paraná, Brazil (25°26'51.6"S and 49°14'09.8"W) between June 2014 and August 2014. Each group was maintained in the laboratory at separate polystyrene containers (26 x 33 x 11 cm) under a 12:12 h (L: D) photoperiod. A substrate consisting of soil, green and senescent grass leaves was placed within each container, which was kept under stable temperature (21 ± 2 °C) and relative humidity of 70% ± 0.5. The soil was kept humid and periodically replaced to prevent the accumulation of feces. Adults emerged in February and March of 2015 (between 9:00 am and 2:00 pm) and were individually transferred to a glass container (11.5 cm x 29 cm x 13 cm) with a white floor (Styrofoam^{TM}), water (watered piece of cotton), green, senescent leaves and healthy grasses. Also, a small wooden platform (60 mm) with humid soil was provided, environmental conditions (temperature and humidity) were maintained the same as the larvae phase. Adult specimens were identified using the taxonomic key provided by Smith (1990).

Preliminary observations were made of isolated adults and groups, and behavioral repertoires were created using the following categories: (1) Movement activity: Inactivity and activity; (2) Individual behavior: Grooming antenna, grooming abdomen, wing spreading and short flight; (3) Thanatosis: Induced and spontaneous thanatosis; and (4) Mating behavior: Copulation and oviposition. Given that spontaneous thanatosis and mating behavior were rare (n < 10 observations), these behaviors were only described qualitatively. All trials started two hours after the emergence of adults, during daylight time (from 11:00 pm to 5:45 pm). A video recorder was used (Everio GZ-MG435BUB) to obtain behavioral data. Behaviors were analyzed in three different sets of trials. First, we recorded individually through focal scan sampling (n = 15 individuals), including both males (n = 9 individuals) and females (n = 6 individuals). Each adult was observed by 1 hour in an interval of 30 seconds (Movement activity) and 10 seconds (Individual behavior) (n = 361 behaviors). During the trials, individuals were placed in Petri dishes (89 mm x 16 mm) with daytime illumination by two LED lightbulbs (≈ 800 lux). Second, we recorded groups of five individuals by scan sampling, with at least one male or female (n = 4). The adults were observed for a total of 8 hours in each trial to describe spontaneous thanatosis, mating and oviposition behavior. Individuals were maintained within the initial glass container. Observations were under natural light conditions. Finally, in order to describe and observe the duration of induced thanatosis in *P. flavipes*, male (n = 3) and female (n = 3) individuals were isolated and mechanically stimulated with a delicate small paintbrush for five seconds every three minutes (n = 60 behaviors). Individuals were placed in Petri dishes (89 mm x 16 mm) with daylight illumination by two LED light bulbs (≈ 800 lux). During all the three set of trials, a period of acclimation was provided for 35 minutes prior to observations. Standard parametric (Two-sample t-test) and non-parametric (G-test goodness of fit) methods were used for the analysis of data, and mean and standard variations were provided when necessary. G-test goodness of fit was used to compare the frequency of behaviors obtained with a hypothetical uniform discrete distribution of the data. Two-sample t-test was used to compare means between males and females in continuous data. Statistical analyses were performed using R 3.2.1 (R Core Team 2015). Detailed descriptions of behavioral acts were made when necessary to explain some unusual behavior.

Results

Data from all individual behavior repertoires resulted in 5.415 recorded behavioral acts. Movement behavior of males and females alternated between activity and inactivity (Table 1), with activity being more common (G = 10.53, df = 1, $p < 0.01$). In addition, when female and male activity levels were considered separately, both were more active than inactive (G = 0.17, df = 1, $p < 0.67$). Interestingly, *P. flavipes* displayed an odd walking behavior with leg extremities (tarsi) being less rigid, sometimes turning the body upside down due to unsynchronized leg movement.

Table 1 Focal scan results of movement behavior of *Perreyia flavipes* adults, males and females. Inactivity (In) and Activity (Ac) were considered

Behavior	Male (n = 9)	Female (n = 6)	Total (n = 15)
In	433 (38.5%)	232 (31%)	665 (52%)
Ac	692* (61.5%)	519* (69%)	1211* (48%)
Total	1.125	751	1.876

Items considered statistically significant ($p < 0.05$) were marked by an asterisk (*). The n value of each item is accompanied by the correspondent percentage (%)

Grooming behavior was performed by the fore and hind pair of legs. The wasp rubs the forelegs between the antennal segments (mean duration in s ± s.d: 12 ± 10) or rubs the hind legs in the abdomen, sometimes above the wings (mean duration in s ± s.d: 14 ± 6.52). Both grooming behaviors studied here were more common than other individual behaviors (Table 2) (G = 88, df = 3, $p < 0.01$). However, males were more prone to perform grooming of the antennal segments than females (G = 154, df = 3, $p < 0.01$). The behavioral act of wing spreading was defined by the act of the female or male to spread its wings voluntarily (2 s ± 1). Also, some males during the individual behavioral trials did a short flight for some seconds (2 s ± 1) with a frenetic movement of the wings, whereas females did not display this behavior. Both wing spreading and short flights behaviors were very rare (Table 2).

Table 2 Focal scan results of *Perreyia flavipes* adults, males and females. Grooming antenna (Ga), grooming abdomen (Gb), wing spreading (Ws) and short flight (Sf) were considered

Behavior	Male (*n* = 9)	Female (*n* = 6)	Total (*n* =15)
Ga	86* (52.1%)	38* (48.1%)	124* (50.8%)
Gb	68 (41.2%)	38* (48.1%)	106* (43.4%)
Ws	9 (5.5%)	3 (3.8%)	12 (5%)
Sf	2 (1.2%)	0	2 (0.8%)
Total	165	79	244

Items considered statistically significant ($p < 0.05$) were marked by an asterisk (*). The *n* value of each item is accompanied by the correspondent percentage (%)

Mating Behavior

In the laboratory, adults seem to seek higher places within the glass container, although all mating behavior was observed at the ground level (*n* = 6). First, the male (see Fig. 1b), approaches the female (Fig. 1a), touching its body with the legs and then climbing on her back. The male then grabs the female using the hind pair of legs (Fig. 2a). In response, the female stays immobile during the whole process, while the male approximates its abdominal apex to the female abdominal sternum IX. Male and female turn back-to-back 180° and join in copula (Fig. 2b). While in copula, the male abdominal apex is placed below the female, sometimes its hind legs remain on the female’s wings (*n* = 2 events). The end of the copulation is initiated either by the female or the male, with one of them rotating its body and completely loosing the contact with the genitalia of the other individual and then walking away (Fig. 2c). The immobile female starts to move a few seconds after the male stopped copulating. The mating behavior lasts for approximately four minutes (mean duration in min ± s.d: 4.25 ± 0.10).

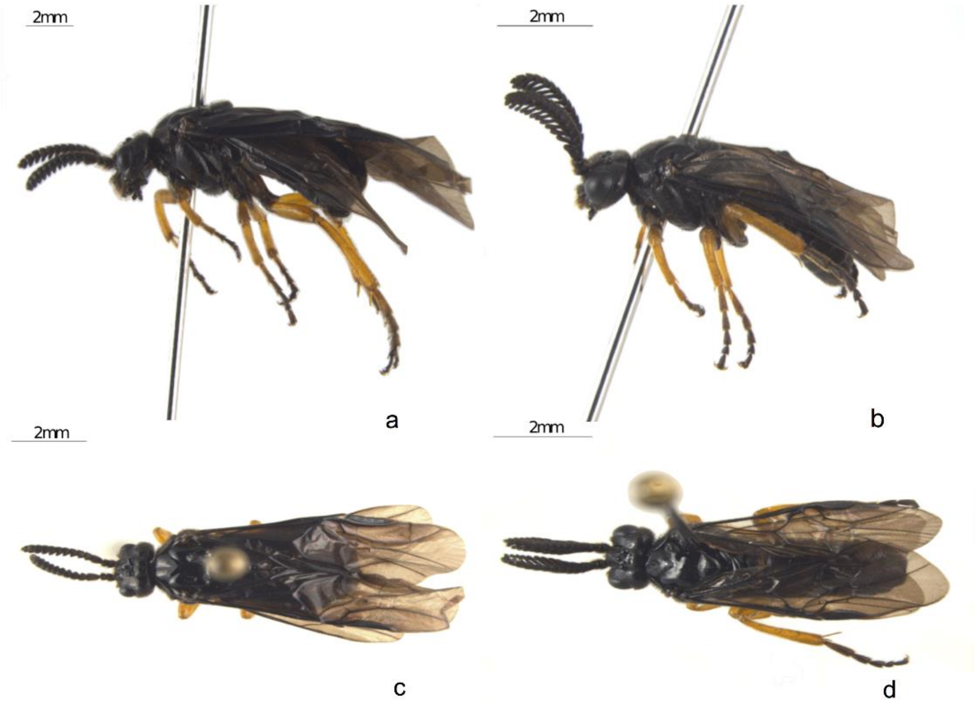

Fig. 1 Dorsal and lateral view of the female (a, c) and male (b, d) of *Perreyia flavipes*

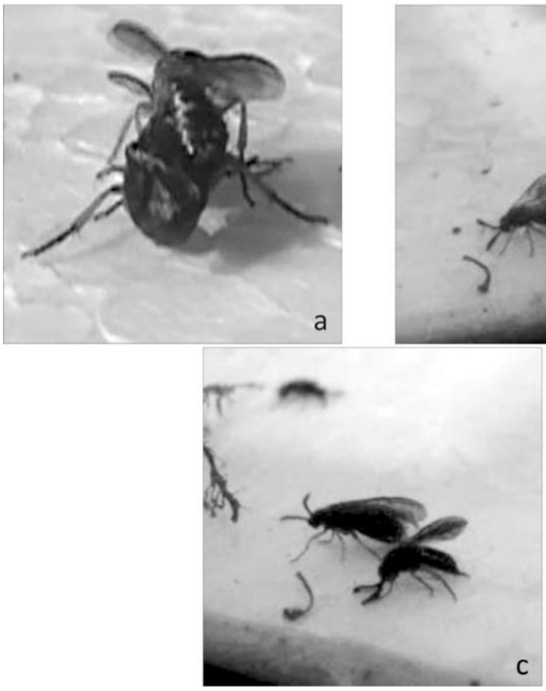

Fig. 2 Mating behavior in *Perreyia flavipes*. The male grabs the female using the hind pair of legs (a). Male and female turn back-to-back 180° and join in copula (b). The end

of the copula is initiated either by the female or the male, with one of them rotating its body and completely loosing the contact with the genitalia of the other one, walking away (c)

Egg laying *P. flavipes* begins by adopting either of two different body positions. In one of them, the body stays in an upward, curved position, with the abdomen elevated and the abdominal apex in contact with the substrate (Fig. 3a). The oviposition lasts at least two hours (*n* = 10) (mean duration in min ± s.d: 120 ± 0.20). Alternatively, the female can remain with her body upside down (Fig. 3b), curving the abdomen upwards. Twelve females, including six that had not mated, deposited eggs. Interestingly, in at least some ovipositions (*n* = 2), females manipulated the batch of eggs, touching it with the first and second pair of legs or all of them (Fig. 3). The eggs are encapsulated with an unknown substance, in a single cluster. Sometimes, watered cotton attracted *P. flavipes* for oviposition (*n* = 2). Afterwards, the female walks away from the masses of eggs and stays motionless, either dying immediately (*n* = 7) or after a few hours (*n* = 3).

Fig. 3 Female body positions during the oviposition in *Perreyia flavipes*. The female stay with the body upside down and a curved abdomen (a) or with the body in a bent-like

position, the abdomen elevated and the last abdominal tergae bending in contact with the substrate (b).

Thanatosis

Thanatosis behavior in *P. flavipes* is characterized by the complete immobility and retracting of body appendages for a few seconds (mean ± s.d: 52 s ± 18.3). The behavior was observed during mechanical stimulation (*n* = 30 events) and social interactions (*n* = 9 events). The duration of thanatosis by mechanical stimulation is longer in males (mean ± s.d: 70 s ± 10.1) than females (mean ± s.d: 22 s ± 9.9) ($t = 15.8$, df = 58, $p < 0.01$). Spontaneous thanatosis happens when one individual meets another and touches it first during walking behavior, only when the first to touch it walked away, the other one started to walk again. The immobility behavior of some females during the mating also could be considered thanatosis-like (*n* = 2), females became immobile after the first contact of the male, remaining in this same state until the end of the copulation.

Discussion

The behavioral acts of *P. flavipes* were few and simple, a lack of elaborate behavioral acts that is expected over the course of such short adult lifespan. However, several behavioral adaptations presented by *P. flavipes* could be considered unusual. The movement activity of *P. flavipes* is limited by inactivity (quiescence) and activity, being the latter more common during the behavioral displays of the adults. One could expect a more limited energetic expenditure due to an absence of energy resupply (*P. flavipes* do not feed during adult phase). The energetic cost could be very severe, particularly because *P. flavipes* has a very deficient gait behavior. The possible explanation could be due to depleted energy supplies by the constant strive to find suitable partners and better mating positions in higher grasses. Legged animals change their gait pattern, such as walk, trot, and gallop, according to locomotion speed, which suggests that an optimal gait pattern is selected so as to minimize the energetic cost of locomotion (Nishii 2000). Deficient gait pattern could be related to limited energy supplies or an extreme reduced energetic cost. Either way, more studies are necessary to understand this process.

Grooming behavior was common in *P. flavipes*. Grooming behavior in insects is usually associated with body maintenance (removal of pathogens or parasites) and homogenization of chemical cues. Cuticular hydrocarbons are known to have a major function in desiccation resistance (Hadley 1981), but are used as sex pheromones in many insects taxa, especially among Diptera (Howard 1993; Singer 1998). As cuticular hydrocarbons are relatively non-volatile, they would not be expected to automatically disperse over the entire body of an individual. Grooming has been considered a means to transfer these hydrocarbons over their bodies (Ayasse et al. 2001). In social insects, there are similarities between the cuticular hydrocarbon composition and the hydrocarbons secreted by the Dufour's gland (i.e. accessory gland of the female reproductive tract) (Dani et al. 1996, 2001). In andrenid bees, species-specific blends of cuticular hydrocarbons serve as sex pheromones attracting males (Schiestl and Ayasse 2000, 2001). The appendages used to groom body segments in *P. flavipes* were specific to those regions: the head, antennae and thorax were groomed by the fore (prothoracic) legs, while the abdomen was exclusively groomed by the hind (metathoracic) legs. Thus, cues on the antenna do not travel to the abdomen or from the abdomen to antenna. This finding raises questions regarding the non-similarity of the hydrocarbons present on the different parts of the wasps, such as the suggested in *Polistes dominulus* (Sumana and Starks 2004). Males compared to females exhibit more antennae grooming in *P. flavipes*, this could be linked with sensorial reception of chemical signals, such as sexual pheromones. For instance, antenna grooming enhances olfactory acuity in insects (Böröczky et al. 2013). Grooming in *P. flavipes* may be an active role in sexual attraction and signaling.

The mating behavior observed in *Perreyia flavipes* is similar to other tenthredinoid species (e.g. Gordh 1975; Avila-Núñez et al. 2007) and to the previous descriptions made by Dutra (2003). This kind of mating has been called "strophandrous" and is exhibited by males of species that have developed the ability of rotating their genitalia in a 180º angle over its middle axis. Copula in other males that do not show this genital torsion is known as "orthandrous" (Gordh 1975). Oviposition is a poorly studied process in Pergidae, which has led authors to suggest that other modes of reproduction predominate within the family, such as parthenogenesis (Carene 1962; Macdonald and Ohmart 1993). Despite the fact that some Tenthredinoidea species present parthenogenesis, there is no clear evidence if parthenogenesis is even one prevalent mode of reproduction in this group. *Perreya flavipes* did oviposition without mating, but parthenogenesis was not observed. Soares et al. (2001) reported that none of the eggs from unmated females hatched. *Perreya flavipes* exhibits a transient maternal care behavior, with some females manipulating the eggs with legs, forming a single compact mass together with the gelatinous substance expelled during the oviposition. In addition, Dutra (2003) observed that *P. flavipes* sometimes dig in the soil to oviposit. Due to the short adult lifespan of *P. flavipes*, maternal care is restricted to the egg stage in this species and only for a few hours. Larger egg clusters may have higher mortality in the egg stage (Hassell 1978). However, as in other sawflies, larval survival can be enhanced through effects originated from aggregation, such as improvement of feeding (e.g. Ghent 1960; Came 1966), protection against the environment (Seymour 1974) and particularly to *P. flavipes*, aggregation could also enhance defense against natural enemies by amplification of the inherent toxicity of individuals. Therefore, the combination of limited maternal care and the benefits of larval aggregation presented by the species, may promoted females to oviposit one large cluster of eggs rather than several small scattered clusters.

