Mechanisms of Individual and Collective Behaviour in Neotropical Termite Species

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submitted by

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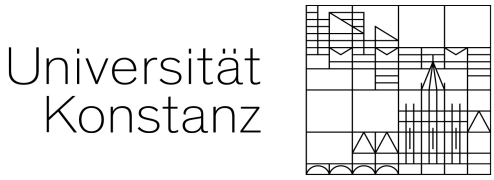

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To my family,
for their unconditional
love and support.

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Summary

How do animals decide about when and where to go? What triggers a specific type of collective motion or lead organisms to move in a particular way? If we are to provide answers to such questions, we first need to understand and characterise the baseline behaviour of individuals. Behavioural estimation, however, can be difficult when field observation is challenging and replication of natural behaviour in laboratory is limited. When working with colonial organisms in laboratory, for instance, vertical walls of more traditional experimental arenas can disrupt free movement of individuals and prevent the detection of collective patterns.

In the **first chapter** of this thesis, to overcome this limitation, I developed an experimental protocol to observe whole colonies of termites under controlled laboratory conditions. In addition to providing details about experimental procedures and discussing factors of relevance when conducting behavioural experiments, this study (in preparation for submission) shows results of movement patterns that corroborate to validate the method as a sufficiently practical tool. For being inexpensive and of easy setup, this approach should allow behavioural observation and recording of thousands of markerless termites, making possible the investigation of several group behaviours and collective patterns of knowledge currently incipient.

In the **second chapter**, I focused on collective motion and investigated how discrete movements of individuals can give rise to emergent patterns at the group-level. Collective behavior has been the subject of a broad general interest for nearly two decades now, yet studies looking at the mechanistic components of coordinated movement in termites are rare. Using the protocol developed in the first chapter, I conducted experiments with whole colonies of the termite *Constrictotermes cyphergaster* to specifically investigate how the structure of groups - characterised by group size and group composition - affects the emergence of coordinated collective motion in termites. This study shows that both group size and group composition influence coordinated collective motion of termites.

The **third chapter** consists of a piece of natural history, in which I described a new potter wasp species from the Brazilian Cerrado, *Montezumia termitophila* sp. nov. (Hymenoptera: Vespidae: Eumeninae). This chapter has been published in *Ecology and Evolution* and provides the very first evidence of association between active colonies of termites and potter wasps. In addition, this work proposes a life cycle for the new wasp species, based on the documentation we reported. This publication highlights the importance of natural history in Biology and reinforces the view of termite societies as an ideal biological system to investigate fundamental questions about animal behaviour.

Finally, in the **fourth chapter**, I shift the focus towards the consequences of evolved morphological traits in the behaviour of eusocial individuals. In termites, caste dimorphism stems not only from behavioural traits and social interactions but also from morphological differences exhibited by individuals, which enables a group of colonial members – namely, a caste - to perform distinct intrinsic colony tasks. Inspired by theoretical concepts of movement ecology and using an interdisciplinary approach, I measured the movement behaviour of *Syntermes molestus* (Burmeister, 1934), a termite known for its accentuated caste dimorphism, in which soldiers are not only larger than workers but also morphologically different. This study, in preparation for submission, demonstrates that, as opposed to a single algorithm of motion operating in both worker and soldier castes, the set of morphological differences exhibited by soldiers allow them to move differently, in a way that reflects their evolutionarily adaptive traits.

In this thesis, although establishing connections with ecological and evolutionary aspects of termite's natural history, I focused primarily on behavioural ecology, looking at the mechanisms behind individual and collective patterns. While the findings I present here fill some of many gaps of termite behavioural ecology, they also raise several interesting questions about the causes and consequences of behavioral mechanisms of socially complex societies. After working with termites for many years, my understanding is that behaviour, performed either individually or collectively, has a pivotal role in the impressive evolutionary success of eusocial systems. With recent technological advances in the field of animal behavior, it is exciting that empirical approaches to simultaneously assess the behaviour of thousands of individuals with accuracy - until recently impossible - are now within our grasp.

Zusammenfassung

Wie entscheiden die Tiere, wann und wohin sie sich bewegen? Was löst eine bestimmte Art von kollektiver Bewegung aus oder veranlasst Organismen, sich auf eine bestimmte Weise zu bewegen? Wenn wir Antworten auf solche Fragen finden wollen, müssen wir zunächst das Grundverhalten von Individuen verstehen und charakterisieren. Die Einschätzung des Verhaltens kann jedoch schwierig sein, wenn die Beobachtung im Freiland schwierig und die Reproduktion des natürlichen Verhaltens im Labor begrenzt ist. Bei der Arbeit mit kolonialen Organismen im Labor können beispielsweise die vertikalen Wände traditioneller Versuchsfelder die freie Bewegung der Individuen stören und die Erkennung von kollektiven Mustern verhindern.

Um diese Einschränkung zu überwinden, habe ich im **ersten Kapitel** ein Versuchsprotokoll entwickelt, mit dem ganze Kolonien von Termiten unter kontrollierten Laborbedingungen beobachtet werden können. Zusätzlich zu den Einzelheiten der experimentellen Verfahren und der Erörterung von Faktoren, die bei der Durchführung von Verhaltensexperimenten von Bedeutung sind, zeigt diese Studie (in Vorbereitung für die Einreichung) Ergebnisse von Bewegungsmustern, die die Methode als ausreichend praktisches Instrument bestätigen. Da diese Methode kostengünstig und einfach einzurichten ist, sollte sie die Verhaltensbeobachtung und -aufzeichnung von Tausenden von Termiten erlauben und die Untersuchung verschiedener Gruppen- und Kollektivverhaltensweisen ermöglichen, deren Kenntnis derzeit noch am Anfang steht.

Im **zweiten Kapitel** habe ich mich auf kollektive Bewegungen konzentriert und untersucht, wie diskrete Bewegungen von Individuen zu neuen Mustern auf Gruppenebene führen können. Obwohl kollektives Verhalten schon seit fast zwei Jahrzehnten Gegenstand eines breiten allgemeinen Interesses ist, sind Studien, die sich mit den mechanistischen Komponenten koordinierter Bewegungen bei Termiten befassen, noch immer selten. Unter Verwendung des im ersten Kapitel entwickelten Protokolls habe ich Experimente mit ganzen Kolonien der Termite *Constrictotermes cyphergaster* durchgeführt, um speziell zu untersuchen, wie die Struktur von Gruppen, gekennzeichnet durch Gruppengröße und Gruppenzusammensetzung, das Auftreten koordinierter kollektiver Bewegungen bei Termiten beeinflusst. Diese Studie zeigt, dass sowohl die Gruppengröße als auch die Gruppenzusammensetzung die koordinierte kollektive Bewegung von Termiten beeinflussen.