There have been no previous studies of thanatosis in Pergidae and it has not been well documented in Hymenoptera. For instance, a list of insect taxa exhibiting thanatosis does not include Hymenoptera (Miyatake 2001a), and a book on parasitoids does not mention thanatosis (Godfray 1994). In recent years, only King and Leaich (2006) studied in detail thanatosis in the species *Nasonia vitripennis*. This phenomenon also occurs in some other pteromalids, e.g., *Spalangia endius* and *S. cameroni* and in a bee (van Veen et al. 1999; King and Leaich 2006). Thanatosis is thought to serve as an antipredator function or an alternative to running away (Prohammer and Wade 1981; Miyatake 2001b). Thanatosis in *P. flavipes* appears to be limited to mechanical stimulation. Thanatosis was enhanced by two factors also affecting other insects groups, mechanical stimulation and falling from higher places (e.g. Holmes 1906; King and Leaich 2006). The response to mechanical stimulation was longer in males than females, males were more inactive than females in general. One different aspect of thanatosis in *P. flavipes* involves social interactions. Individuals also exhibit thanatosis and thanatosis-like behavior when touching each other. Two situations demonstrated this kind of behavior in *P. flavipes*: activity behavior (e.g. moving) and mating. This is the first time that this sort of thanatosis behavior is noticed in animal behavior. The challenge provided by this particular species is to deal with a wide array of life history traits that require the application of appropriate techniques in a small frame of time (i.e. short life span of adults). Therefore, there is still a considerable lack of basic behavioral and ecological data in the literature. Here, we seek to better understand the natural history of *P. flavipes* by using techniques to obtain behavioral data in the laboratory and we hope this study provides insight into the natural history of such intriguing but poorly studied species of neotropical sawfly.

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Bibliography

Avila-Núñez JL, Daniel OL, Silmi S, Calcagno-Pisarelli MP (2007) Life history of *Aneugmenus merida* Smith (Hymenoptera: Tenthredinidae) in the Venezuelan Andes. Neotrop Entomol 36:22–27

Ayasse M, Paxton RJ, Tengo J (2001) Mating behavior and chemical communication in the order Hymenoptera. Annu Rev Entomol 46:31–78

Boevé J-L, Rozenberg R, Shinohara A, Schmidt S (2014) Toxic Peptides Occur Frequently in Pergid and Argid Sawfly Larvae. PLoS One 9(8):e105301. doi:10.1371/journal.pone.0105301

Böröczky K, Wada-Katsumata A, Batchelor D, Zhukovskaya M, Schal C (2013) Insects groom their antennae to enhance olfactory acuity. P Natl A Sci USA 110:3615–3620

Camargo OR (1955) Contribuição ao estudo do Tenthredinideo Mata Porcos, *Paraperreyia dorsuaria* (Know, 1899), no Rio Grande do Sul. Dissertation, Universidade Federal do Rio Grande do Sul

Carene PB (1962) The characteristics and behavior of the Saw-Fly *Perga affinis affinis* (Hymenoptera). Aust J Zool 10:1–34

Came PB (1966) Primitive forms of social behaviour, and their significance in the ecology of gregarious insects. Proc Ecol Soc Aust 1:75-78

Costa LA (1941) Bichos “Mata Porcos”. Chácaras e quintais 63:43

Costa JT (2006) Hymenoptera: Symphita In: Costa JT (ed) The other insect societies. Belknap Press of Harvard University Press, Cambridge, pp 631-653

Dadswell LP, Abott WD, McKenzie RA (1985) The occurrence, cost and control of sawfly larval (*Lophyrotoma interrupta*) poisoning of cattle in Queensland 1972-81. Aust Vet J 62:94–97

Dutra F, Riet-Correa F, Mendez MC, Paiva N (1997) Sawfly larval poisoning in cattle and sheep in Uruguay. Vet Hum Toxicol 39:281–286

Dutra F (2003) Intoxicación por larvas de *Perreyia flavipes* en bovinos y ovinos, caracterización de la enfermedad y biología del insecto. Veterinaria 38:7–24

Flores C, Ugalde-Gómez J, Hanson-Snortum P, Gauld I, Austin AD (2000) The biology of perreyiine sawflies (Hymenoptera: Pergidae) of the *Perreyia* genus-group. In: Austin AD, Dowton M (eds) Hymenoptera: evolution, biodiversity and biological control. CSIRO Publishing, Collingwood, pp 258-266

Ghent AW (1960) A study of the group-feeding behaviour of larvae of the jack pine sawfly, *Neodiprion pratii banksianae* Roh. Behaviour 16:110-148.

Godfray HCJ (1994) Parasitoids. Princeton University Press, Princeton, New Jersey

Gordh G (1975) Sexual behavior of *Hemitaxonous dubitatus* (Norton) (Hymenoptera: Tenthredinidae). Entomol News 86:161–166

Hadley NF (1981) Cuticular lipids of terrestrial plants and arthropods: A comparison of their structure, composition, and waterproofing function. Biol Rev 56:23–47

Hassell MP (1978) The Dynamics of Arthropod Predator-Prey Systems, Princeton University Press, Princeton, New Jersey

Holmes J (1906) Death-feigning in *Ranatra*. J Comp Neurol Psycho 16:200–216

Howard RW (1993) Cuticular hydrocarbons and chemical communication. In: Stanley-Samuelson DW, Nelson DR (eds) Insect Lipids: Chemistry, Biochemistry and Biology.

University of Nebraska Press, Lincoln, pp 179–226

Jonck F, Casagrande RA, Froehlich DL, Ribeiro Junior DP, Gava A (2010) Intoxicação espontânea por larvas de *Perreyia flavipes* (Pergidae) em suínos no estado de Santa Catarina. Pesquisa Vet Brasil 30:1017–1020

King BH, Leaich HR (2006) Variation in propensity to exhibit thanatosis in *Nasonia vitripennis* (Hymenoptera: Pteromalidae). J Insect Behav 19: 241–249

Macdonald J, Ohmart CP (1993) Life history strategies of Australian pergid sawflies and their interactions with host plants. In: Wagner MR, Raffa KF (eds) Sawfly Life History Adaptations to Woody Plants. Academic Press Inc., San Diego, pp 485–502

Miyatake T (2001a) Effects of starvation on death-feigning in adults of *Cylas formicarius* (Coleoptera: Brentidae). Ann Entomol Soc Am 94:612–616

Miyatake T (2001b) Diurnal periodicity of death-feigning in *Cylas formicarius* (Coleoptera:Brentidae). J Insect Behav 14:421–431

Nishii J (2000) Legged insects select the optimal locomotor pattern based on the energetic cost. Biol cybern 83:435–442

Oelrichs PB, MacLeod JK, Seawright AA, Moore MR, Ng JC, Dutra F, Riet-Correa F, Mendes MC, Thamsborg SM (1999) Unique toxic peptides isolated from sawfly larvae in three continents. Toxicon 37:537–544

Thamsborg SM (1999) Unique toxic peptides isolated from sawfly larvae in three continents. Toxicon 37:537–544

Prohammer LA, Wade MJ (1981) Geographic and genetic variation in death-feigning behavior in the flour beetle, *Tribolium castaneum*. Behav Genet 11:395–401

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https:www.R-project.org/

Raymundo DL, Bezerra Jr PS, Bandarra PM, Pedroso PMO, Oliveira EC, Pescador CA, Driemeier D (2008) Spontaneous poisoning by larvae of *Perreyia flavipes* (Pergidae) in sheep. Pesquisa Vet Brasil 28:19–22

Raymundo DL, Bezerra Junior PS, Bandarra PM, Santos AS, Sonne L, Pavarini SP, Côrrea AMR, Dias MM, Driemeier D (2009) Intoxicação espontânea pelas larvas de *Perreyia flavipes* em bovinos no Estado de Santa Catarina, Brasil. Ciência Rural 39:163–166

Riet-Correa F, Dutra F, Soares MP, Mendez MC (1998) Sawfly (*Perreyia flavipes*) larval poisoning of cattle, sheep and swine. In: Garland T, Barr AC (eds) Toxic Plants and Other

Natural Toxicants, CAB International Press, pp 291–296

Schiestl FP, Ayasse M (2000) Post-mating odor in females of the solitary bee, *Andrena nigroaenea* (Apoidea, Andrenidae), inhibits male mating behavior. Behav Ecol Sociobiol 48:303–307

Schiestl FP, Ayasse M (2001) Post-pollination production of a repellent compound in a sexually deceptive orchid: a new mechanism for maximizing reproductive success? Oecologia 126:531–534

Seymour RS (1974) Convective and evaporative cooling in sawfly larvae. J Insect Physiol 20:2447-2457.

Singer TL (1998) Roles of hydrocarbons in the recognition systems of insects. Am Zool 38:394–405

Smith DR (1990) A synopsis of the sawflies (Hymenoptera, Symphyta) of the America South of the United States: Pergidae. Rev Bras Ent 34:7–200

Smith DR, Wagner MR, Raffa KF (1993) Systematics, life history, and distribution of sawflies. Sawfly life history adaptations to woody plants. In: Wagner MR, Raffa KF (eds) Sawfly life history adaptations to woody plants. Academic Press Inc., San Diego, pp. 3–32

Soares MP, Riet-Correa F, Smith DR, Pereira Soares M, Mendez MC, Brandolt AL (2001) Experimental intoxication by larvae of *Perreyia flavipes* Know, 1899 (Hymenoptera: Pergidae) in pigs and some aspects on its biology. Toxicon 39:669–678

Soares MP, Quevedo OS, Schild AL (2008) Intoxicação por larvas de *Perreyia flavipes* Konow, 1899 (Hymenoptera: Pergidae) em bovinos na região sul do Rio Grande do Sul. Pesquisa Vet Brasil 28:169–173

Sumana A, Starks PT (2004) Grooming patterns in the primitively eusocial wasp *Polistes dominulus*. Ethology 110:825–833

van Veen JW, Sommeijer MJ, Aguilar MI (1999) Behavioural development and abdomen inflation of gynes and newly mated queens of *Melipona beecheii* (Apidae, Meliponinae). Insect Soc 46: 361–365

CAPÍTULO III

Wall following behavior in larvae of the sawfly *Perreyia flavipes*

(Hymenoptera: Pergidae)

Artigo preparado pelas normas da revista Journal of Theoretical Biology

Wall following behavior in larvae of the sawfly *Perreyia flavipes* (Hymenoptera: Pergidae)

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Abstract

The attraction of animals to walls is a phenomenon very well known in the study of animal behavior. Its impact in the spatial movement dynamics and how it works could bring interesting information about movement dynamics and the self-organization of social animals. *Perreyia flavipes* (Hymenoptera: Pergidae) is a sawfly species that presents aggregation behavior during its larval phase, sporadically moving (foraging behavior) or in a moveless aggregate, isolated individuals are found moving randomly in the field, along natural borders. We hypothesized that the role of boundaries in the movement of a high gregarious species such *P. flavipes* is fundamental for the onset of aggregation behavior, being present in its individual movement dynamics. Here, we use movement data of the species *P. flavipes* to understand individual movement behavior. First, we identify and quantify the behavioral distribution of the larvae in bounded spaces. Second, we build a statistical model of individual motion to verify if these behavioral rules can explain the spatial distribution of the individuals in an enclosed area. Our results indicate that the spatial distribution of *Perreyia flavipes* is affected by the presence of edges through thigmotaxis behavior, the movement behavior of the species suggest interesting properties with a power law distribution of intermittent behavior.

Key words: Data tracking, movement, activity, foraging, behavior.

Introduction

Of all possible physical influences driving the formation of spatial patterns in animal behavior, a well known mechanism is the reliance on spatial heterogeneity (Jeanson et al., 2003). Even with simple heterogeneities, such as hard boundaries, animal displacement along a confined space might exhibit thigmotactic behavior (from the Greek *thigma*=touch*; taxis*=movement) (Fraenkel and Gunn, 1961). In natural environments, boundaries may separate microhabitats, given the structural changes created by rocks, crest lines or grooves (Jander and Daumer, 1974; Klotz and Reid, 1992, 1993; Klotz et al., 2000). Boundaries may also separate habitats and microhabitats (Fagan et al. 1999; Ovaskainen and Cornell, 2003). In addition, interactions between individuals can be influenced by spatial structures, as in the case of wall-following behavior leading to an increase the probability of encountering conspecifics close to the edges. Aggregation behavior is more likely closer to the edges of an arena in the heteropteran *Dysdercus cingulatus* (Farine and Lobreau, 1984). The same is true for the schooling behavior when fishes are introduced in a water tank (Suzuki et al., 2003), the spatial distribution of cockroaches (Jeanson et al., 2003, 2005), and in the influence of trail foraging along boundaries on the behavior of ants (Dussotour et al., 2005). In this study we investigate the influence of spatial heterogeneity on the movement of the South American sawfly *Perreyia flavipes* (Hymenoptera: Pergidae).

This species is characterized by a strong tendency for aggregation behavior in its larval phase. During the winter season, from June to September, clusters of *P. flavipes* larvae are commonly found in open grasslands, forming an orderly moving column of approximately 100 individuals (varying from six individuals to nearly 200), particularly after rainfalls and on cloudy days (Soares et al., 2001, Chapter II). Such collective behavior involving the orderly moving aggregation is known to occur only in few species from phylogenetically distinct insect lineages, including other sawflies (i.e. *Lophyrotoma interrupta* and *Perreyia lepida;* Dadswell, 1985; Costa, 2006) and caterpillars (*Malacosoma* spp.; Dussutour et al., 2007. Interestingly, when *P. flavipes* is not foraging, isolated individuals are commonly found in natural cracks and along natural boundaries, such as clear patches on grasslands (F.M.N., pers. obs.). One approach to understanding how group patterns emerge in this species is to investigate the behavior of individuals in relation to their local environment, without reference to the global structure (Gueron et al. 1996), given that self-organized behaviors can be able to produce clusters starting from a homogeneous distribution of animals in a uniform environment (Jeanson et al., 2005, Theraulaz et al., 2002 and Depickère et al., 2004).

Models of boundary-following behaviors in animals usually assume a switch between free-field movements (within two or three dimensions) and movement along a boundary (which is restricted to one dimension) (Jeanson et al., 2003). Under wall-following behavior, animals probably are not attracted toward physical heterogeneities by long-range stimuli (as it is the case in phototaxis or chemotaxis), but rather move randomly in the environment until establishing a physical contact with an obstacle that will guide their motion. We hypothesized that the role of boundaries in the movement of a high gregarious species such *P. flavipes* is fundamental for the onset of aggregation behavior, being present in its individual movement dynamics. Here, we use movement data of the species *P. flavipes* to understand individual movement behavior of the species under the influence of walls. First, we identify and quantify the behavioral distribution of the larvae in bounded spaces. Second, we build a mathematical model of individual motion to verify if these behavioral rules can explain the spatial distribution of the individuals in an enclosed area.

Methods and Results

Data collection

Experiments were performed with last instar larvae of *P. flavipes*. At this stage of development, the mean body length is approximately 10 mm and the body width is 2.5 mm. Three groups of the *P. flavipes* larvae (42 - 150 individuals) were collected in the campus of the Universidade Federal do Paraná, Curitiba, Paraná, Brazil (25°26'51.6"S, 49°14'09.8"W) between May and June of 2015. Each group was maintained in the laboratory within separate polystyrene containers (26 x 33 x 11 cm) under conditions of 12 h light: 12 h dark (LD 12:12). A substrate consisting of soil, green and senescent grass leaves was placed within each container, which was kept under stable temperature (21 ± 2 ^{o}C) and relative humidity of 70 % ± 0.5. The soil was kept humid and periodically replaced to prevent the accumulation of feces.

The trials began at least one week after the individuals were placed under the laboratory conditions to allow for their acclimatization. The experimental arena was circular with 13.3 cm in diameter and 1.8 cm in height, with a glass cover plate. Individuals were manually placed within the arena under controlled environmental conditions (21 ± 2 ℃ and 65 % ± 10 relative humidity). An individual was randomly chosen among the population of each group for the trials. After the end of each trial, the respective chosen individuals were separated from the main group for at least 24 h to avoid pseudoreplicates. The color of the arena floor was opaque white for better image contrast, consisting of a white paper above a rubber odorless substrate. After each trial, the arena was cleaned, washed in bleach (80%), dried and not used for at least five hours before a new experiment, and the arena floor was always changed. We made experiments in two different light conditions, one replicating daylight (880≈ lux; daylight) and other one darkness (300≈ lux; redlight). Both regimes were also made in agreement to the natural light of the day, with trials being made during the morning, afternoon and evening (9:00 AM to 10:00 PM). A total of 15 replicates were made for each light condition (N=30).

The behavior of the larvae was recorded with a high definition camera (Sony HDR CX220) placed above the arena. Each trial lasted for one hour (N=3.600 frames) with data interval of one second between frames. The paths were then digitized at a sample rate of one point every 1 s with an automatic video-tracking software (FIJI software, v. 1.90). The sampling rate was chosen so as to avoid both undersampling (which induces a loss of information due to low temporal resolution) and oversampling (which introduces some noise due to the wobbling movement of the larva). The paths were converted as a series of Cartesian coordinates and were characterized by a series of parameters.

Wall-following behavior of *P. flavipes*

When introduced into the arena, the larvae spent most of their time walking close to the edge, with their bodies dragging along the wall. The radial distribution of larvae during the experiments showed that the individuals tended to reach the periphery of the arena and stay in physical contact with the arena wall for more than 90% of their time (Figure 1). In our experiments, we considered that a larva displayed wall-following behavior when it was less than 10 mm from the boundaries. This corresponds to the minimal distance required for a larva to establish contact with the wall.

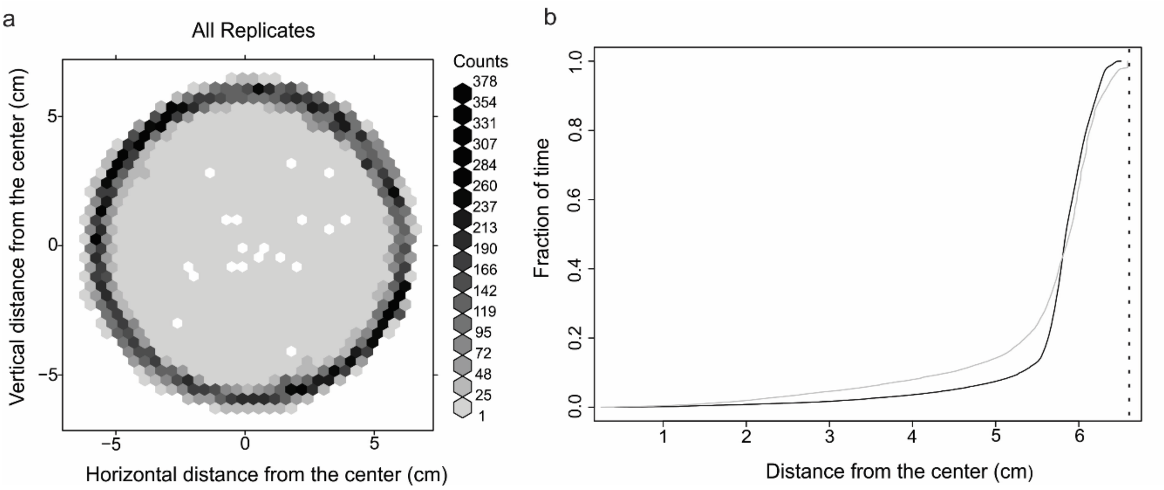

Figure 1. Radial distribution of the larvae along the surface of the circular arena. Distribution of Cartesian points of all the replicates (*n* = 30), the counts data are equivalent to a path of the larva along the arena (a), the graphic scale of paths is from white grey to black (0 to 378). Distance from the center of the arena of the individuals as a fraction of time (b), the black color line is equivalent to individuals of the diurnal and the grey color line is from the nocturnal trials, the dashed line represents the end of the arena.

We assumed that larvae remained inactive when two following conditions were fulfilled: (1) its displacement between two successive steps was less than 30 mm/s (the wobble movement of the larvae) and (2) the stop duration was at least 1 s (corresponding to one observation). The beginning of a path was determined by whenever a motionless larva started a new displacement, or when a moving larva stopped. A path ended each time a larva stopped or started to move. Since the displacement of the larvae into the central zone was uncommon (10 % in average), we disregarded the effects of the central displacement in our model. The movement behavior was uniform along the wall, individuals moved counter or clockwise. To determine whether the final distribution of individuals was influenced by spatial heterogeneity, phototaxis or residual chemical cues, we pooled the coordinates of all Individuals. The spatial distribution of individuals was uniform (random: Rao spacing test: 20 larvae, Z = 137.61, NS; Batschelet, 1981). We also observed if the individuals get tired over time, we plotted the inactivity and activity times of the individuals by a LOESS (locally weighted scatterplot smoothing) regression fit, there was no abrupt or larger differences among the intermittency between activity and inactivity (Appendix A). We conclude that the probability of one individual to stop or move was constant within the experiments.

In order to quantify the changing states between movement and inactivity we used a survival curve analysis (Haccou and Meelis 1992). A survival curve analysis consists of plotting on a log-linear or log-log scale the proportion of individuals that remained in a given state as a function of the time (or distance) elapsed since the beginning of this state. We observed the survival curve analysis for the moving time and stopping time of individuals. We tested whether the data fit a power-law distribution by plotting on a log-log scale, if the distribution follows a power law distribution, the distribution in a log-log scale should be represented by a straight line, which was confirmed (Figure 2) (Arnold 1983). Our survival curves suggest that the duration of the stops and movements was either short and frequent or very long and few. We therefore assumed that each larva could be in two separately states that control the duration of the stops and movements. This hypothesis is supported by behavioral observations. Thus, when a larva stops, it may remain either with low activity and display only body movements ("awake" state) or inactive without performing any movement (‘‘resting’’ state). The ‘‘awake’’ state is characterized by short stops (1 s ± 8) and the ‘‘resting’’ state by long stops (66 s ± 37.5). Similarly, during the movement behavior, the larvae have short and frequent movements and long and few ones. The short movement behavior ("transitional" state; 1 s ± 1.8) could be interpreted as a transitional phase between the start or the end of the movement state and the change of state to a stop state (long or short); long movements ("active" state; 61 s ± 79) were characterized by the non-stop displacement of the larvae in the arena. The probability $1/\tau$ per unit time of performing either a stop or a movement was given by the slope τ of the straight line fitting the log-log survival curve. Fifty per cent (*n* = 17718) of the paths ended with a stop and fifty per cent (*n* = 17854) with a move during the day. Thirty per cent (*n* = 10555) of the paths ended with a stop and 70 % (*n* = 24866) with a move during the night. Therefore, the probability p per unit time to stop (P_s) or to move (P_m) on the periphery was:

$$P_s = \frac{1}{\tau Stop,p},$$

$$P_m = \frac{1}{\tau Move,p},$$

We measure the angular difference θ_i of the position of the larvae at the beginning and the end of each path with respect to the center of the arena (arena radius: *r*) and its relative duration t_i. Taking into account all the paths collected for all individuals, the average speed (S) (separately, for day and night) was computed as follows:

$$S = \frac{\sum_{i=1}^{n} \Delta s}{\sum_{i=1}^{n} t_i},$$

Where Δs is the displacement, t_i is total time and n is the number of possible states (i.e. time steps over time) or the total of individuals. Using this formula, we found that the average velocity at the peripheral zone was 0.6 mm s^{-1} during the day and 0.4 mm s^{-1} during the night. In our model, we also incorporated the probability of turn behavior, we categorized the radians as binary when counter (1) or clock wise (0), the final result (x_i) was pooled for all the individuals. From the average number of x_i (ax_i), we extracted the turn rate value (TR) as follows:

$$TR = 1 - ax_i$$

The results for the turn rate of the day was 0.49 and for the night 0.33. All the statistical quantification of individual behaviors compiled from the movement behavior of *P. flavipes* were used in the modelling of the individuals. Therefore, the model parameters were defined and used in our simulations (Table 1).

Table 1. Movement parameters obtained for *Perreyia flavipes*.

Parameters	**Day (N = 35572)**	**Night (N = 35421)**
Mean speed (S)	0.66 mm s^{-1}	0.40 mm s^{-1}
Probability to stop (P_s)	0.24 s^{-1}	0.20 s^{-1}
Probability to move (P_m)	0.30 s^{-1}	0.30 s^{-1}
Turn rate (TR)	0.49	0.33

Description of numerical individual-based model

The spatially explicit numerical model was written in R software (v. 3.2.3) with help of the circular package (Agostineli and Lundi 2003). The model was based entirely on the experimental measures of individual behavior, its design was inspired in previous attempts to model wall following behavior (Jeanson et al., 2003; Jeanson et al., 2005). In this model, the larvae moved in only one dimension preserving time and spatial scales of experiments with time steps of 1 s/ cycle. We assumed that since the movements of individuals along the center of the arena were few and sporadic, they did not have influence on the dynamics of movement behavior presented by *P. flavipes*. The basic units in the model were individual larva that were characterized by their spatial location along the walls, speed, motion state (moving or stopped) and orientation (counter or clockwise; - or +). At the beginning of a simulation run, individuals were randomly initialized in a stopped or move state on an also randomly determined radian value along the π value (0 to 6.28), representing a point along the wall. Each larva then adjusted its behavior according to the behavioral rules derived from experiments (Table 1). We compared the survival curves of the data with the model by computer simulations (*n* =

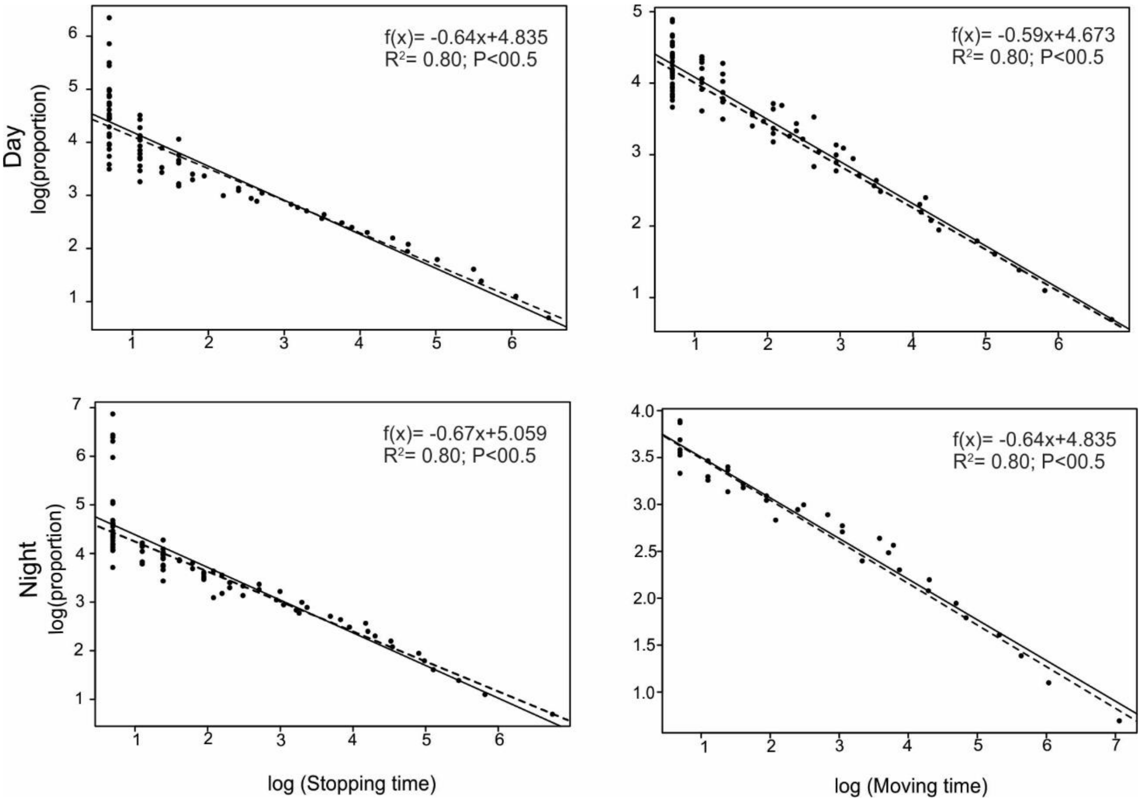

Figure 2. Survival curves of the stopping and moving times in a log-log scale from the diurnal (Day) and nocturnal (Night) trials, with the fitted regression line (solid) of the data and the model fitted regression line (dashed).

35572 run simulated steps for day and 35421 run simulated steps for night). The model successfully emulated the survival curve distribution of the empirical data (Figure 2).

Discussion

This study confirms that the spatial distribution of *Perreyia flavipes* is affected by the presence of edges through thigmotaxis behavior. We investigated how the movement displacement of *P. flavipes* works, extracting a series of parameters from its movement dynamics. In order to characterize the behavior of the species in a bounded space, we proposed a method of statistical modeling of individual motion. Compared to the standard procedures used to model animal wall-following behavior (Jeanson et al., 2003, Jeanson et al., 2005), our method is simpler and yet is able to demonstrate the observed dynamics.

In our experiments, the time that the individual spent in the contact with the wall was greater than that expected when calculated from the total surface of the circular arena. This result suggests that individuals exhibit an active tendency to walk along the walls. *Perreyia flavipes* has a stronger wall-following behavior, with more than 90% of the time staying close to the wall, than other empirical studies. For instance, cockroaches stay in contact with boundaries only 65% of the time within the arena periphery (Jeanson et al., 2003). In foraging ants, on the other hand, workers can be actively engaged in trail-following behavior, but nonetheless they exhibit a constant probability per unit of distance to leave the trail (Calenbuhr and Deneubourg, 1992). Differently, *P. flavipes* is known to be a gregarious species, with a constant physical contact among conspecifics. We suspect that the individuals of *P. flavipes* use natural boundaries more frequently to find other individuals to aggregate; this could be the main reason to explain the stronger attraction to boundaries compared to other species.

Perreyia flavipes also presented more activity and higher speed values during the day than the night trials. The behavior of the larvae of *P. flavipes* according to diurnal or nocturnal cycles of activity is unknown. Nonetheless, we observe in the field (n = 10, F.M.N., pers. obs.) that *P. flavipes* aggregations commonly moves during the day after rainfalls. During the night, individuals stay in scattered groups in the field, either stationary or at least under the same surface area. We assume that single individuals are not foraging when alone in the field, but looking for other individuals. An aggregation may form initially by random encounter and grow by density-dependent interactions (Parrish and Keshet 1999). Based on this assumption, and based in our results, *P. flavipes* appears to be prone to be more proactive to find conspecifics during the day. This behavior does not have higher influence in the intermittent movement behavior of the species, since the proportion of moving and stop times stay similar for both day and night regimes.

The frequency of intermittent walking behavior of the species follows a power law distribution. Power law distributions have mathematical properties that are common features of many complex systems and could be produced by endogenous processes, such as feedback loops, self-organization, and network effects (Maye et al., 2007). In general, this result is different from other instances of wall-following behavior, which can follow an exponential distribution (e.g. Jeanson 2012, Jeanson 2013). This suggests that individual behavior observed would not be influenced by larger time trials (disregarding the effects of lack of energy). The group movement behavior of *P. flavipes* could be additive; one key mechanism of self-organization in social systems is the existence of positive feedbacks (Bonabeau et al., 1997). Thus, observed movement patterns could be modified by step truncation and increased turning. Aggregation of individuals relies on the amplification of a dynamic signal provided by other individuals; more aggregated individuals provide a stronger tendency for aggregation. For instance, in the bark beetle, *Dendroctonus micans* (Deneubourg et al. 1990), or in the social amoebae *Dictyostelium discoideum* (Marée and Hogeweg, 2001), aggregates emerge from the attraction between individuals and amplification processes mediated by the production and diffusion of chemicals (aggregation pheromone and cAMP, respectively).

We analyzed only a fraction of the data extracted from our empirical experiments. The approach developed here might be extended to other experimental contexts such as the analysis of the collective behavior of highly thigmotactic animals confined to a finite space or within ecological contexts, such as the modeling of the foraging patterns of species. It is possible that the inclusion of a few more parameters in our model, derived from the data, such as a perception radius and the probability to stop or move in an aggregate, the emergence of aggregation on *P. flavipes* could be an easy process to describe. Aggregation patterns result from interactions between individuals that follow simple rules based on local information, without reference to the global pattern (Deneubourg et al., 2002). By this approach, we could verify the wall-following behavior of individuals of *P. flavipes*, its implication on the arising of self-organization of aggregates and movement dynamics within a power law distribution.