Das **dritte Kapitel** besteht aus einem Stück Naturgeschichte, in dem ich eine neue Töpferwespenart aus dem brasilianischen Cerrado beschreibe, Montezumia termitophila sp. nov. (Hymenoptera: Vespidae: Eumeninae). In diesem Kapitel, das in Ecology and Evolution veröffentlicht wurde, lieferte ich den allerersten Beweis für eine Verbindung zwischen aktiven Termitenkolonien und Töpferwespen. Darüber hinaus habe ich auf der Grundlage der von uns vorgelegten Dokumentation einen Lebenszyklus für die neue Art vorgeschlagen. Diese Arbeit unterstreicht die Bedeutung der Naturgeschichte in der Biologie und bestärkt die Ansicht, dass Termitengesellschaften ein ideales biologisches System zur Untersuchung grundlegender Fragen zum Verhalten von Tieren darstellen.

Im **vierten Kapitel** schließlich verlagere ich den Schwerpunkt auf die Auswirkungen entwickelter morphologischer Merkmale auf das Verhalten eusozialer Individuen. Bei Termiten ergibt sich der Kastendimorphismus nicht nur aus Verhaltensmerkmalen und sozialen Interaktionen, sondern auch aus morphologischen Unterschieden zwischen den Individuen, die es einer Gruppe von Koloniemitgliedern - einer Kaste - ermöglichen, unterschiedliche Aufgaben innerhalb der Kolonie zu erfüllen. Inspiriert von theoretischen Konzepten der Bewegungsökologie und unter Verwendung eines interdisziplinären Ansatzes habe ich das Bewegungsverhalten von *Syntermes molestus* (Burmeister, 1934) gemessen, einer Termite, die für ihren ausgeprägten Kastendimorphismus bekannt ist, bei dem die Soldaten nicht nur größer sind als die Arbeiterinnen, sondern sich auch morphologisch unterscheiden. Die Studie, die für die Einreichung vorbereitet wird, zeigt, dass im Gegensatz zu einem einzigen Bewegungsalgorithmus, der sowohl in der Arbeiterinnen- als auch in der Soldatenkaste funktioniert, die morphologischen Unterschiede der Soldaten es ihnen ermöglichen, sich auf eine Art und Weise zu bewegen, die ihre evolutionär angepassten Eigenschaften widerspiegelt.

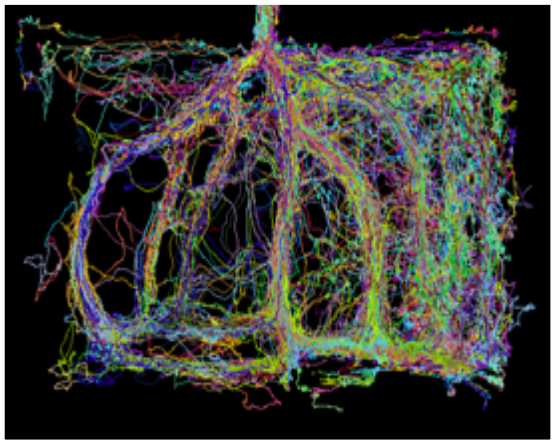
Obwohl ich Verbindungen zu ökologischen und evolutionären Aspekten der Naturgeschichte der Termiten herstellte, konzentrierte ich mich in dieser Arbeit in erster Linie auf die Verhaltensökologie und untersuchte die Mechanismen hinter individuellen und kollektiven Mustern. Die hier vorgestellten Ergebnisse füllen nicht nur einige der vielen Lücken in der Verhaltensökologie der Termiten, sondern werfen auch einige interessante Fragen zu den Ursachen und Folgen der Verhaltensmechanismen sozial komplexer Gesellschaften auf. Nachdem ich nun seit sieben Jahren mit Termiten arbeite, bin ich zu der Überzeugung gelangt, dass das Verhalten - ob individuell oder kollektiv - eine zentrale Rolle für den beeindruckenden evolutionären Erfolg eusozialer Systeme spielt. Es ist aufregend, dass mit den jüngsten technologischen Fortschritten auf dem Gebiet des Tierverhaltens empirische Ansätze zur gleichzeitigen Bewertung des Verhaltens von Tausenden von Individuen mit einer Genauigkeit, die bis vor kurzem unmöglich war, nun in greifbare Nähe gerückt sind.

Preface
About /bɪˈheɪvjər/

Understanding how to behave in a complex world is presumably one of the very first things animals do in life. It seems reasonable to say that learning a behaviour is a constant struggle, in the good sense of the word, obviously. But what is behaviour?

***Behaviour as a concept**, for instance, has been defined as "the way in which one acts or conducts oneself, especially towards others". This definition, though intuitively linkable to human societies, could be easily applied to every single animal that has ever lived in or interacted with groups, societies, and communities; from solitary to highly social species. As a matter of fact, for simplicity, one may think of an animal's actions as reactions to either biotic or abiotic components in space over time. Such an objective notion seems to hold irrespective of the animal type and regardless of the environment they are in. Yet, to put it simply, animals interacting in the wild seem to be inherently limited to two options: whenever interacting, they are doing so either with another living-being or with something else not alive.*

*When put it that way, 'behaviour' may seem not so difficult to grasp. If we think of **behaviour as a response**, however, things get more complicated. Generally, behaviours may occur individually or in groups, at completely different time scales, and from local to global scenarios. With such a range of intrinsic and extrinsic factors, it is not surprising that a behavioural response may be fairly easy to observe, yet difficult to predict. To complicate matters, variation of behaviour will occasionally appear as diverse as possible in the real world (believe me!). This is likely because animal behaviour, as opposed to morphological or physiological traits, can change 'on the fly' and more rapidly, not to mention that it can adapt almost immediately in response to both internal and external stimuli. It is perhaps for this reasons that, in my opinion, understanding proximal and ultimate causes of behavioural mechanisms and their plasticity, is at the heart of understanding life itself.*



In science, trying to make sense of behavioural variation (in all its madness) is hardly an easy task. Luckily, the idea of **behaviour as an evolutionarily adaptive trait** that varies under the umbrella of natural selection seems logical and straightforward. In fact, to date, evolutionary theory remains as elegant as ever when dealing with natural complexity (or 'weird biological idiosyncrasies', if you will). Evolution provides utterly logical explanations to even the most chaotic observations of nature. In this sense, in terms of behaviour variation and its adaptive value, since Darwin's seminal work (1859) and the "survival of the fittest" it became clear that the way organisms behave, such as responding to stimuli and situations, may eventually contribute to determine their fate in evolution. From an evolutionary perspective, therefore, **behaviour as a novelty** is something that is constantly shaped by unique selective pressures, as any other life-history trait.