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References

Agostinelli C. and Lund. 2013. R package 'circular': Circular Statistics (version 0.4-7).

Arnold, B. C., 1983 Pareto Distributions. International Cooperative Publishing House, Fairland, Maryland.

Batschelet, E. 1981.Circular Statistics in Biology. New York: Academic Press.

Bonabeau, E., Theraulaz, G., Deneubourg, J.L., Aron, S. and Camazine, S. 1997. Self-organization in social insects. *Trends in Ecology and Evolution*, 12, 188–193.

Calenbuhr, V., Deneubourg, J.L., 1992. A model for osmotropic orientation (I). *J. Theor. Biol.* 158, 359–393.

Costa J.T., 2006 Hymenoptera: Symphita. In: Costa J. T., (ed) *The other insect societies*, pp. 631–653. Belknap Press of Harvard University Press, Cambridge.

Dadswell L.P., Abott W.D., McKenzie R.A., 1985 The occurrence, cost and control of sawfly larval (*Lophyrotoma interrupta*) poisoning of cattle in Queensland 1972-81. *Australian Veterinary Journal* **62**, 94–97.

Deneubourg J.L., Camazine S and Detrain C. 1990 Self-organization or individual complexity: a false dilemna or a true complementarity In: *Information processing in social insects*. Detrain C., Deneubourg J.L. et J.M. Pasteels (eds), Birkhauser Verlag, 401-408

Deneubourg, J. L., Lioni, A., Detrain, C. 2002 Dynamics of aggregation and emergence of cooperation. *The Biological Bulletin*, *202*(3), 262-267.

Dussutour, A., Deneubourg, J. L., and Fourcassié, V. 2005 Amplification of individual preferences in a social context: the case of wall-following in ants. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1564), 705-714.

Fagan, W. F., Cantrell R. S., and Cosner C. 1999. How habitat edges change species interactions. *The American Naturalist* **153:**165–182.

Farine, J. P. and Lobreau, J. P., 1984. Le gregarisme chez *Dysdercus cingulatus* Fabr. (Hateroptera:Pyrrhocoridae): nouvelle methode d'interpretation statistique. *Insectes Soc.* 3, 277–290.

Fraenkel, G. S. and Gunn, D. L. 1961. The Orientation of Animals: Kineses, Taxes and Compass Orientation. New York: Dover

Gueron, S., Levin, S. A., and Rubenstein, D. I. (1996). The dynamics of herds: from individuals to aggregations. *Journal of Theoretical Biology*, *182*(1), 85-98.

Haccou, P., and Meelis, E. 1992. Statistical analysis of behavioural data: An approach based on time structured models. Oxford, UK: Oxford University Press

Jander, R. and Daumer, K., 1974. Guideline and gravity orientation of blind termites foraging in the open (Termitidae: Macrotermes, Hospitalitermes). *Insectes Soc*. 21, 45–69.

Klotz, J.H., Reid, B.L., 1992. The use of spatial cues for structural guideline orientation in *Tapinoma sessile* and *Camponotus pennsylvanicus* (Hymenoptera: Formicidae). J. Insect Behav. 5, 71–82.

Klotz, J.H., and Reid, B.L., 1993. Nocturnal orientation in the black carpenter ant *Camponotus pennsylvanicus* (DeGeer) (Hymenoptera: Formicidae). Insectes Soc. 40, 95–106.

Klotz, J.H., Reid, B.L., Hamilton, J., 2000. Locomotory efficiency in ants using structural guidelines (Hymenoptera: Formicidae). Sociobiology 35, 79–88.

Marée, A. F., and Hogeweg, P. 2001. How amoeboids self-organize into a fruiting body: multicellular coordination in Dictyostelium discoideum. *Proceedings of the National Academy of Sciences*, *98*(7), 3879-3883.

Maye, A., Hsieh, C. H., Sugihara, G., & Brembs, B. 2007. Order in spontaneous behavior. *PloS one*, *2*(5), e443.

Ovaskainen, O., Cornell, S.J., 2003. Biased movement at a boundary and conditional occupancy times for diffusion processes. *J. Appl. Probab*. 40, 557–580.

Parrish, J. K., and Edelstein-Keshet, L. 1999. Complexity, pattern, and evolutionary trade-offs in animal aggregation. *Science*, *284*(5411), 99-101.

R Core Team. 2015 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (url:http://www.R-project.org/)

Schindelin, J., Arganda-Carreras, I., Frise, E., et al. 2012 Fiji: an open-source platform for biological-image analysis, *Nature methods* 9(7): 676-682, PMID

Suzuki, K., Takagi, T., Hiraishi, T., 2003 Video analysis of fish schooling behavior in finite space using a mathematical model. *Fish. Res.* 60, 3–10.

Soares, M.P., Riet-Correa F, Smith D.R., Pereira S.M., Mendez M.C., Brandolt A.L., 2001 Experimental intoxication by larvae of *Perreyia flavipes* Know, 1899 (Hymenoptera: Pergidae) in pigs and some aspects on its biology. *Toxicon* **39**, 669–678.

Theraulaz, G., Bonabeau, E., Nicolis, S. C., Sole´, R. V., Fourcassie´, V., Blanco, S., Fournier, R., Joly, J.L., Fernandez, P., Grimal, A., Dalle, P. and Deneubourg, J.L. 2002. Spatial patterns in ant colonies. *Proceedings of the National Academy of Sciences*, U.S.A., 99, 9645–9649.

Appendix A. Plots of the inactivity and activity times of the individuals (*n* = 10 replicates) by a LOESS (locally weighted scatterplot smoothing) regression fit. We use this information to observe if there is temporal influence on the pattern of activity and inactivity of the individual. There were no abrupt or larger differences among the intermittency behavior between activity and inactivity.

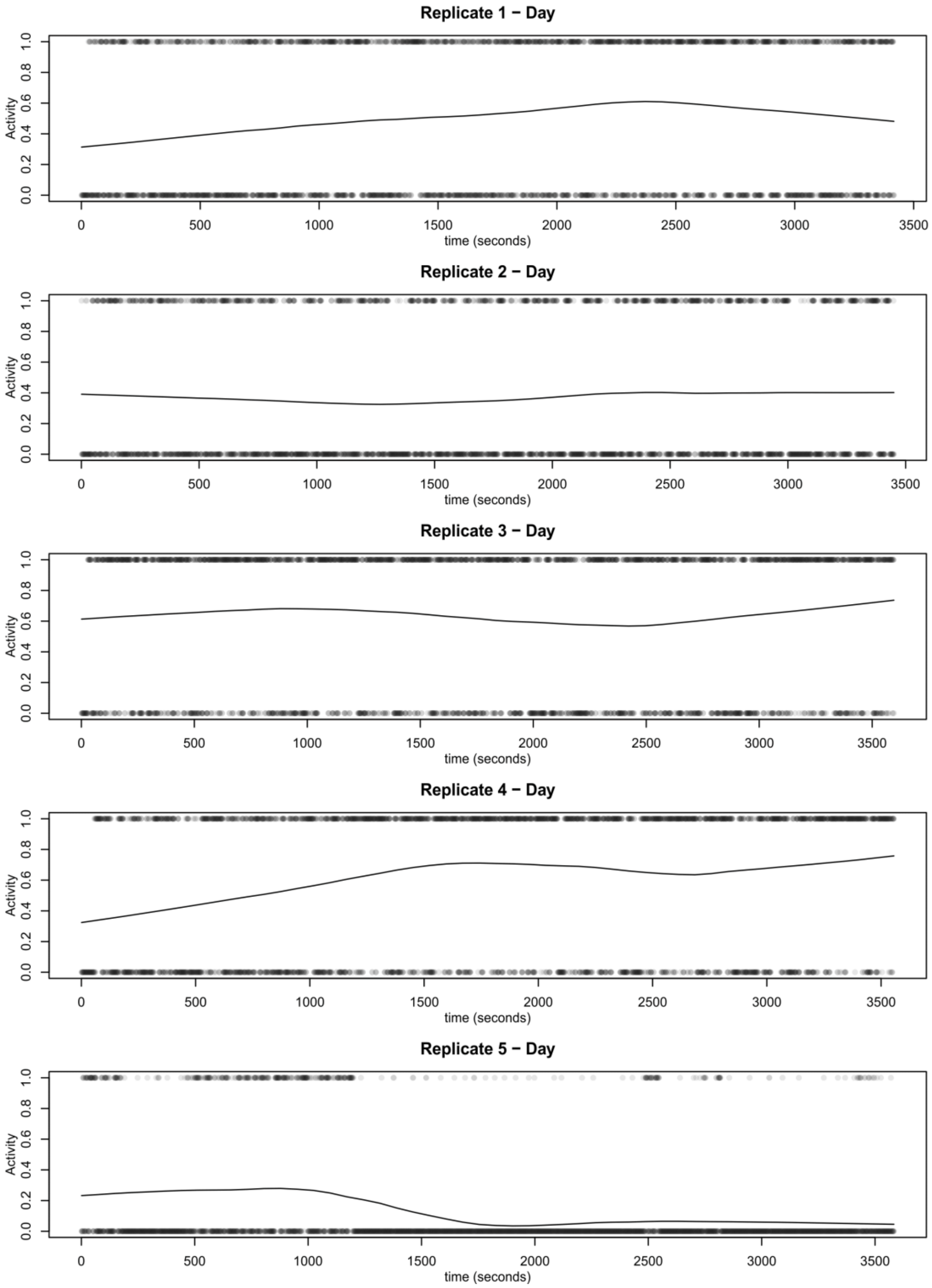


Figure 1 Plots of the inactivity (0.0) and activity (1.0) times of the individuals for diurnal

trials (*n* = 10 replicates; one to five) by a LOESS (locally weighted scatterplot smoothing) regression fit.

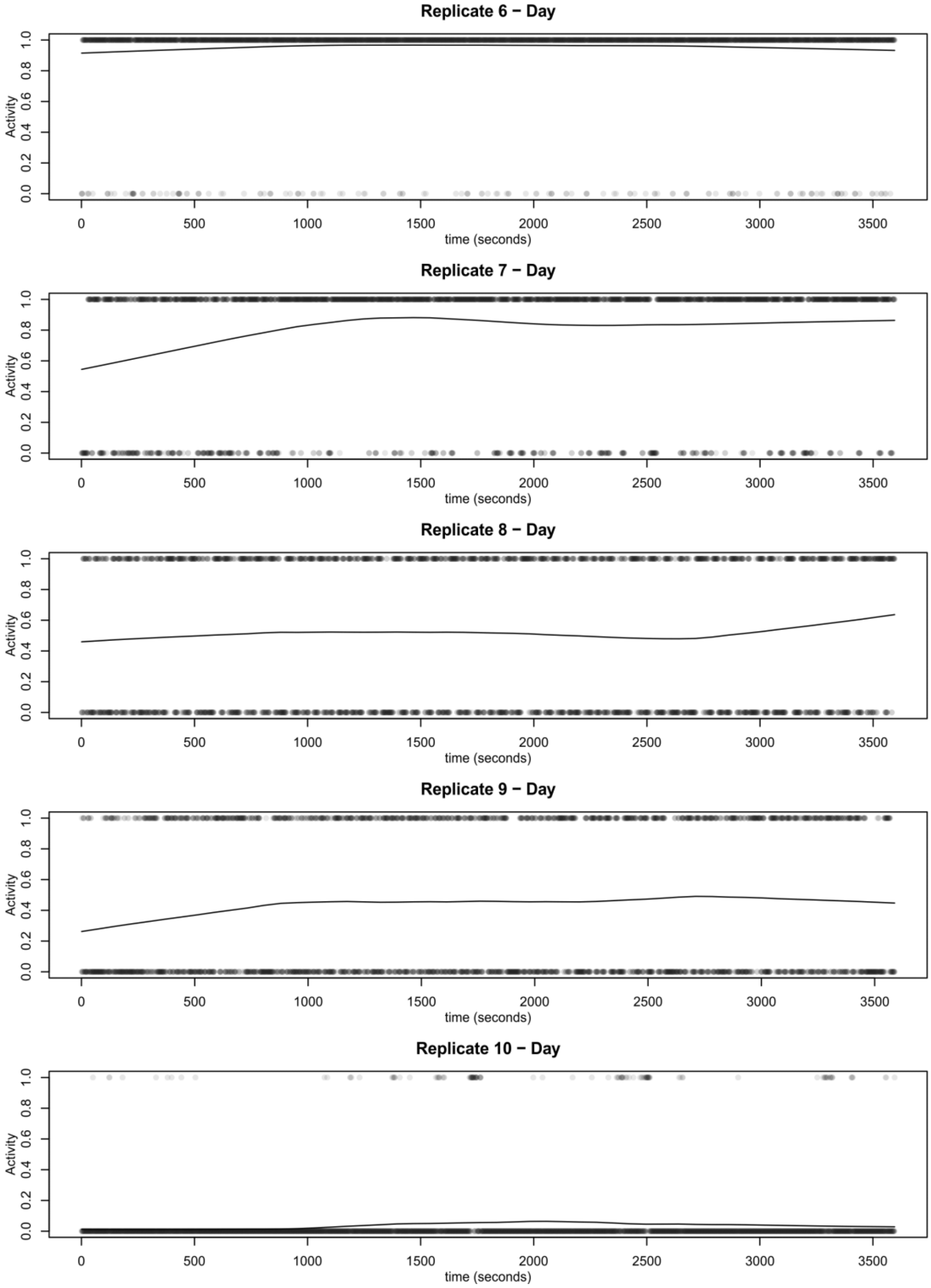


Figure 2 Plots of the inactivity (0.0) and activity (1.0) times of the individuals for diurnal trials (*n* = 10 replicates; six to 10) by a LOESS (locally weighted scatterplot smoothing) regression fit.