There is also the idea of **behaviour as a state**: it typically relates to a living organism, yet it can be employed when referring to non-living systems too. I will explain: for instance, it is common to empirically observe and describe in detail the way a natural phenomenon occurs. In a similar fashion, one may empirically observe and describe how a sophisticated machine operates, exposing its component parts. Behavioural descriptions of living systems often provide the first steps toward understanding mechanisms underlying biological patterns. For machines, on the other hand, descriptive reports of behaviour are intrinsic to tell, for example, whether a piece of equipment is operating properly or has a malfunction. My point is: without knowing the parts, or at least the basics of it, it seems fair to say that it is impossible to tell anything about a system, whether living or not. A key difference between synthetic and biological systems is nonetheless self-evident: machines, obviously, lack any sort of biological life. One could consider a machine "alive" metaphorically, as long as it functions in line with a given purpose (and do not conflict with Asimov's first and second laws, but I digress). What I am trying to say here is that, interestingly, for both a machine and an organism, it is undeniable that behaviour will most certainly have not only an impact on daily routines but also a major role throughout their existence.

P.S.: This thesis is not about writing. Yet, writing is the craft I use to explain methods, describe patterns, discuss findings and, of course, convey messages. As someone that have always enjoyed reading and writing, I decided to include this letter as a preface of the thesis. This letter is perhaps addressed to my future self - more than to anyone else - and it served as a place where I could write about behaviour - a core topic of this thesis - in a more relaxed way. I hope that, even if my passion for biology, behaviour, and the marvellous group of termites is not shared, the reader may enjoy this read, as much as I have enjoyed writing it.

General Introduction

How can the astonishing ability of eusocial organisms to navigate through complex environments without any sort of central control be explained? Drawing on theoretical concepts, empirical practice, and experimental evidence, in this thesis I outline the fundamental principles of ecology and evolution underlying this phenomenal collective behaviour.

Understanding the proximate and ultimate causes underpinning animal behavior is of paramount importance in biology. Along the development of this thesis, I put efforts to reconcile different disciplines to properly investigate mechanisms of individual and collective behaviour in termite species. Although, theory and modelling served as conceptual foundations throughout the development of many aspects of my research with termites, my framework is primarily based on experimentation and detailed observation of behaviour. As such, along with several collaborators, I developed methods to acquire and analyse large-scale behavioral data of termites.

In this general introduction, I briefly discuss some of the core subjects related to the main topic of this thesis. In doing so, I try to conciliate both novel and classic literature to provide not only a general introduction about behavioural ecology and evolution of social insects, but also a broad picture of the current state of art, with comparative remarks about the main eusocial taxa.

This thesis contains four chapters, which I developed during my years as a doctoral student in the Department of Collective Behaviour of the Max Planck Institute of Animal Behaviour, at the University of Konstanz, Germany. This work was conducted under the supervision of Prof Dr Iain D. Couzin and is a result of a collaborative effort with different scientists, with whom I worked and had the pleasure to share co-authorship in the resulting published material. Those researchers who have been involved in projects but have not met the criteria for authorship, are properly mentioned in the acknowledgements section. By exploring biological mechanisms, this work aims to fill in important gaps in the knowledge about the behavioural ecology of termites.

I. Movement ecology in eusocial insects

Over the past half century, eusocial insects have been subject of several studies about behaviour (e.g., Noirot, 1970; Hölldobler & Wilson, 1990; Schmid-Hempel, 1990; Rosengaus & Traniello, 1993; Pinter-Wollman et al., 2012; Jandt et al., 2012; Mersch et al., 2013; Bertelsmeier et al., 2016), with many research approaches focusing on unveiling less exposed traits of the group. In fact, for displaying both individual adaptive responses to stimuli (e.g., nestmate recognition, caste detection) and sophisticated behaviours elicited by cooperative interactions between nestmates (e.g., nest building, defensive actions, foraging), many eusocial species have emerged as models for examining various aspects of behaviour (e.g., Yan et al., 2014; Feinerman et al., 2018; Lemanski et al., 2019; Revely et al., 2021). One of these behavioral aspects, movement, is arguably one of the most important elements throughout the life of individuals and, as such, has become of particular interest in the study of animal behaviour (Holyoak et al., 2008). Not surprisingly, recent conceptual and technological advances on how to measure and analyse behaviour have helped to consolidate 'movement ecology' as one of the fundamental research fields in Biology (Nathan et al., 2008).

An important question arising from observing behaviour of animals is why individuals decide to move in a particular way or another. In theory, an individual's decision to move from one place to another is not fundamentally trivial: on top of deciding when and where to go, there is invariably a matter of how to move when doing so (see '*movement ecology paradigm*' in Nathan et al., 2008). In highly social contexts, such as in the middle of eusocial colonies, a suite of smaller discrete decisions taken individually is said to ultimately influence collective outcomes, many times, reaching consensus (Conradt & Roper, 2005). As such, when performing task-focused or target-oriented behaviours (e.g., foraging and nest defence, respectively), the way how actions are performed by individuals is expected to matter as much as the collective actions themselves.

II. Collective motion in eusocial colonies

In eusocial insects, collective movement has been examined in several species and across different scales. In ants, for instance, following pioneering observations of feeding habits by naturalists (reviewed in Wheeler & Bailey, 1920), many studies have provided valuable insight on ant behaviour with the publication of detailed ethograms and behavioural repertoires (e.g., Wilson & Fagen, 1974; Wilson, 1976). Over the past 50 years or so, behavioural aspects specifically related to ant movement have been thoroughly investigated, either through mathematical modelling and simulations or empirically with field and laboratory experiments. Topics such as colony activity (Da Silva, 1977), searching behaviour (Wehner & Srinivasan, 1981), foraging organisation (Deneubourg et al., 1986), seed dispersal (Brew et al., 1989), trail following (Edelstein-Keshet et al., 1995), traffic dynamics (Burd et al., 2002; Couzin & Franks, 2002) and decision making (Franks et al., 2003), have all been elucidated to a certain degree.

Other hymenopterans have been explored too: bees, for instance, have been incrementally investigated, perhaps due to their role in many ecological interactions (e.g., pollination). The waggle dance of bees, known since the time of Aristotle, is arguably one of the most iconic examples of movement patterns seen in the group. Since its formal description in the literature (von Frisch & Lindauer, 1956), the behaviour has been further investigated (e.g., Beekman et al., 2004) and reviewed several times (Grüter & Farina, 2009; Couvillon, 2012; Couvillon & Ratnieks, 2015; Ratnieks & Visscher, 2015; Collett, 2019, Ai et al., 2019). Other movement aspects of bees that have been investigated include for example social behaviour (Michener, 1969), visual communication (Warrant, 2008), and detrimental effects of pesticides (Thompson, 2003).

Other than Hymenoptera, a third eusocial organism of general interest in terms of collective motion is the group of termites (Dyctiopera: Blattodea; formerly known as Isoptera). Although often praised for their fascinating abilities to build striking nests (Emerson, 1938; Noirot, 1970), many species can exhibit equally impressive motion patterns (Traniello 1981, Miura & Matsumoto, 1998). The number of studies about movement in termites, however, is far less impressive that those about bees and ants. In fact, because of a small group of invasive species known to cause severe economic damages every year (Su & Scheffrahn, 2000; Evans, 2010), a staggering number of papers with termites is mostly concerned about how to kill them. Also, it is difficult to tell whether the smaller proportion of young scientists interested in termites is caused by the fact that people unfamiliar with the group mistakenly assume that every termite species is a pest. But given how popular science often portrays the group, it would not be surprising if that is the case. Of course, studies about pest

control involving termite species represent only a portion of the literature. The other side of the coin includes a great deal of studies focused on exploring aspects other than pest management. These reports with both invasive and non-invasive species, have helped to foster a growing interest for termites, not because of their economic impact, but for their many interesting biological features.

III. The chemistry behind movement patterns

A number of movement patters are triggered by chemical compounds such as pheromones. The term 'pheromone' - derived from the Greek *pherein* (to carry) and *horman* (to stimulate) - was first coined by Karlson and Lüscher (1959). In their study, they proposed the replacement of previous inaccurate terms such as 'ectohormones' (Bethe, 1932) and described pheromones as "substances secreted by an animal to the outside that cause a specific reaction in a receiving individual of the same species". Historically, the idea of chemicals playing a key role in social interactions started to populate the literature at least more than half century ago (Wilson, 1965). Since then, our understanding about the chemical ecology of social insects has grown exponentially. Pheromones involved with movement are among the most studied chemical compounds, particularly in termites and ants.

In the past half century, research with termites has incrementally clarified the nature of chemical communication in the group. In Isoptera, trail-laying behaviour was originally described for *Zootermopsis nevadensis* (Lüscher and Müller, 1960; Stuart, 1961) and *Nasutitermes corniger* (Stuart, 1961). A recent review of termite pheromones compiled a list with 68 species known to have evolved trail-following components (see Mitaka & Akino, 2021). More than half of these (37 species) belong to the family most studied by far, Termitidae, while other species are distributed across different families (e.g., Mastotermitidae, Archotermopsidae, Serritermitidae, Kalotermitidae and Rhinotermitidae). Nevertheless, the list of species exhibiting trail pheromones is likely grow over time, as several species correlated to those listed in the review have not been fully investigated yet. For instance, *C. cyphergaster* – one of the biological systems studied in this thesis - is the only listed species of *Constrictotermes* (Holmgren 1910), but the genus includes a number of other termites (e.g. *C. cacaoensis, C. cavifrons, C. electroconstrictus, C. guantanamensi, C latinotus, C.parviceps, C. rupestris*). It is plausible, therefore, that species not listed in that study either share similar components (i.e., 3-6-8-dodecatrienol and Neocembrene: Cristaldo et al., 2014) or present novel chemicals.

IV. Recent advances in animal tracking

Since the first known ethological studies our understating of animal behaviour has progressed extensively, perhaps as a direct consequence of the flourishment of modern techniques to quantifying animal movement. Yet, only recently with the advent of computational methods (e.g., based on machine learning), advances in the study of animal behaviour have reached another level, in which it is possible to quantify the behaviour of hundreds - if not thousands - of individuals simultaneously. Of course, working in experiments with that many individuals comes with challenges. One of the these, for instance, is how to properly keep the identity of individuals over time without having to manually intervene the process of tracking.

Several image-based tracking software claim to be capable of automatically extracting data from videos (for a review, see Dell et al., 2014). With most of these tracking methods, however, when working with videos containing a dynamic background or in situations where object occlusion is frequent, the task of tracking individuals while keeping identities becomes challenging. To make matters worse, developing custom solutions for tracking specific animal systems from scratch can be laborious and time-consuming. Also, when dealing with large number of individuals simultaneously, many of the open-source tracking solutions currently available are incipient. In a video, to keep individual identities consistently over time, it is necessary to extract and maintain the positional information (x-y coordinates) for each one of the individuals from one frame to another. Although object detection from frames can be achievable, for example, by subtracting moving pixels from the static pixels of a video background, matching the identities of detected moving elements (obstacles) requires more complex calculations.

One approach of computer science that has been largely adopted for animal tracking is the Kuhn-Munkres algorithm (Kuhn, 1955; Munkres, 1957), also known as Hungarian algorithm. In this method, the idea is to define bounding boxes for each of the obstacles detected in a current frame and then compare the position of bonding boxes of obstacles in the next frame. The matching process relies on Intersection Over Union (IOU) scores that vary from zero to one: scores close to one mean that bounding boxes in two consecutive frames are overlapping, which means that they probably refer to the same object. Likewise, a low IOU score means that there is little overlapping between two bounding boxes, and thus a lower probability of having the same object in the respective positions. Additionally, in two-dimensional setups, shape scores may be used to improve the identification and matching between two consecutives frames (e.g., associating higher scores for objects varying less in shape or size).

Another important method, frequently used in combination with the Hungarian algorithm, is the Kalman filter (Kalman, 1960), which allows the prediction of current and future positions of tracking obstacles. As a Bayesian filter implementation, the method consists of a sequence of alternations between (i) a prediction phase, in which an estimated state is used to predict the current state and its uncertainty, and (ii) an update phase, when observation of sensors is used to correct the predicted state and obtain more accurate estimates.

V. Collective behaviour in complex systems

A useful way to interpret a given complex system is the idea of it as an assemblage of simpler units capable of executing non-trivial, large-scale behaviours. Repeated interactions performed by groups of these 'simpler units' are known to scale to representative and visually impressive collective behaviours. In fact, rather than intrinsically driven by intricate cognitive processes, many collective behaviours have been revealed as emergent phenomena arising from local interactions among the individual units (Sumpter, 2006). In science, self-organization and collective behaviour have been consistently investigated over the last decades both in vertebrate (Couzin & Krause, 2003) and invertebrate (Goss et al., 1989; Deneubourg et al., 1999; Millor et al., 1999; Camazine et al., 2001; Detrain et al., 2001; Boomsma & Franks, 2006) animal groups.

From optimization of processes and functions, such as enhanced foraging and improved locomotion efficiency, to eye-catching collective patterns of synchronous movement, collective behaviour seems to be 'imprinted' on complex systems. For instance, animal groups facing uncertainty are often required to respond quickly when making decisions (Couzin, 2009). This is especially crucial in species reliant upon non-visual cues, such as in termites.