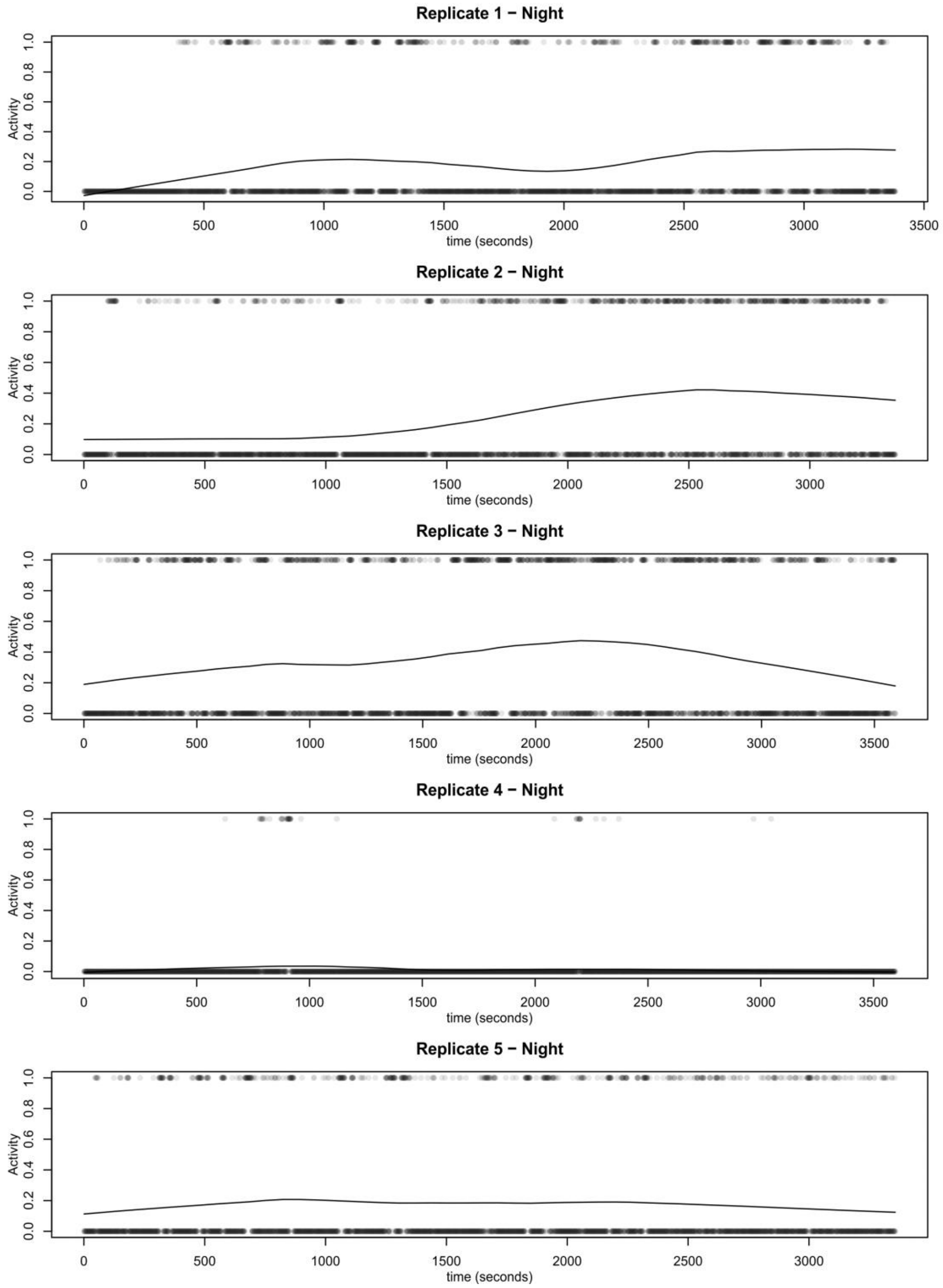
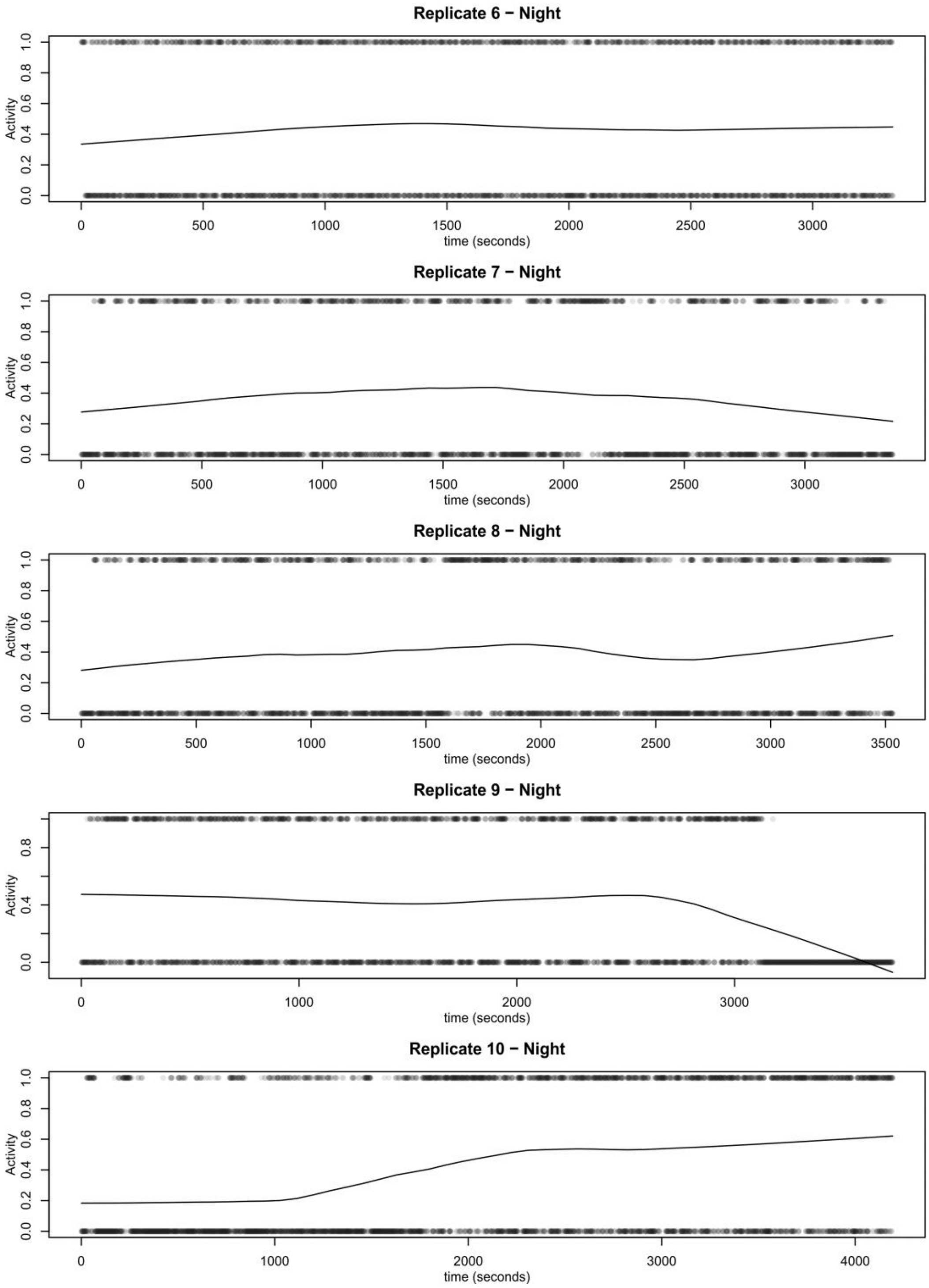


Figure 3 Plots of the inactivity (0.0) and activity (1.0) times of the individuals for nocturnal trials (*n* = 10 replicates; one to five) by a LOESS (locally weighted scatterplot smoothing) regression fit.


Replicate 6 − Night

Replicate 7 − Night

Replicate 8 − Night

Replicate 9 − Night

Replicate 10 − Night

Figure 4 Plots of the inactivity (0.0) and activity (1.0) times of the individuals for nocturnal trials (*n* = 10 replicates; six to ten) by a LOESS (locally weighted scatterplot smoothing) regression fit.

CONCLUSÃO

Como podemos mensurar a complexidade de sistemas biológicos quantitativamente e qualitativamente? Esta talvez seja uma das grandes questões abertas no estudo de sistemas complexos. Nesta tese utilizamos análises de recorrência (i.e. Plots de recorrência e RQA – Recurrence quantification analysis), descrição de comportamentos de movimento, incluindo modelagem, para obtermos algumas respostas sobre como ocorre o comportamento complexo individual de insetos. O estudo dos sistemas complexos durante as duas últimas decadas vem avançado exponencialmente através do surgimento de novas tecnologias baseadas em ferramentas computacionais (Mitchell 2012). Em geral, formigas estão sendo usadas como um dos principais sistemas modelo para este tipo de estudo na literatura (e.g. Deneuborg et al. 1993, Miramontes 1992, Christensen et al. 2014). Embora, formigas possuam grande diversidade de comportamento e organização social em diversos níveis, a história natural de diversos outros insetos possibilita questionamentos intrigantes sob a ótica do estudo da complexidade e emergência organizacional, tais como agregações de insetos (Deneubourg et al. 2002). Apesar de diversos avanços no estudo do forrageio de espécies, estrutura complexa de ninhos, padrões de auto-organização nos sistemas biológicos, existe um vácuo na interpretação do estudo da complexidade individual de organismos e sua emergência na auto-organização (Deneubourg et al. 1990).

Os resultados da tese indicam que o comportamento individual complexo varia em grande escala de espécies diferentes, mas também dentro da mesma espécie (em castas diferentes, no caso das formigas). Porém este padrão pode ser comparável inter e intra-espécie pelos métodos propostos. No caso de estudo do papel individual de agregações de *Perreyia flavipes*, através de alguns parâmetros, podemos prever a movimentação de indivíduos que demonstram possuir também um padrão de movimento com propriedades de auto similaridade e aditividade, o que pode ser parte do processo natural de auto-organização de agregações. Acreditamos que estes resultados possuam no mínimo duas implicações: um aumento no conhecimento deste tipo de fenômeno comportamental e como mensurá-lo (1), além de informações de história natural da desconhecida espécie *P. flavipes*, resultados empíricos que podem ser utilizados para descrever comportamento semelhantes, através de modelagem matemática e postulados teóricos (2).

Durante a tese, foi acumulado um número muito grande de dados, gerando mais de quatro terabytes de informação em formato de vídeos, dos quais podem ser extraídos, em uma estimativa primária, cerca de 10 milhões de dados (incluíndo quantidade de farmes analisados por vídeos e atos comportamentais). Primeiramente pretendiamos extrair o máximo de dados, representando a emergência de auto-organização, de indivíduos a grupos, em todas as espécies utilizadas na tese. Porém, tivemos grande dificuldades na extração de dados para algumas espécies (especialmente em formigas), relacionadas a movimentação e ao tamanho dos indíviduos (e.g. *Linepithema micans*). Não obstante, o processo de extração de dados em densidades maiores através de tracking computacional é problemático. É necessário um trabalho manual de grande intensidade para corrigir possíveis erros de tracking relacionados com a perda de identidade dos indíviduos em grupo ao longo do tempo. Durante a execução da tese conseguimos começar a extrair os dados *a priori* para somente um indivíduo, mas há perspectivas intrigantes para expandir estas análises para conjuntos de indivíduos. Espero que nosso estudo contribua para avanços na área de mensuração comportamental em testes empíricos de sistemas complexos.

REFERÊNCIAS

Christensen K, Papavassiliou D, Figueiredo A de, Franks N R, Sendova-Franks A B. 2014 Universality in ant behaviour , *J. R. Soc. Interface* **12** , 20140985.

Deneubourg JL, Pasteels JM & Verhaeghe JC. 1983 Probabilistic behaviour in ants: a strategy of errors*? J. Theor. Biol.* 105, 259{271.79).

Deneubourg JL, Camazine S & Detrain C. 1990 Self-organization or individual complexity: a false dilemna or a true complementarity In: *Information processing in social insects*. Detrain C., Deneubourg J.L. et J.M. Pasteels (eds), Birkhauser Verlag, 401-408

Deneubourg JL, Lioni A, & Detrain C. 2002 Dynamics of aggregation and emergence of cooperation. *The Biological Bulletin*, 202(3), 262-267.

Mitchell, M. 2009 *Complexity: A guided tour*. Oxford University Press.

Miramontes, O. 1992. Complexity and Behaviour in *Leptothorax* Ants. Master Thesis, Open University, U.K.

ANEXO I

Métodos para a extração de dados de atividade em insetos através de sistemas de *tracking* de movimento

No desenvolvimento desta tese, dois programas computacionais foram utilizados de um modo extensivo para a extração do *tracking* do movimento de indivíduos: ImageJ/Fiji (Schindelin et al. 2002) e Ctrax (Branson et al. 2009). Observamos na literatura uma escassez de descrições de procedimentos para análise de vídeos em softwares de tracking no comportamento animal. Como os passos de extração a obtenção dos dados finais podem ser obtusos e ter diversas variações, neste anexo esclarecemos como os dados desta tese foram extraídos através dos programas escolhidos, de forma que possa servir de um roteiro para pesquisadores que estejam tendo dificuldades de extrair dados semelhantes. A seguir, descrevo a finalidade dos programas utilizados, seus métodos e parâmetros.

Pré-processamento de vídeos

Os vídeos extraídos através dos testes empíricos nesta tese são de extensão MOD. (i.e. Module file format). Infelizmente, é raro encontrar filmadoras no mercado nacional que possuam opção de gravação com extensões de vídeo mais comuns a softwares de tracking ou manipulação de vídeos (e.g. MPEG, AVI, MOV). O formato MOD não é suportado pelos programas utilizados em nosso trabalho (Ctrax e FIJI). Desta forma, os vídeos originais tiveram que ser convertidos em outros formatos de vídeo. Para o software Ctrax, primeiramente os arquivos foram convertidos através do programa VirtualDub MPEG2 (v. 1.6.19) para o formato de vídeo MPEG. Depois disso, os arquivos foram convertidos para o formato AVI (AVI não comprimido) pelo mesmo programa, com uma taxa de fps menor (20 fps) para aumentar a velocidade computacional do programa. No uso do Ctrax, os vídeos foram analisados através de um único arquivo AVI (Extensão de vídeo de duas horas). No uso do software Fiji, os vídeos foram convertidos em AVI com plugins diferentes pelo programa Xilisoft (v. 5.1) e divididos em uma série de vídeos menores de 10 minutos (de um tempo total de 1 hora) devido à restrição de memória JAVA no sistema que exige alta poder de computação. No fim do experimento, os resultados extraídos em cada pedaço de vídeo foram compilados novamente em um único arquivo.

Ctrax

Ctrax é um programa especialmente feito para o *tracking* de movimentos, a *priori* desenvolvido para o estudo do comportamento locomotor da mosca *Drosophila melanogaster.* É utilizado para estimar as posições e orientações de um indivíduo ou muitos em um longo período de tempo, em uma média de 1 hora e meia de modo automático, segundo os autores, com mínima supervisão necessária (Branson et al. 2009). Além disto possibilita análises quantitativas do comportamento de objetos livres. Apesar do seu objetivo primário, que foi o estudo da mosca *Drosophila melanogaster*, é facilmente adaptável para outros *setups* experimentais e espécies, como em estudos realizados nos últimos anos com baratas e formigas (Bender et al. 2011; Reid et al. 2011). A cada nova versão do programa, notamos uma melhora em sua estabilidade. Conjuntamente com o software que é disponibilizado gratuitamente, os desenvolvedores disponibilizam um pacote de correção de erros via Matlab chamado de “FixErrors” que identifica sequências suspeitas de frames e permite ao usuário corrigir manualmente qualquer erro de *tracking*.

O *tracking* pelo Ctrax em nossos dados foi robusto até certo ponto, certas espécies aqui escolhidas foram extremamente problemáticas para o *tracking* de movimento, no sentido de possuírem dinâmicas de movimento complicadas (e.g. mudança de angulação alta, paradas demasiadamente longas). Em nosso caso, apesar da existência de *software* para correção, várias espécies que utilizamos na tese apresentaram problemas crônicos que não podiam ser remediados em pequena escala. Deve-se lembrar também que o nosso *tracking* foi ininterrupto por duas horas, até onde se sabe, um *tracking* dessa magnitude e com o intervalo de tempo determinado em nossos experimentos (sendo o menor de intervalo de tempo de cerca de 1 s) é inexistente na literatura. Aparentemente, a origem da complexidade de padrões de movimentação dos indivíduos, seja pela mudança brusca de angulação, ou de dinâmica de atividade a inatividade, prejudica o modelo de *tracking* automático dos programas.

Desta maneira, o modo mais parcimonioso que encontramos de corrigir os problemas, embora, muitas vezes laborioso e dispendioso de tempo, foi através da correção manual. Esta correção manual consiste em parar o *tracking* automático nos trechos em que ele começa a apresentar falhas, e mudar os parâmetros de identificação, predição de velocidade do modelo, a taxa de identificação e elementos espúrios ou perdidos (Figura 3). Após a mudança dos parâmetros, inicia-se o processo de *tracking* novamente, do frame em que foi parado. Outro método mais brusco, consiste em mudar os parâmetros de estimativas de background e reiniciar o *tracking*. Em ambos os casos, a conversão automática para o arquivo SBFMF (i.e. *Static Background Fly Movie Format*) feita pelo programa é perdida. Este arquivo possibilita o uso do *toolbox* de correção, além de ser uma cópia bem menor do arquivo original em AVI. Na próxima seção, brevemente explano os passos necessários e as configurações necessárias de se configurar para o *tracking*. No Ctrax, as configurações básicas envolvem os seguintes passos: Configurações de ajuste de *background* (1), nível de ajuste do *background* (2) e parâmetros de *tracking* (3).

Parâmetros do Ctrax

1.1 Configurações de ajuste de fundo (i.e. *Background estimation settings*)

O primeiro passo para iniciar o processo de *tracking* no Ctrax é definir os parâmetros de computação do modelo de fundo (O que pode ser facilmente ser feito através de sua interface gráfica). Os parâmetros são os seguintes:

Algoritmo (i.e. *Algorithm*): Se refere a definição do algoritmo que será utilizado para estimar a imagem de *background* e seu desvio padrão**.** Existem basicamente dois métodos. O método de Média/Mediana de diferença absoluta, estima o centro da imagem como a mediana de frames amostrados e o desvio padrão da diferença média absoluta desta mediana. Através do método de Média/Desvio Padrão, estima-se a imagem central como a média dos quadros incluídos na amostra e o desvio como o desvio padrão dos quadros incluídos na amostra. Em nossos experimentos, utilizamos apenas o primeiro método.