Chapter I

Using semi-folded arenas to replicate spontaneous emergence of collective trails in whole colonies of termite species

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ABSTRACT

Behaviour is intrinsically linked to the ecology and evolution of organisms, yet it may be difficult to measure in species with cryptic habits. We demonstrate that semi-folded arenas can be used as a controlled device to replicate natural, spontaneous collective motion of an entire termite colony. Being relatively inexpensive, this method can be applied with adaptations to capture individual and collective behaviour of number of other eusocial species. To demonstrate the utility of semi-folded arenas and test whether our protocol is suitable to replicate self-organized trails in termites, we recorded the behaviour of 16 natural colonies of *Constrictotermes cyphergaster* (Silvestri 1901) (Termitidae: Nasutitermitinae), a Neotropical termite species currently not listed as endangered or threatened species. Using high-resolution cameras to record and computer vision techniques to extract the termites' motion, we managed to capture the onset of spontaneous self-organized trails, as well as to characterize a variety of collective patterns that dynamically emerge and fade out over time. The ability to track and analyse the dynamics of self-organized trails of whole colonies in controlled environments can aid in addressing historically neglected aspects of collective motion in complex societies.

Keywords: Collective behavior, Trail formation, Self-organization, Termitidae, Nasutitermitinae, *Constrictotermes cyphergaster*

1. INTRODUCTION

Understanding the behavioural ecology of organisms requires a good balance between detailed observation and unbiased experimentation. Combined, these approaches should provide, in theory, good estimates for any baseline behaviour of interest. Behavioural estimation, however, may be difficult to obtain when field observation is challenging and replication of natural behaviour in laboratory is limited, as in species with cryptic habits. When studying eusocial insects, for instance, measuring behaviour can be somewhat troublesome, since colonial members spend a non-negligible amount of time being inactive (Hölldobler & Wilson, 1990; Schmid-Hempel, 1990; Rosengaus & Traniello, 1993, Pinter-Wollman et al., 2012; Jandt et al., 2012) or performing behaviours inside a closed nest (Noirot, 1970; Noirot & Darlington, 2000; Mersch et al., 2013; Bertelsmeier et al., 2016). Interactions and cooperative tasks performed by colony members within nest walls are inadvertently hidden from naked-eye observation. As a result, despite being relatively easy to locate in the wild, the physical structure of eusocial nests could be interpreted, to some extent, as a "black box" encrypting the colony's behaviour. It seems therefore inevitable that methods attempting to expose these cryptic behaviours would arise as solutions to access the full behavioural repertoire of eusocial species.

In behavioural research with termites, while some work has been done in the field (e.g., Shellman-Reeve, 1997; Evans et al., 2011) or sometimes with a combination of both field and laboratory procedures (Ferreira et al., 2018), most studies describe results found in strictly controlled conditions. A common practice in laboratory studies with termites is the design of experimental arenas using containers of various shapes and sizes. With Petri dishes, for instance, a large body of research has been done investigating topics such as aggressiveness (Eisner et al., 1976; Haverty & Thorne, 1989; Su & Haverty, 1991; Delphia et al., 2003; Florane et al., 2004; Ishikawa & Miura, 2012), mating (Mizumoto & Dobata, 2019; Valentini et al., 2020; Mizumoto et al., 2020), social response to infection (Davis et al., 2018), alarm reaction to pheromones (Reinhard & Clément, 2002) and regulatory mechanisms of caste differentiation (Matsuura et al., 2010). Evidently, not everything comes down to using Petri dishes in laboratory, and it is not surprising that specialized solutions have been thought with certain groups in mind. Studies with invasive termite species, for example, have used multiple interconnected planar arenas, a design seemingly more appropriate to investigate foraging patterns of subterranean species (e.g., *Coptotermes formosanus*: Su et al., 2017). In addition, early studies looking at the effect of air movement on termite behaviour have described the use of glass tubes to simultaneously contain termite individuals and facilitate behavioural observation (Howse, 1966). Such examples illustrate that the species' habits may have

a direct influence in the type of setup established. But besides the model species itself, other reasons may affect the final design of experiments. Some protocols have been designed such as to allow the observation of behaviours triggered in specific situations (e.g., during interspecific interactions). In a previous study investigating nonaggressive behaviour in termites (Hugo et al., 2020), for instance, we designed an observational protocol to increase the probability of encounter between nest-sharing termite species: a host (*Constrictotermes cyphergaster* Silvestri 1901) and its obligatory inquiline (*Inquilinitermes microcerus* Silvestri 1901). Similarly, but investigating *Coptotermes gestroi* (a pest species), Mizumoto et al. (2020) developed a protocol combining behavioral observation in experimental arenas with computer simulations to elucidate movement patterns during mating behavior. These two independent studies share similarities in their methodology: as in several other studies, in both reports experimental arenas composed of Petri dishes have been used to increase the probability of encounter between termite individuals and facilitate the observation and measurement of context-dependent behaviours.

Comparatively, experiments with whole colonies of eusocial insects are less frequently conducted but can be nonetheless found in the literature. Studies with bees (e.g., Smith et al., 2016; Wild et al. 2021) and ants (e.g., Mersch et al., 2013; Bertelsmeier et al., 2016) using whole colonies have been recurrently proposed and validated in recent years. In contrast, advances in the development of methods and protocols to deal with whole colonies of termites in controlled conditions are still rare. To the best of our knowledge, little is the number of publications that manage to replicate natural termite collective behaviours in laboratory. This study aims to overcome this limitation, by proposing a protocol that allows the observation of whole colonies of termites under controlled conditions. Our method is based on the usage of semi-folded arenas as a platform to record various aspects of termite's behavioural ecology. This inexpensive, easy-to-setup approach allows behavioural observation and recording of colonies with hundreds of thousands of individuals, making possible the investigation of several individual and collective behaviours of knowledge currently incipient.

2 MATERIAL & METHODS

2.1 Biological species

We worked with *Constrictotermes cyphergaster* (Silvestri 1901) (Termitidae: Nasutitermitinae), a Neotropical termite with nocturnal foraging with occurrence in South America (Mathews, 1977; Constantino 1998; Krishna et al., 2013), but also present in dryland areas of Brazil (Godinho et al., 1989; Moura et al., 2006; Vasconcellos et al., 2007; Bezera-Gusmão et al., 2013). Recently, *C.*

cyphergaster has been increasingly studied due to nest-sharing and association with secondary termite species (e.g., *Inquilinitermes microcerus*; Cristaldo et al., 2012; Cristaldo et al., 2014; Florencio et al., 2014; Cristaldo et al., 2016; DeSouza et al., 2016; Rodrigues et al., 2018; Hugo et al., 2020a) and other organisms, such as rove beetles (e.g., *Corotoca melantho*; Rosa et al., 2008; Rosa et al., 2018; Pisno et al., 2019) and, so far, one potter wasp species (e.g., *Montezumia termitophila*; Hugo et al., 2020b).

2.2 Study site and sampling

To conduct experiments using semi-folded arenas, 16 termite nests were collected in the Brazilian Cerrado (Ratter et al., 1997), in areas currently classified as 'equatorial savannas with dry winters' (Kottek et al., 2006). Sampling occurred in 2019 and 2020, at the beginning of the wet season (November). Nests were collected near the municipality of Divinópolis (20º08'20"S, 44º53'02"W), in the State of Minas Gerais (South-eastern Brazil).