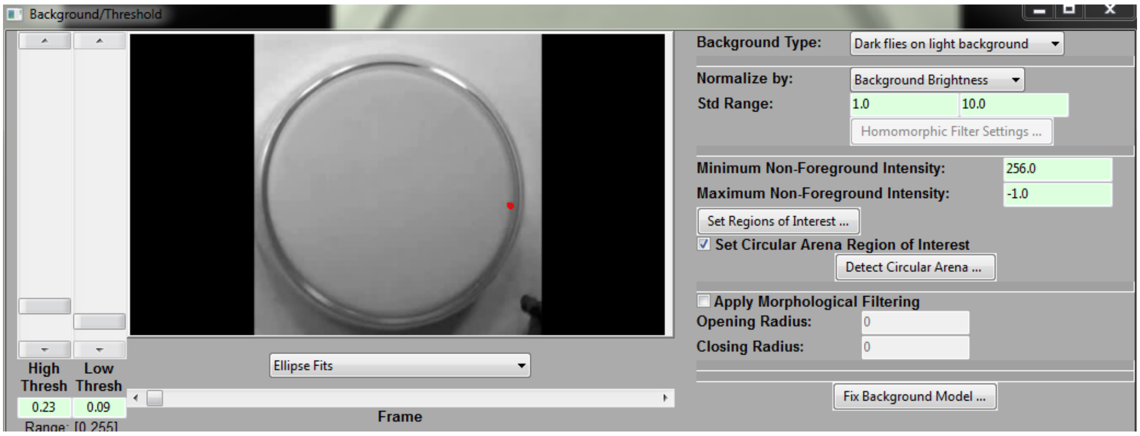
Número de *frames* (i.e. *Number of frames*): O *background* é estimado de amostras contínuas do primeiro, ao último frame do vídeo em análise. Em todos os nossos experimentos, foram utilizados cerca de 300 frames.

Primeiro Frame e Último Frame (i.e. *First Frame and Last Frame*): Intervalo específico dos quais as amostras de frames são extraídas para estimar o *background*. Utilizamos toda a extensão do vídeo (duas horas). O indivíduo quando permanece parado por muito tempo, pode interferir na estimativa da definição de *background*. Em nossos experimentos utilizamos todos os frames do vídeo, para evitar influências de longos intervalos de imobilidade (tais como ocorrem em *Tenebrio molitor*).

1.2 Ajustes de nível de imagem de fundo (i.e. *Background/Threshold*)

O ajuste do nível da imagem de fundo é um passo crucial para o programa reconhecer os pixels do indivíduo selecionado, comparados com os pixels da imagem de fundo definida anteriormente (Figura 1). A diferenciação entre imagem de fundo (i.e. *background*) e indivíduo envolve a diferença entre o frame atual e a média da imagem de fundo, dividida pela normalização da imagem. Desta forma, o primeiro passo é definir qual o tipo de fundo utilizado. Três tipos de imagem de fundo estão pré-definidos pelo programa: Indivíduos de cor clara em imagem de fundo escuro (i.e. *Light flies on the background*), Indivíduos escuros em imagem de fundo claro (i.e. *Dark flies on light background*) e "outros". No primeiro, Ctrax somente procura por pixels que são mais claros do que a imagem de *background*. No segundo, Ctrax somente procura por pixels que são mais escuros que o fundo. Se o tipo de imagem de fundo é outro, então Ctrax procura por qualquer tipo de diferença da imagem do fundo e nivela sua diferença absoluta.

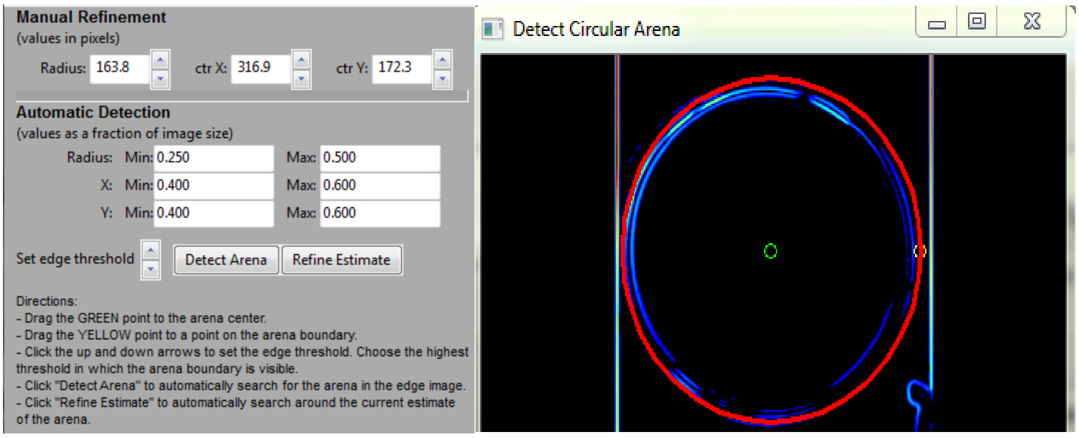
A normalização da imagem pode ser definida de diversas maneiras. Através do desvio padrão, Ctrax normaliza pelo nível do desvio padrão, tal como computado no processo de modelagem da imagem de fundo. A imagem de desvio padrão é mais suscetível a erros em estimar os indivíduos parados por um longo período de tempo. Uma maneira de impedir ou diminuir este problema é definir um alcance específico paro o desvio padrão. Em nossos testes, quando utilizamos o desvio padrão como normalização, o alcance máximo do desvio padrão definido foi três vezes maior do que o valor mínimo. A normalização de background por brilho, normaliza o background pela sua média de imagem. Esta é uma aproximação razoável, devido ao fato de quanto mais claro é o pixel, mais alto é a sua taxa de ruído. A normalização por desvio padrão e por brilho foram as únicas usadas em nossos testes. O uso de cada normalização foi definido para cada indivíduo, já que notamos diferenças individuais (entre as séries temporais) grandes.



Background Type: Dark flies on light background
Normalize by: Background Brightness
Std Range: 1.0 10.0
Minimum Non-Foreground Intensity: 256.0
Maximum Non-Foreground Intensity: -1.0
☑ Set Circular Arena Region of Interest
☐ Apply Morphological Filtering
Opening Radius: 0
Closing Radius: 0
Ellipse Fits
High Thresh: 0.23
Low Thresh: 0.09
Range: [0 255]

Figura 1. Tela de ajuste do nível de fundo (*Background/Threshold*) da interface gráfica do Ctrax.

Os valores restantes foram mantidos como o padrão definido pelo programa. Regiões onde deve ocorrer o *tracking* podem ser pré-estabelecidas, seja em formato de polígonos ou em formato de uma arena circular (i.e. *detect circular arena*). Uma vez que estas regiões sejam selecionadas, todas as outras regiões excluídas da seleção são consideradas imagem de fundo pelo programa. Estas configurações são selecionadas manualmente pelo cursor do mouse ou pela definição numérica de radiano e medidas cartesianas x e y (Figura 2). Para obter uma classificação de pixels como de primeiro ou segundo plano, Ctrax usa como limiar a diferença normalizada entre a imagem atual e do fundo. Para um pixel ser do primeiro plano, a sua distância a partir do fundo deve estar acima de um limiar inferior (i.e. *Low Thresh*), e não deve ter um pixel acima de um limiar superior (i.e. *High Thresh*) que apenas passa por pixels acima do limite inferior. Ou seja, Ctrax encontra todos os pixels que estão acima do limiar inferior, em seguida, remove todos os componentes conectados de *pixels*, tal que nenhum *pixel* no componente conectado está acima do limiar superior. Os limiares podem ser definidos com as barras de rolagem do lado esquerdo da interface gráfica. O limiar inferior não pode se sobrepor ao limiar superior. Alterar o algoritmo de normalização muda consideravelmente a gama de distâncias, de modo que a interface gráfica tenta compensar essas mudanças na magnitude.



Manual Refinement
(values in pixels)
Radius: 163.8
ctr X: 316.9
ctr Y: 172.3

Automatic Detection
(values as a fraction of image size)
Radius: Min: 0.250 Max: 0.500
X: Min: 0.400 Max: 0.600
Y: Min: 0.400 Max: 0.600

Directions:
- Drag the GREEN point to the arena center.
- Drag the YELLOW point to a point on the arena boundary.
- Click the up and down arrows to set the edge threshold. Choose the highest threshold in which the arena boundary is visible.
- Click "Detect Arena" to automatically search for the arena in the edge image.
- Click "Refine Estimate" to automatically search around the current estimate of the arena.

Figura 2. Parâmetros para a detecção de arena circular da interface gráfica do programa Ctrax.

Se houver indivíduos não detectados, um nível de limiar mais baixo ajuda em sua detecção. Se houver objetos além do indivíduo (e.g. barreiras físicas), o limiar deve ser maior. Um limiar mais baixo resultará em uma área de detecção maior e sua elevação irá resultar em uma área de indivíduos detectados menor. Definir o limiar muito baixo irá resultar em ruído instrumental. Os benefícios de uma área maior é a melhor estimação do ponto central (centróide) do indivíduo analisado, assim como outras informações mais precisas, tais como angulação correta. O ruído da câmera e artefatos de compressão podem, potencialmente, ser compensados pela aplicação de filtragem morfológica (i.e. *morphologic filtering*). Nós não usamos a filtragem morfológica em nossos experimentos.

1.3 Ajuste de *tracking*

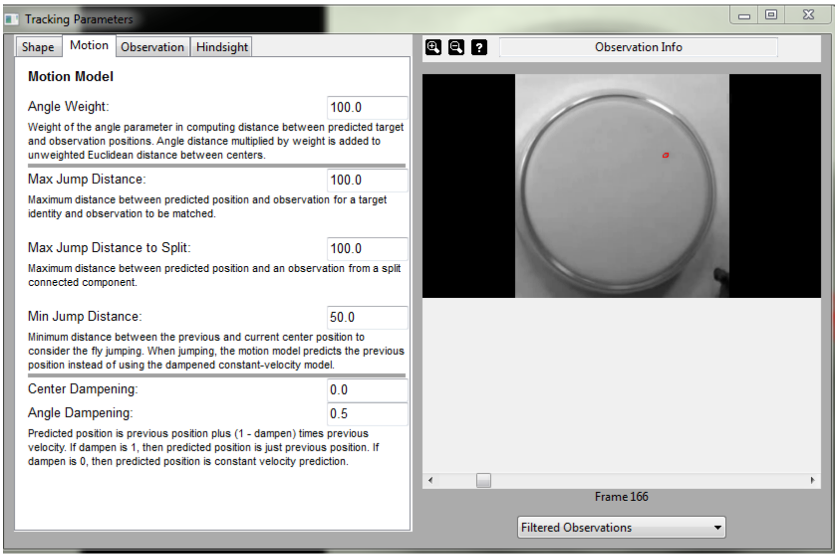
Esta etapa se refere ao controle dos parâmetros de observação, detecção e identidade utilizados pelo programa para detectar e acompanhar o comportamento de movimentação das espécies. No programa, uma tela com quatro abas existe, referente a forma (i.e. *Shape*), movimento (i.e. *Movement*), observação (i.e. *Observation*) e retrospectiva do modelo de movimento do programa (i.e. *Hindsight*).

1.4 Parâmetros de forma

Nesta seção define-se os tamanhos morfológicos esperados de cada elipse (contorno físico considerado pelo software) correspondentes ao objeto estudado. Isto é computado automaticamente pelo programa ou pode ser manualmente indicado na interface gráfica. As definições abrangem tamanho mínimo, esperado, máximo, entre outros. Ressalto que no caso das espécies utilizadas na tese, muitas vezes o tamanho mínimo não correspondia a realidade, devido a inúmeras falhas do *tracking* automático, por isso o valor mínimo foi extraído manualmente através de um pequeno trecho de vídeo (e.g. 5 min).

1.5 Parâmetros de movimento

O programa assinala identidades para a observação detectada, de modo que as elipses detectadas são baseadas nos dois frames anteriores e no modelo de movimento do programa. Esta distância é baseada tanto na posição central da elipse quanto na sua orientação. Neste item tivemos uma grande dificuldade, pois o programa não reconhecia por muitas vezes um indivíduo com a mesma identidade, mas trocava-as simultaneamente. Na prática, isto tem pouca repercussão, uma vez que com um indivíduo, baste conectar todas as identidades em pontos cartesianos em uma só. Esta distância é determinada com base na posição central da elipse e a sua orientação. O parâmetro do peso de ângulo (i.e. *angle weight*) especifica a importância relativa da orientação da elipse em relação à posição central. O erro total é o quadrado da distância entre as posições centrais previstas e detectadas mais os tempos do peso de ângulo o quadrado da distância entre as orientações previstas e detectadas (que são todos entre 0 e 180 graus). O erro na posição central é medida em pixels ao quadrado e o erro na orientação é medido em radianos ao quadrado. Assim, o peso do ângulo deve ser superior a 1, mesmo que o efeito desejado é o de que a orientação seja menos importante do que a posição central correspondente. Em todas as experiências, utilizou-se o peso de valor 100.



Motion Model

Angle Weight: 100.0

Weight of the angle parameter in computing distance between predicted target and observation positions. Angle distance multiplied by weight is added to unweighted Euclidean distance between centers.

Max Jump Distance: 100.0

Maximum distance between predicted position and observation for a target identity and observation to be matched.

Max Jump Distance to Split: 100.0

Maximum distance between predicted position and an observation from a split connected component.

Min Jump Distance: 50.0

Minimum distance between the previous and current center position to consider the fly jumping. When jumping, the motion model predicts the previous position instead of using the dampened constant-velocity model.

Center Dampening: 0.0

Angle Dampening: 0.5

Predicted position is previous position plus (1 - dampen) times previous velocity. If dampen is 1, then predicted position is just previous position. If dampen is 0, then predicted position is constant velocity prediction.

Figura 3. Interface gráfica relativa aos parâmetros de *tracking* do programa Ctrax.

A distância de pulo máximo (i.e. *Max Jump Distance*) é a raiz quadrada do erro máximo permitido entre as posições previstas e observadas. A distância de pulo mínimo é a mínima distância entre a posição prévia e atual. O modelo de movimento prediz a posição prévia ao invés do uso do modelo de velocidade constante. Apesar de não estarmos lidando com indivíduos que executam saltos de movimento, a manutenção teórica do modelo se faz de útil, uma vez que ocorreram perdas fragmentadas de *tracking*. Em nosso trabalho, utilizamos o valor 10 no pulo máximo e 1 no pulo mínimo. Existem duas formas com as quais o Ctrax calcula a posição dos indivíduos, através do centro do indivíduo (i.e. *Center Dampening*) ou do ângulo (i.e. *Angle Dampening*). O algoritmo controla a previsão da posição do indivíduo dada suas duas posições prévias. O algoritmo prediz que a posição central irá ser a posição central prévia juntamente com a velocidade*(1-*Center Dampening*), e que a orientação irá ser a posição prévia juntamente com a velocidade angular*(1-*Angle Dampening*). Em nossos experimentos, por muitas vezes deixamos o coeficiente de ambos em 0.5. Concluímos que no caso das espécies estudadas, principalmente formigas, o modelo que se baseia em ângulos é um pouco deficiente, e o modelo baseado na velocidade constante é mais eficiente, desta forma igualamos o valor do coeficiente para ambos os casos.

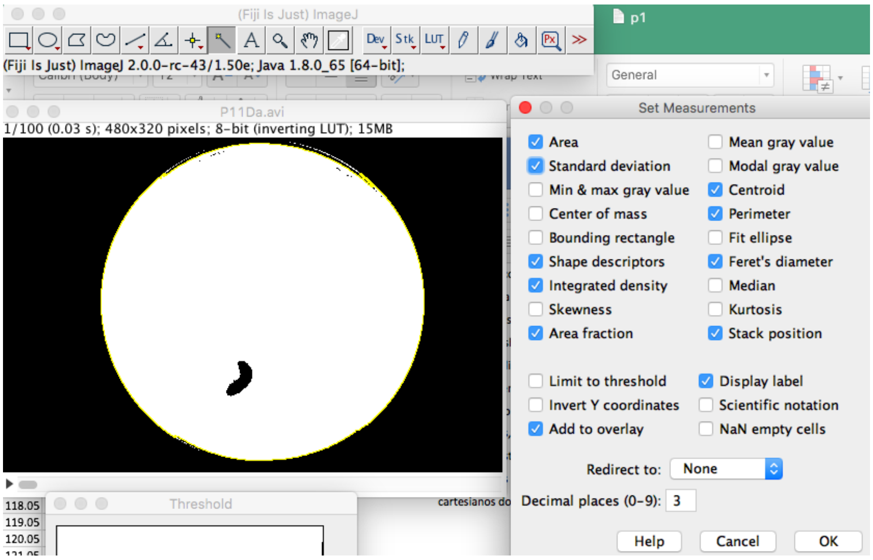
Os parâmetros de observação afetam o processamento de componentes conectados. Se por ventura, o indivíduo for reconhecido pelo programa como dois componentes (elipses) ao invés de um, o usuário poderá controlar o número de agrupamentos (i.e. *clusters*) que o programa pode reconhecer, ou se clusters podem ser unidos se suficientemente próximos. O último conjunto de ferramentas, se baseia na revisão de erros dos modelos de movimentação do programa. O usuário tem opções como concertar detecções divididas (que pertencem ao mesmo indivíduo), a junção destas detecções, detecções espúrias e perdidas. O valor que escolhemos para esses parâmetros variavam muito de espécie para espécie e até mesmo entre indivíduos da mesma espécie. De um modo geral, deixamos um valor maior sempre para a detecção de *tracking* perdidos por um período de tempo. É aconselhável deixar para todos os itens um valor maior do que o pré-definido pelo programa, uma vez que as definições primárias são estipuladas de experimentos dos autores apenas com *Drosophyla melanogaster*.

Fiji

O desenvolvimento do software Fiji foi inspirado em seu predecessor computacional ImageJ. Tal como ImageJ, FIJI é um projeto *Open Source* presente em repositórios online (Git - http://imagej.net/Git), em código aberto, com diversas *libraries* e *plugins* disponíveis. FIJI é um programa licenciado sob a licença de uso geral pública (em inglês: *GNU General Public License*). É construído através do núcleo do programa ImageJ. ImageJ é um programa que possibilita o processamento de imagens em sequência. Difere de outros programas devido ao fato de oferecer diversas ferramentas de mensuramento de *pixels*. Devido a sua abrangência, é extensamente utilizado no meio científico, como nas áreas de física e medicina. ImageJ pode exibir, editar, analisar, processar e salvar imagens em 8-bit, 16-bit e 32-bits ou vídeos em formato compatível com imagens. Além disso, tem a capacidade de calcular o número de pixels de determinado objeto (e.g. inseto), sua área, perímetro e/ou distância. Além disso, suporta muitas funções padrões de processamento de imagens, incluindo ajuste de contraste, detecção de bordas e funções de filtragem de imagens. ImageJ é um software de código livre com a possibilidade de oferecer vários plug-ins. O desenvolvimento do ImageJ foi coordenado por Wayne Rasband da National Institute of Mental Health, Bethesda, Maryland, USA. Tendo como premissa uma plataforma mais estável e com mais recursos, Fiji possui um leque de plug-ins maior do que ImageJ, e devido a sua versatilidade foi escolhido por nós após breves testes pilotos.

2.1. Parâmetros do FIJI

Comparado com o Ctrax, FIJI possui um número menor de configurações, porém é menos intuitivo e exige uma capacidade de improvisação e aprendizado por tentativa e erro do usuário. Isto se deve a um fato simples, FIJI é a priori um programa de análise de imagens e não um programa de *tracking* de vídeo, como o Ctrax e outros. Porém, sua flexibilidade se torna uma grande vantagem quando analisamos o movimento não usual de indivíduos. No caso de *Perreyia flavipes* e seu movimento mais ondulatório, ele se torna mais preciso, além de que se considerarmos agregações, deve existir algum tipo de mensuramento da área da superfície da agregação, o que o programa dispõe através de mensuramentos diversos da imagem, no caso *frame* por *frame*. O primeiro passo é escolher converter em escala de cinza o vídeo, isto normaliza a imagem para o fundo branco e o indivíduo de cor negra, criando o contraste necessário para o *tracking*. Após isso, se seleciona a área de fundo (no menu; *Oval*, *elliptical selections*), que neste caso é o círculo correspondente a arena. No menu principal, na aba de edição (*edit*), seleciona-se a opção *clear outside*. Existe a opção de melhor o contraste, através de slides manuais. Na aba imagem, abre o menu de imagem (i.e. image) e seleciona ajuste e o nível (*threshold*), o método default do programa foi o suficiente para conseguirmos separar o indivíduo da imagem de fundo, mas existem diversos métodos dentro do programa e em *plugins* disponibilizados livremente. Com as ferramentas de desenho, seleciona-se o diâmetro da arena e calcula-se através do menu de análise a escala da arena. Depois, analisa-se quais parâmetros considerar para a extração de dados. Existe uma série destes que se interelacionam e podem trazer questionamentos importantes para estudos envolvendo movimentação de insetos. Pode-se extrair os dados cartesianos do objeto estudado, área, medidas relacionadas a circularidade do objeto, entre outros (Figura 4).



(Fiji Is Just) ImageJ 2.0.0-rc-43/1.50e; Java 1.8.0_65 [64-bit];

1/100 (0.03 s); 480x320 pixels; 8-bit (inverting LUT); 15MB

☑ Area
☑ Standard deviation
☐ Min & max gray value
☐ Center of mass
☐ Bounding rectangle
☑ Shape descriptors
☑ Integrated density
☐ Skewness
☑ Area fraction
☐ Mean gray value
☐ Modal gray value
☑ Centroid
☑ Perimeter
☐ Fit ellipse
☑ Feret's diameter
☐ Median
☐ Kurtosis
☑ Stack position

☐ Limit to threshold
☐ Invert Y coordinates
☑ Add to overlay
☑ Display label
☐ Scientific notation
☐ NaN empty cells

Redirect to: None

Decimal places (0-9): 3

Figura 4. Interface gráfica do programa FIJI, mostrando a imagem em fundo branco e com indivíduo em coloração negra, ajustada para o *tracking*. Ao lado, os mensuramentos disponíveis (i.e. *Set mensuraments*).

Arquivos brutos de dados.

Ambos os programas utilizados geram outputs numéricos de dados. Os formatos de dados gerados são em formato de texto tabulado (.txt) e formatos de arquivo de leitura via o programa *Matlab* (mat). Ctrax cria colunas com valores de ângulos em radianos, velocidade, eixos de coordenadas X, Y cartesianos. Utilizamos os valores de X e Y para extrair o deslocamento de cada indivíduo. FIJI cria uma planilha de dados (.xls) com diversos dados relativos ao *tracking* do indivíduo. Tais como: medidas cartesianas, área corporal ocupada pelo indivíduo no espaço, circularidade (medida de quão circular é um objeto, de escala inicial de 0 a 1), perímetro, diâmetro de *feret* (i.e. distância mínima entre dois pontos), entre uma série de outras medidas interessantes para análise comportamental.

Referências

Bender JA, Simpson EM, Tietz BR, Daltorio KA, Quinn RD, Ritzmann RE. 2011 Kinematic and behavioral evidence for a distinction between trotting and ambling gaits in the cockroach, *Blaberus discoidalis. J. Exp. Biol.* 214, 2057–2064. (doi: 10.1242/jeb.056481)

Branson K, Robie AA, Bender J, Perona P, Dickinson, MH. 2009 High-throughput ethomics in large groups of *Drosophila*. *Nature Methods* **6**:451-457.DOI:10.1038/nmeth.1328

Reid CR, Sumpter DJT, Beekman M. 2011 Optimisation in a natural system: Argentine ants solve the Towers of Hanoi. *J. Exp. Biol.* 214, 50–58. (doi: 10.1242/jeb.048173.)

Schindelin J, Arganda-Carreras I, Frise E. et al. 2012. Fiji: an open-source platform for biological-image analysis, *Nature methods* 9(7: 676-682, PMID 2274377)

MATLAB version 7.10.0. Natick, Massachusetts: The MathWorks Inc., 2010.

REFERÊNCIAS GERAIS

Agostinelli C, Lund U (2013) R package 'circular': Circular Statistics (version 0.4-7)

Arnold, BC (1983) Pareto Distributions. International Cooperative Publishing House, Fairland, Maryland.

Avila-Núñez JL, Daniel OL, Silmi S, Calcagno-Pisarelli MP (2007) Life history of *Aneugmenus merida* Smith (Hymenoptera: Tenthredinidae) in the Venezuelan Andes. Neotrop Entomol 36:22-27

Ayasse M, Paxton RJ, Tengo J (2001) Mating behavior and chemical communication in the order Hymenoptera. Annu Rev Entomol 46:31-78

Batschelet, E (1981) Circular Statistics in Biology. New York: Academic Press

Bender JA, Simpson EM, Tietz BR, Daltorio KA, Quinn RD, Ritzmann RE (2011) Kinematic and behavioral evidence for a distinction between trotting and ambling gaits in the cockroach, *Blaberus discoidalis.* J Exp Biol 214:2057-2064. doi: 10.1242/jeb.056481

Biro PA, Beckmann C, Stamps JA (2010) Small within-day increases in temperature affects boldness and alters personality in coral reef fish. Proc R Soc Lond B. 277(1678), 71e77 (doi:10.1098/rspb.2009.1346)

Boi S, Couzin ID, Del Buono N, Franks NR, Britton NF (1999) Coupled oscillators and activity waves in ant colonies. Proc Royal Soc B Bio Sci 266:371-378. doi: 10.1098/rspb.1999.0647

Bonabeau, E., Theraulaz, G., Deneubourg, J.L., Aron, S. and Camazine, S (1997) Self-organization in social insects. Trends in Ecology and Evolution, 12, 188-193

Boevé J-L, Rozenberg R, Shinohara A, Schmidt S (2014) Toxic Peptides Occur Frequently in Pergid and Argid Sawfly Larvae. PLoS One 9(8):e105301. doi:10.1371/journal.pone.0105301

Böröczky K, Wada-Katsumata A, Batchelor D, Zhukovskaya M, Schal C (2013) Insects groom their antennae to enhance olfactory acuity. P Natl A Sci USA 110:3615-3620

Branson K, Robie AA, Bender J, Perona P, Dickinson MH (2009) High-throughput ethomics in large groups of *Drosophila*. Nat methods 6:451-457. doi*:* 10.1038/nmeth.1328

Brodin T (2009) Behavioral syndrome over the boundaries of life-carryovers from larvae to adult damselfly. Behav Ecol 20, 30e37 (doi: 10.1093/beheco/arn111)

Calenbuhr V, Deneubourg JL (1992) A model for osmotropic orientation (I). J. Theor. Biol. 158, 359-393

Camargo OR (1955) Contribuição ao estudo do Tenthredinideo Mata Porcos, *Paraperreyia dorsuaria* (Know, 1899), no Rio Grande do Sul. Dissertation, Universidade Federal do Rio Grande do Sul

Came PB (1966) Primitive forms of social behaviour, and their significance in the ecology of gregarious insects. Proc Ecol Soc Aust 1:75-78

Carene PB (1962) The characteristics and behavior of the Saw-Fly *Perga affinis affinis* (Hymenoptera). Aust J Zool 10:1-34

Chapman BB, Hegg A, Ljungberg P (2013) Sex and the syndrome: individual and population consistency in behaviour in rock pool prawn, *Palaemon elegans*. PLoS One, 8(3), e59437. (doi: 10.1371/journal.pone.0059437)

Christensen K, Papavassiliou D, Figueiredo A de, Franks N R, Sendova-Franks A B (2014) Universality in ant behaviour , J R Soc Interface 12 , 20140985.

Costa LA (1941) Bichos “Mata Porcos”. Chácaras e quintais 63:43

Costa JT (2006) Hymenoptera: Symphita In: Costa JT (ed) The other insect societies. Belknap Press of Harvard University Press, Cambridge, pp 631-653

Cole BJ (1991a) Is animal behaviour chaotic? Evidence from the activity of ants. Proc Roy Soc Lond B Bio 244:253-259. doi*:* 10.1098/rspb.1991.0079

Cole BJ (1991b) Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. J Insect Behav 4:129-137. doi: 10.1007/BF01054607

Cole BJ (1992) Short-term activity cycles in ants: age-related changes in tempo and colony synchrony. Behav Ecol Sociobiol 31:181-188. doi: 10.1007/BF00168645

Cole BJ (1994) Chaos and behavior: the perspective of Nonlinear Dynamics In: *Behavioral Mechanisms in Evolutionary Ecology* (Ed. by L. Real), pp. 423-443. Chicago: University of Chicago Press.

Cole BJ (1995) Fractal time in animal behaviour: the movement activity of *Drosophila*. Anim Behav 50:1317-1324. doi*:* 10.1016/0003-3472(95)80047-6

Creed Jr RP, Miller JR (1990) Interpreting animal wall-following behavior. Experientia, 46(7), 758-761.

Cotton RT (1927) Notes on the biology of the meal worms, *Tenebrio molitor* Linne and *T. obscurus* Fab. Ann Entomol Soc Am 20:81-86. doi: 10.1093/aesa/20.1.81

Dadswell LP, Abott WD, McKenzie RA, (1985) The occurrence, cost and control of sawfly larval (*Lophyrotoma interrupta*) poisoning of cattle in Queensland 1972-81. Australian Veterinary Journal 62, 94-97

Dell AI, Bender JA, Branson K, Couzin ID, Polavieja de GG, Noldus LPJJ, Pérez-Escudero A, Perona P, Straw AD, Wikelski M, Brose U (2014) Automated image-based tracking and its application in ecology, Trends Ecol Evol 29:417-428. doi: 10.1016/j.tree.2014.05.004

Deneubourg JL, Camazine S, Detrain C (1990) Self-organization or individual complexity: a false dilemna or a true complementarity In: *Information processing in social insects*. Detrain C, Deneubourg JL. and JM Pasteels (eds), Birkhauser Verlag, 401-408

Deneubourg JL, Pasteels JM, Verhaeghe JC (1983) Probabilistic behaviour in ants: a strategy of errors*? J* Theor Biol 105, 259{271.79).

Deneubourg JL, Aron S, Goss SAPJM, Pasteels JM, Duerinck G (1986) Random behaviour, amplification processes and number of participants: how they contribute to the foraging properties of ants. Physica D 22(1):176-186

Deneubourg JL, Camazine S, Detrain C (1990) Self-organization or individual complexity: a false dilemna or a true complementarity In: *Information processing in social insects*. Detrain C., Deneubourg J.L. et J.M. Pasteels (eds), Birkhauser Verlag, 401-408

Deneubourg JL, Lioni A, Detrain C (2002) Dynamics of aggregation and emergence of cooperation. The Biological Bulletin, 202(3), 262-267.

Detrain C, Pasteels JM (1991) Caste differences in behavioral thresholds as a basis for polyethism during food recruitment in the ant, *Pheidole pallidula*. J Insect Behav 4(2):157-176. doi: 10.1007/BF01054609

Detrain C, Pasteels JM (1992) Caste polyethism and collective defense in the ant *Pheidole pallidula*: the outcome of quantitative differences in recruitment. Behav Ecol Sociobiol 29:405-412. doi: 10.1007/BF00170170

Dussutour A, Deneubourg JL, Fourcassié V (2005) Amplification of individual preferences in a social context: the case of wall-following in ants. Proceedings of the Royal Society of London B: Biological Sciences, 272(1564), 705-714.

Dutra F, Riet-Correa F, Mendez MC, Paiva N (1997) Sawfly larval poisoning in cattle and sheep in Uruguay. Vet Hum Toxicol 39:281-286

Dutra F (2003) Intoxicación por larvas de *Perreyia flavipes* en bovinos y ovinos, caracterización de la enfermedad y biología del insecto. Veterinaria 38:7-24

Eckmann JP, Kamphorst SO, Ruelle D (1987) Recurrence plots of dynamical systems. Europhys Lett 5:973-977. doi: 10.1209/0295-5075/4/9/004

Fagan WF, Cantrell RS, Cosner C (1999) How habitat edges change species interactions. The American Naturalist 153:165-182

Farine JP, Lobreau JP (1984) Le gregarisme chez *Dysdercus cingulatus* Fabr. (Hateroptera:Pyrrhocoridae): nouvelle methode d'interpretation statistique. Insectes Soc. 3, 277-290.