Termite nests were collected by a single collector (HH), using a picola hammer to detach the nests from tree trunks and plastic boxes to transport all nest material. As nests of this species tend to be attached to tree trunks by a small section (**Fig. S1**, Supplementary Material), a few insertions with the picola hammer in the part of the nest connected to the tree are enough to detach the whole nest without causing much damage. Immediately after collection, nests were transported to the recording room and colonies were kept in their original nests. To provide suitable conditions of relative humidity (75 -80%) and temperature (24-26ºC) (Collins, 1969), nests were placed inside nest boxes with the exposed section (i.e., originally attached to trees) facing down (**Fig. 1: C-D**).

2.3 Design of semi-folded arenas

To allow free-walking of termites and minimise the effect of physical barriers in observable collective behaviours, we designed a circular arena with a semi-folded section at the edge of the main surface: the circular edge of the arena folds in a u-grove shape, providing an infinite closed-loop space where termites can walk continuously during the whole behavioural observation (**Fig. 1: A-B**). The folded landscape is intended to allow individual termites to move back and forth within the recording area without immediate obstacles (e.g., vertical walls).

To prevent individuals from climbing vertically the wall and scaping the arena, the outer surface of the folded section had a smooth texture, slippery enough to contain *C. cyphergaster* termites. Work conducted with species able to climb vertical smooth surfaces effortlessly, such as ants equipped with tarsal claws (Endlein & Federle, 2015), may require additional features to create an even more slippery surface. In this sense, application of talc particles, a method previously used in experiments involving termites and ants (Cornelius & Grace, 1996) and generally employed for rearing captive colonies of ants (e.g., Banks et al., 1981), could confer non-stick properties to surfaces and prevent individuals from scaping. For instance, in addition to solutions of Polytetrafluoroethylene (commercially known as Fluon® PTFE. AGC Chemicals Inc), a mixture of talc powder and ethanol has been suggested as an alternative when dealing with ant species of small size (Ning et al., 2019).

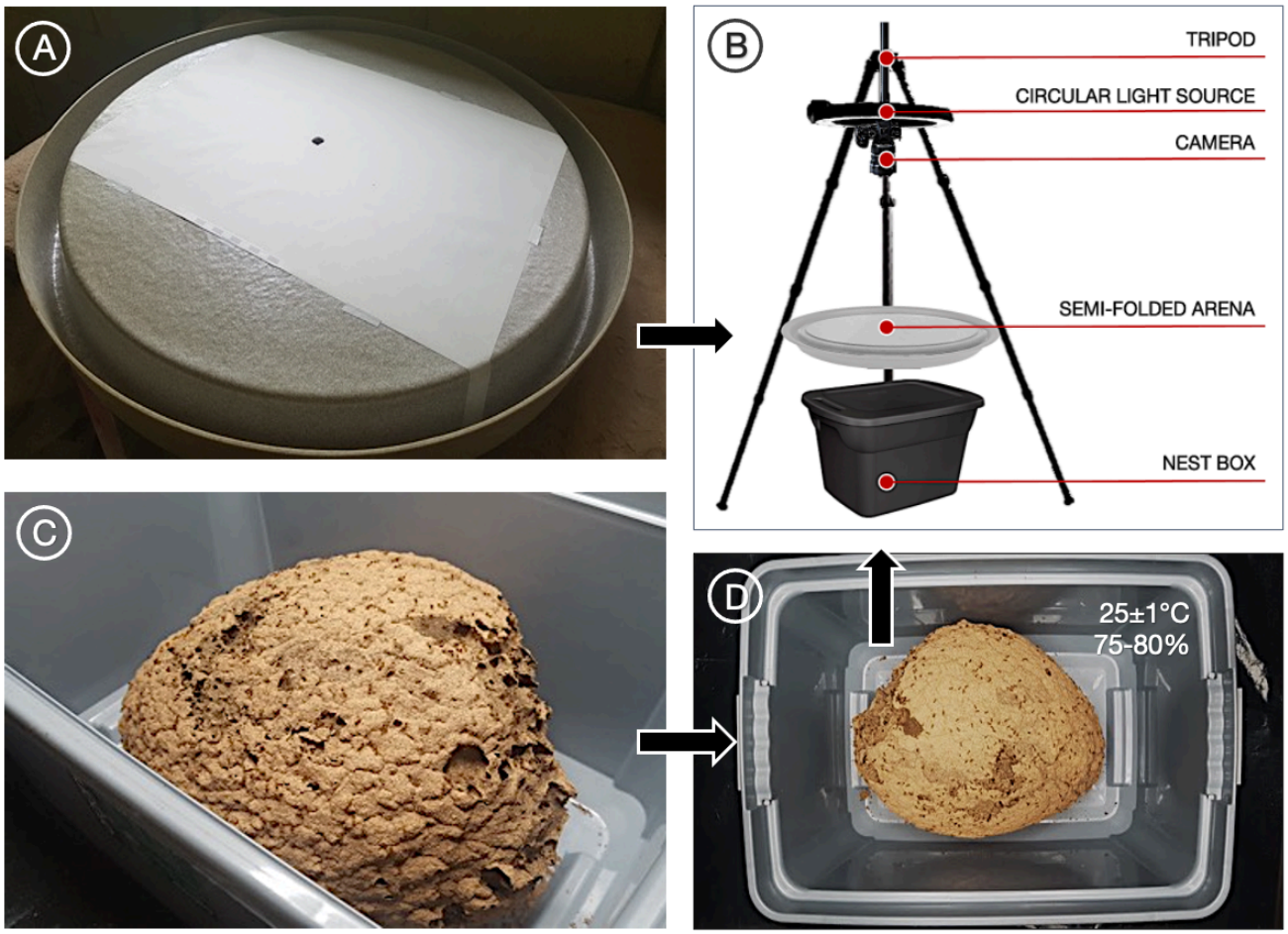

Figure 1 | Experimental design: Semi-folded circular arena (diameter=42cm) lined with tracing paper (DIN A3) and graphic scale (top-lateral view) (A); Schematic diagram of with components of recording setup [tripod, circular light source, semi-folded arena, nest box] (B); Whole colony of termites [*C. cyphergaster*] (C); Nest box to accommodate colonies under controlled conditions of temperature and humidity [25±1°C, 75-80%].

2.4 Arena preparation

We tested different surface solutions, aiming to provide ideal conditions for the movement of termites. From our pilot experiments, we found that lining semi-folded arenas with sheets of tracing paper provides the best performance, with termites forming collective paths very quickly (**Fig. 2**). Commercially, the maximum dimension of most tracing paper brands is DIN A0 (841 × 1189 mm = $33^{1/8} \times 46^{19/24}$ in), but it can be difficult to find. In Brazil, where we conducted the study, the maximum paper dimension commercially available at the time of experiments was DIN A3 (297 × 420 mm = $11^{17/24} \times 16^{13/24}$ in). As for the quality of the tracing paper itself, higher grammage is highly recommended (190 g/m² or superior, often marketed as 'premium') since it prevents paper wabbling from airborne moisture, which indeed affected the motion of termites during pilots. Because the tracing paper dimension was DIN A3, our semi-folded arenas were designed with 420mm in diameter, so the paper would fit nicely inside the arena circumference (**Fig. 2: 1**).