Fraenkel GS, Gunn DL (1961) The Orientation of Animals: Kineses, Taxes and Compass Orientation. New York: Dover

Finlay BJ., et al (2006) Self-similar patterns of nature: insect diversity at local to global scales. Proc. R. Soc. Lond. B. 273, 1596:1935-1941

Flores C, Ugalde-Gómez J, Hanson-Snortum P, Gauld I, Austin AD (2000) The biology of perreyiine sawflies (Hymenoptera: Pergidae) of the *Perreyia* genus-group. In: Austin AD, Dowton M (eds) Hymenoptera: evolution, biodiversity and biological control. CSIRO Publishing, Collingwood, pp 258-266

Fondacaro JD, Butz A (1970) Circadian rhythm of locomotor activity and susceptibility to methyl parathion of adult *Tenebrio molitor* (Coleoptera: Tenebrionidae). Ann Entomol Soc Am 63(4):952-955. doi: 10.1093/aesa/63.4.952

Frisch KV, *The Dancing Bees: An Account of the Life and Senses of the Honey Bee*, Harvest Books New York (1953)

Giraud T, Blatrix R, Poteaux C, Solignac M, Jaisson P (2000) Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. Mol Ecol 9:1835-1841. doi: 10.1046/j.1365-294x.2000.01085.x

Ghent AW (1960) A study of the group-feeding behaviour of larvae of the jack pine sawfly, *Neodiprion pratii banksianae* Roh. Behaviour 16:110-148

Gueron S, Levin SA, Rubenstein DI (1996). The dynamics of herds: from individuals to aggregations. Journal of Theoretical Biology, 182(1), 85-98

Gordon DM, Goodwin BC, Trainor LE (1992) A parallel distributed model of the behaviour of ant colonies. J Theor Biol 156(3):293-307

Grimaldi D, Agosti D (2000) A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants. P Natl Acad Sci USA 97:13678-13683. doi: 10.1073/pnas.240452097

Guimarães-Filho ZO, Caldas IL, Viana RL, Kurths J, Nascimento IC, Kuznetsov YK (2008) Recurrence quantification analysis of electrostatic fluctuations in fusion plasmas. Phys Lett A 372:1088-1095. doi: 10.1016/j.physleta.2007.07.088

Godfray HCJ (1994) Parasitoids. Princeton University Press, Princeton, New Jersey

Gordh G (1975) Sexual behavior of *Hemitaxonous dubitatus* (Norton) (Hymenoptera: Tenthredinidae). Entomol News 86:161-166

Haccou P, Meelis E (1992) Statistical analysis of behavioural data: An approach based on time structured models. Oxford, UK: Oxford University Press

Hadley NF (1981) Cuticular lipids of terrestrial plants and arthropods: A comparison of their structure, composition, and waterproofing function. Biol Rev 56:23-47

Hassell MP (1978) The Dynamics of Arthropod Predator-Prey Systems, Princeton University Press, Princeton, New Jersey

Holmes J (1906) Death-feigning in *Ranatra*. J Comp Neurol Psycho 16:200-216

Hölldobler B, Wilson EO (1990) The ants. Harvard, MA: The Belknap Press of Harvard University Press

Howard RW (1993) Cuticular hydrocarbons and chemical communication. In: Stanley-Samuelson DW, Nelson DR (eds) Insect Lipids: Chemistry, Biochemistry and Biology. University of Nebraska Press, Lincoln, pp 179-226

Holyst JA, Zebrowska M, Urbanowicz K (2001) Observation of deterministic chaos in financial time series by Recurrence Plots, can one control chaotic economy? Eur Phys J B 20:531-535. doi: 10.1007/PL00011109

Jander R, Daumer K (1974) Guideline and gravity orientation of blind termites foraging in the open (Termitidae: Macrotermes, Hospitalitermes). *Insectes Soc* 21, 45-69

Jonck F, Casagrande RA, Froehlich DL, Ribeiro Junior DP, Gava A (2010) Intoxicação espontânea por larvas de *Perreyia flavipes* (Pergidae) em suínos no estado de Santa Catarina. Pesquisa Vet Brasil 30:1017–1020

Kennel MB, Brown R, Abarbanel HDI (1992) Determining embedding dimension for phase-space reconstruction using a geometrical construction. Phys Rev A 45:3403 (1992). doi: 10.1103/PhysRevA.45.3403

King BH, Leaich HR (2006) Variation in propensity to exhibit thanatosis in *Nasonia vitripennis* (Hymenoptera: Pteromalidae). J Insect Behav 19: 241-249

Klotz JH, Reid BL (1992) The use of spatial cues for structural guideline orientation in *Tapinoma sessile* and *Camponotus pennsylvanicus* (Hymenoptera: Formicidae). J Insect Behav 5, 71-82

Klotz JH, Reid BL (1993) Nocturnal orientation in the black carpenter ant *Camponotus pennsylvanicus* (DeGeer) (Hymenoptera: Formicidae). Insectes Soc. 40, 95-106

Klotz JH, Reid BL, Hamilton J (2000) Locomotory efficiency in ants using structural guidelines (Hymenoptera: Formicidae). Sociobiology 35, 79-88

Korb J, Heinze J (2004) Multilevel selection and social evolution of insect societies. Naturwissenschaften 91:291-304. doi: 10.1007/s00114-004-0529-5

Kruskal WH (1952) Use of ranks in one-criterion variance analysis. J Am Stat Assoc 47:583-621. doi: 10.1080/01621459.1952.10483441

Lattke JE (1990) Revisión del género *Gnamptogenys* Roger en Venezuela (Hymenoptera: Formicidae). Acta Terramaris 2:1-47

Lilliefors H (1967) On the Kolmogorov–Smirnov test for normality with mean and variance unknown. J Am Stat Assoc 62:399-402. doi: 10.1080/01621459.1967.10482916

Lommelen E, Johnson CA, Drijfhout FP, Billen J, Wenseleers T, Gobin B (2006) Cuticular hydrocarbons provide reliable cues of fertility in the ant *Gnamptogenys striatula*. J Chem Ecol 32:2023-2034. doi: 10.1007/s10886-006-9126-8

Lone SR, Sharma VK (2011) Timekeeping through social contacts: Social synchronization of circadian locomotor activity rhythm in the carpenter ant *Camponotus paria*. Chronobiol Int 28:862-872. doi*:* 10.3109/07420528.2011.622676

Lubbock, J. 1881 Observations on ants, bees, and wasps. IX. Color of flowers as an attraction to bees: Experiments and considerations thereon. J Linn Soc Lond (Zool.) 16: 110-112

Macdonald J, Ohmart CP (1993) Life history strategies of Australian pergid sawflies and their interactions with host plants. In: Wagner MR, Raffa KF (eds) Sawfly Life History Adaptations to Woody Plants. Academic Press Inc., San Diego, pp 485-502

Mann HB, Whitney DR (1947) On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. Ann Math Stat 18:50-60. doi: 10.1214/aoms/1177730491

Marée AF, and Hogeweg P (2001) How amoeboids self-organize into a fruiting body: multicellular coordination in Dictyostelium discoideum. Proceedings of the National Academy of Sciences, 98(7), 3879-3883

Marwan N, Thiel M, Nowaczyk NR (2002) Cross Recurrence Plot Based Synchronization of Time Series. Nonlinear Proc Geoph 9:325-331. doi: 10.5194/npg-9-325-2002

Marwan N, Trauth MH, Vuille M, Kurths J (2003) Comparing modern and Pleistocene ENSO-like influences in NW Argentina using nonlinear time series analysis methods. Clim Dynam 21:317-326. doi: 10.1007/s00382-003-0335-3

Marwan N, Kurths J (2005) Line structures in recurrence plots. Phys Lett A 336:349-357. doi: 10.1016/j.physleta.2004.12.056

Marwan N**,** Romano MC, Thiel M, Kurths J (2007) Recurrence Plots for the Analysis of Complex Systems. Phys Rep 438:237-329. doi: 10.1016/j.physrep.2006.11.001

Marwan N, Donges JF, Zou Y, Donner RV, Kurths J (2009) Complex network approach for recurrence analysis of time series. Phys Lett A 373:4246-4254. doi: 10.1016/j.physleta.2009.09.042

Marwan N (2011) How to avoid potential pitfalls in recurrence plot based data analysis. Int J Bifurcat Chaos 21:1003-1017. doi: 10.1142/s0218127411029008

Maye A, Hsieh CH, Sugihara G, Brembs B (2007) Order in spontaneous behavior. *PloS one*, 2(5), e443.

MATLAB version 7.10.0 (2010) The MathWorks Inc, Natick, Massachusetts

Miramontes O (1992) Complexity and Behaviour in Leptothorax Ants. Master Thesis, Open University, UK

Mitchell, M. 2009 *Complexity: A guided tour*. Oxford University Press.

Miyatake T (2001a) Effects of starvation on death-feigning in adults of *Cylas formicarius* (Coleoptera: Brentidae). Ann Entomol Soc Am 94:612-616

Miyatake T (2001b) Diurnal periodicity of death-feigning in *Cylas formicarius* (Coleoptera:Brentidae). J Insect Behav 14:421-431

Nicolis SC, Deneubourg JL (1999) Emerging patterns and food recruitment in ants: an analytical study. J Theor Biol 198:575-592. doi: 10.1006/jtbi.1999.0934

Nishii J (2000) Legged insects select the optimal locomotor pattern based on the energetic cost. Biol cybern 83:435-442

Neves FM, Pie MR, Viana RL (2012) Self-organization in the movement activity of social insects (Hymenoptera: Formicidae), AIP Conf Proc 1479:658-661. doi: 10.1063/1.4756220

Oelrichs PB, MacLeod JK, Seawright AA, Moore MR, Ng JC, Dutra F, Riet-Correa F, Mendes MC, Thamsborg SM (1999) Unique toxic peptides isolated from sawfly larvae in three continents. Toxicon 37:537-544

Öster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton.

Parrish JK, Edelstein-Keshet L (1999) Complexity, pattern, and evolutionary trade-offs in animal aggregation. *Science*, 284(5411), 99-101.

Ovaskainen O, Cornell SJ (2003) Biased movement at a boundary and conditional occupancy times for diffusion processes. *J. Appl. Probab*. 40, 557-580.

Pie MR, Traniello JFA (2007) Morphological evolution in a hyperdiverse clade: the ant genus *Pheidole*. J Zool 271(1):99-109 doi: 10.1111/j.1469-7998.2006.00239.x

Prohammer LA, Wade MJ (1981) Geographic and genetic variation in death-feigning behavior in the flour beetle, *Tribolium castaneum*. Behav Genet 11:395-401

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (url:http://www.R-project.org/)

Raymundo DL, Bezerra Jr PS, Bandarra PM, Pedroso PMO, Oliveira EC, Pescador CA, Driemeier D (2008) Spontaneous poisoning by larvae of *Perreyia flavipes* (Pergidae) in sheep. Pesquisa Vet Brasil 28:19-22

Raymundo DL, Bezerra Junior PS, Bandarra PM, Santos AS, Sonne L, Pavarini SP, Côrrea AMR, Dias MM, Driemeier D (2009) Intoxicação espontânea pelas larvas de *Perreyia flavipes* em bovinos no Estado de Santa Catarina, Brasil. Ciência Rural 39:163-166

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos T R Soc B 365(1560):4051-4063. doi: 10.1098/rstb.2010.0208

Reid CR, Sumpter DJT, Beekman M (2011) Optimisation in a natural system: Argentine ants solve the Towers of Hanoi. J Exp Biol 214:50-58. doi: 10.1242/jeb.048173

Riet-Correa F, Dutra F, Soares MP, Mendez MC (1998) Sawfly (*Perreyia flavipes*) larval poisoning of cattle, sheep and swine. In: Garland T, Barr AC (eds) Toxic Plants and Other Natural Toxicants, CAB International Press, pp 291-296

Riley MA, Balasubramaniam R, Turvey MT (1999) Recurrence quantification analysis of postural fluctuations. Gait Posture 9:65-78. doi: 10.1007/s10439-013-0790-x

Schiestl FP, Ayasse M (2000) Post-mating odor in females of the solitary bee, *Andrena nigroaenea* (Apoidea, Andrenidae), inhibits male mating behavior. Behav Ecol Sociobiol 48:303–307

Schiestl FP, Ayasse M (2001) Post-pollination production of a repellent compound in a sexually deceptive orchid: a new mechanism for maximizing reproductive success? Oecologia 126:531-534

Schindelin J, Arganda-Carreras I, Frise E, et al. (2012) Fiji: an open-source platform for biological-image analysis, *Nature methods* 9(7): 676-682, PMID

Seymour RS (1974) Convective and evaporative cooling in sawfly larvae. J Insect Physiol 20:2447-2457.

Singer TL (1998) Roles of hydrocarbons in the recognition systems of insects. Am Zool 38:394–405

Smith DR (1990) A synopsis of the sawflies (Hymenoptera, Symphyta) of the America South of the United States: Pergidae. Rev Bras Ent 34:7-200

Smith DR, Wagner MR, Raffa KF (1993) Systematics, life history, and distribution of sawflies. Sawfly life history adaptations to woody plants. In: Wagner MR, Raffa KF (eds) Sawfly life history adaptations to woody plants. Academic Press Inc., San Diego, pp. 3-32

Soares MP, Riet-Correa F, Smith DR, Pereira Soares M, Mendez MC, Brandolt AL (2001) Experimental intoxication by larvae of *Perreyia flavipes* Know, 1899 (Hymenoptera: Pergidae) in pigs and some aspects on its biology. Toxicon 39:669-678

Soares MP, Quevedo OS, Schild AL (2008) Intoxicação por larvas de *Perreyia flavipes* Konow, 1899 (Hymenoptera: Pergidae) em bovinos na região sul do Rio Grande do Sul. Pesquisa Vet Brasil 28:169-173

Solé RV, Miramontes O, Goodwin BC (1993) Oscillations and chaos in ant societies. J Theor Biol 161: 343-357. doi: 10.1006/jtbi.1993.1060

Sumana A, Starks PT (2004) Grooming patterns in the primitively eusocial wasp *Polistes dominulus*. Ethology 110:825–833

Suzuki K, Takagi T, Hiraishi T (2003) Video analysis of fish schooling behavior in finite space using a mathematical model. Fish Res 60, 3-10.

Sweeney K, Cusack B, Armagost F, O'Brien T, Keiser CN, Pruitt JN (2013) Predator and prey activity levels jointly influence the outcome of long-term foraging bouts. Behav Ecol 24:1205-1210. doi: 10.1093/beheco/art052

Theiler J, Eubank S, Longtin A, Galdrikian B, Farmer JD (1992) Testing for nonlinearity in time series: the method of surrogate data. Physica D 58(1-4):77-94. doi: 10.1016/0167-2789(92)90102-S

Theraulaz G, Bonabeau E, Nicolis SC, Solé RV, Fourcassié V, Blanco S, Fournier R, Joly JL, Fernandez P, Grimal A, Dalle P, Deneubourg JL. 2002. Spatial patterns in ant colonies. Proceedings of the National Academy of Sciences, U.S.A., 99, 9645-9649.

van Veen JW, Sommeijer MJ, Aguilar MI (1999) Behavioural development and abdomen inflation of gynes and newly mated queens of *Melipona beecheii* (Apidae, Meliponinae). Insect Soc 46: 361-365

Webber Jr. CL, Zbilut JP (1994) Dynamical assessment of physiological systems and states using recurrence plot strategies. J Appl Physiol 76:965-973

Webber Jr. CL, Marwan N, Facchini A, Giuliani A (2009) Simpler methods do it better: Success of Recurrence Quantification Analysis as a general purpose data analysis tool. Phys Lett A 3753:3756. doi: 10.1016/j.physleta.2009.08.052

Wild AL (2007) Taxonomic revision of the ant genus *Linepithema* (Hymenoptera: Formicidae). Univ of California Press, 126

Wild AL (2009) Evolution of the Neotropical ant genus *Linepithema*. Syst Entomol 34:49-62. doi: 10.1111/j.1365-3113.2008.00435

Wilson EO (1985) The sociogenesis of insect colonies. Science 228:1489-1495. doi: 10.1126/science.228.4707.1489

Wilson EO (2003) *Pheidole* in the New World: a dominant, hyperdiverse ant genus. Harvard University Press, Cambridge, MA

Zbilut JP (1992) Embeddings and delays as derived from quantification of recurrence plots. Phys Lett A 171:199-203. doi: 10.1016/0375-9601(92)90426-M