2.5 Recording setup and calibration

To record high-resolution videos of termites (4k @25 fps), we used a Sony Alpha 7SII camera equipped with a Zeiss Batis 25mm f/2 lens. One of the main advantages of this model - and newer versions of the same series - is that the camera's image sensor is more sensitive in low-light conditions, a particularly useful feature if experimental conditions require darker environments. In our case, with *C. cyphergaster*, we recorded behaviours under visible spectrum of light (with LEDs), since it is an established protocol for video-recording the species. We used a lighting source composed by a single 18" LED ring light with adjustable colour temperature and brightness (Neewer ® RL-18II), aligned symmetrically to the camera lens position (**Fig. 1: B**). This was by far the best solution for recording videos with semi-folded circular arenas, as it provides an evenly distributed and diffusive light that prevents shadows and facilitate posterior animal tracking.

As for the camera+lens device, because the subject distance (i.e., distance between camera and surface of arenas) was 50 cm and the focal length/aperture was 25-f/2.0, the 'depth of field' (DoF) parameter was the following: DoF near limit=47.82 cm, DoF far limit=52.39 cm, Depth of field=4.57 cm, Depth of field in front=2.18 cm (47.72%), Depth of field behind=2.39 cm (52.28%). We set the focus using the auxiliary camera setting, zooming in to make sure the image at the level of the arena surface was sharp. To set the camera axis and define the image size, as to cover the observation arena with some margin (**Fig. 2: 2**), we used the graphical scale and calibration marks on the sides on the tracing paper. With all set in its place, each whole-colony observation was then recorded

using a unique termite nest collected in either one of the field expeditions (November 2019, November 2020). We reiterate that it is of foremost importance to calibrate the system before starting recordings, since the quality of the video recorded will determine the acquisition of tracking data.

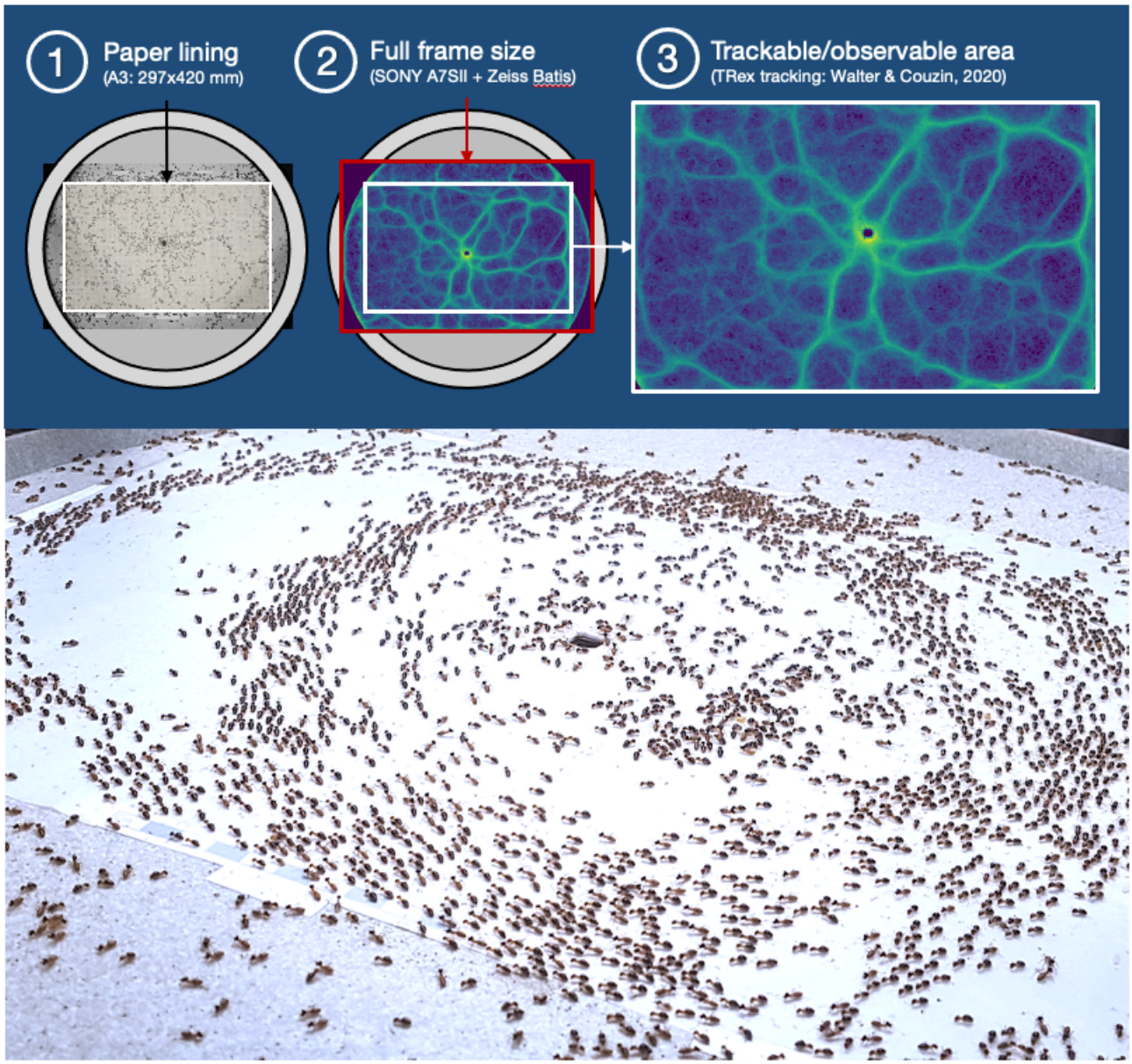

Figure 2 | Data acquisition from high-resolution videos: Semi-folded circular arena lined with and graphic scale (1); (B); Whole colony of termites [*C. cyphergaster*] (C); Nest box to accommodate colonies under controlled conditions of temperature and humidity [25±1°C, 75-80%]

2.5 Control of temperature and humidity

Effects of temperature and humidity on behavioural and physiological responses of termites have been reported for different species (e.g., Collins 1969; Steward, 1982; Carey et al., 2019), but more

frequently in economically significant ones (with pest status), such as *Coptotermes formosanus* (Nakayama et al., 2004; Gautam & Henderson, 2011; Wiltz, 2015; Zukowski & Su, 2017) and *Reticulitermes flavipes* (McManamy et al., 2008, Wiltz, 2015). Generally, compared to eusocial Hymenopterans, such as bees (Radchenko & Pesenko, 1994) and ants (Bujan et al., 2016), termites are more susceptible to water loss. Evidence suggests that except for drywood termites, which have evolved to produce increased amounts of metabolic water in lower humidity, and thus require little water to survive (Steward, 1982), most termites cannot tolerate low airborne moisture. Excessive desiccation is, therefore, expected to compromise research with termites, and for this reason it is crucial to provide laboratory conditions as to avoid detrimental effects. Dampness (i.e., slightly wet condition), on the other extreme, is equally a problem. In laboratory, high humidity in combination with warmer temperatures comes with a price: these are ideal conditions for the growth of many fungi and bacteria (Nevalainen et al., 2015). As a result, one challenge when dealing with captive colonies of termites indoors is to simultaneously keep adequate conditions, humidity- and temperature-wise, and prevent external contamination from the proliferation of opportunistic microorganisms. In this regard, customised humidity chambers have been proposed previously as a method for maintaining subterranean termite species in laboratory (Grace, 1986). In fact, for years, variations of such method have been generally adopted by researchers in protocols for rearing captive termite colonies. Obviously, different termite species require different conditions and only by systematically testing and adjusting the conditions it is possible to establish a protocol that works in the long term. In species without established rearing protocols (e.g., many Neotropical termites), a solution is to sample either whole nests or individuals from natural colonies and, in the best-case scenario, immediately conduct experimental procedures. Nonetheless, even working with termites from natural colonies, controlling the pair humidity-temperature during experiment procedures is mandatory to obtain results with quality. In general, it is possible to mitigate effects of termites' dehydration by (i) keeping individuals within their original nests prior to bioassays, (ii) limiting periods of behavioural observation and video recordings and, most importantly, (iii) controlling humidity inside containers used to do experimental procedures with individuals. In this study, we took all these measures to provide suitable conditions for the emergence of collective trails. We recommend that other studies eventually using our protocol, with termites or other eusocial insects, do the same.

Tracking individuals and validation of protocol

In theory, videos containing static backgrounds illuminated by homogenous and calibrated light source, can be tracked using a variety of available tracking algorithms (Dell et al., 2014). In this study, to acquire movement data from high-resolution of videos of termites, we used TRex, a software

ideally suited for tracking multiple markerless individuals (for details, see Walter & Couzin, 2021). To provide evidence that our protocol is suitable for its intended purpose (i.e., replicate spontaneous emergence of collective trails in termites) and validate the method, we used the first six minutes of tracking data acquired with TRex and plotted individual trajectories using Python basic libraries (e.g., matplotlib). Additionally, we used Kernel Density Estimate (KDE) methods to compile for each colony a comparative graphical representation of speed profiles per caste. These results should be extended and analysed in detail in a companion paper.

3 RESULTS

3.1 Spontaneous emergence of collective behaviour

Spontaneous emergence of collective termite trails was observed in all whole-colony observations with *C. cyphergaster* (**Fig. 3**). For most termites in videos, we were able to extract positional data and plot individual trajectories (**Fig. 4**).

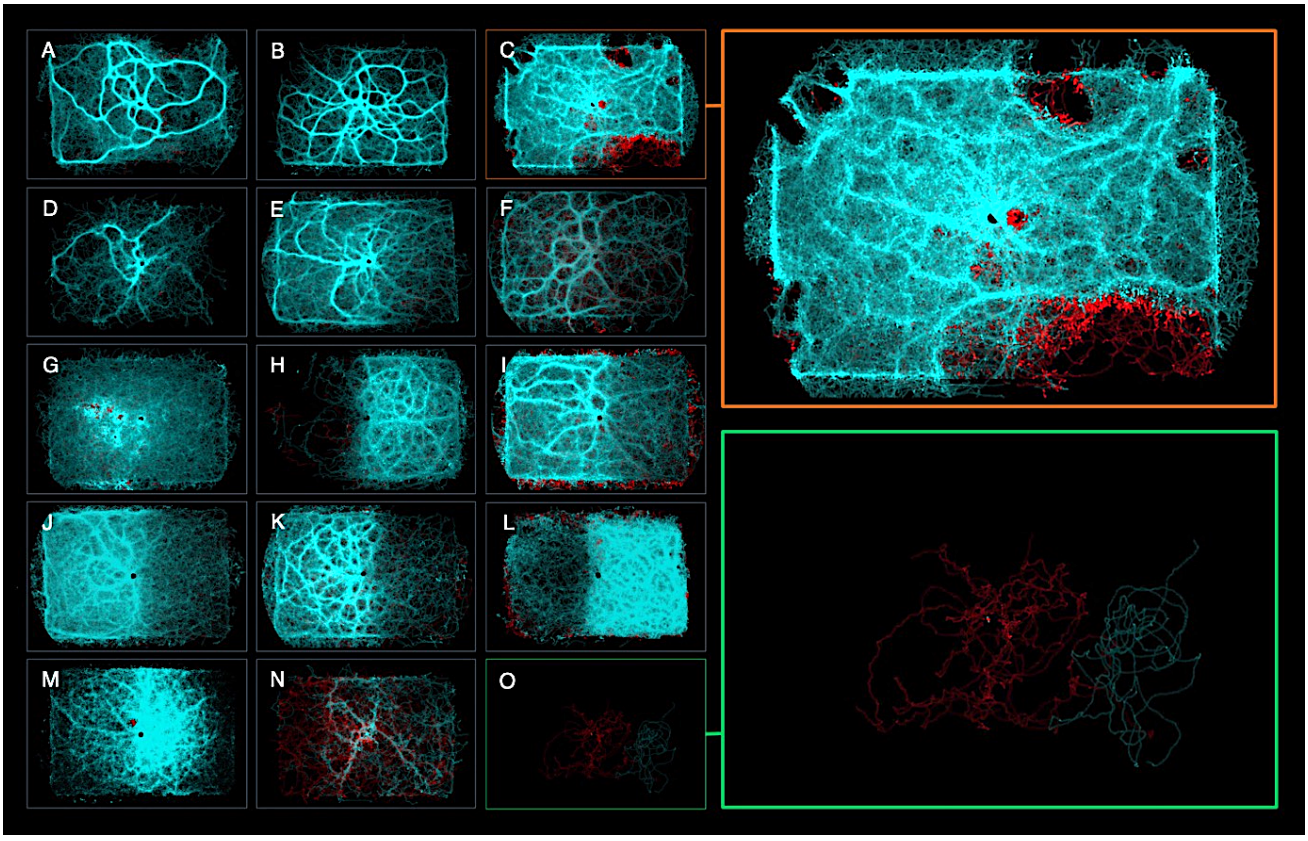

Figure 3 | Spontaneous emergence of collective trails: Behavioural observations recorded using semi-folded arenas. Each exploration activity was recorded using a unique termite colony and consisted of a video with 900000 frames on average.

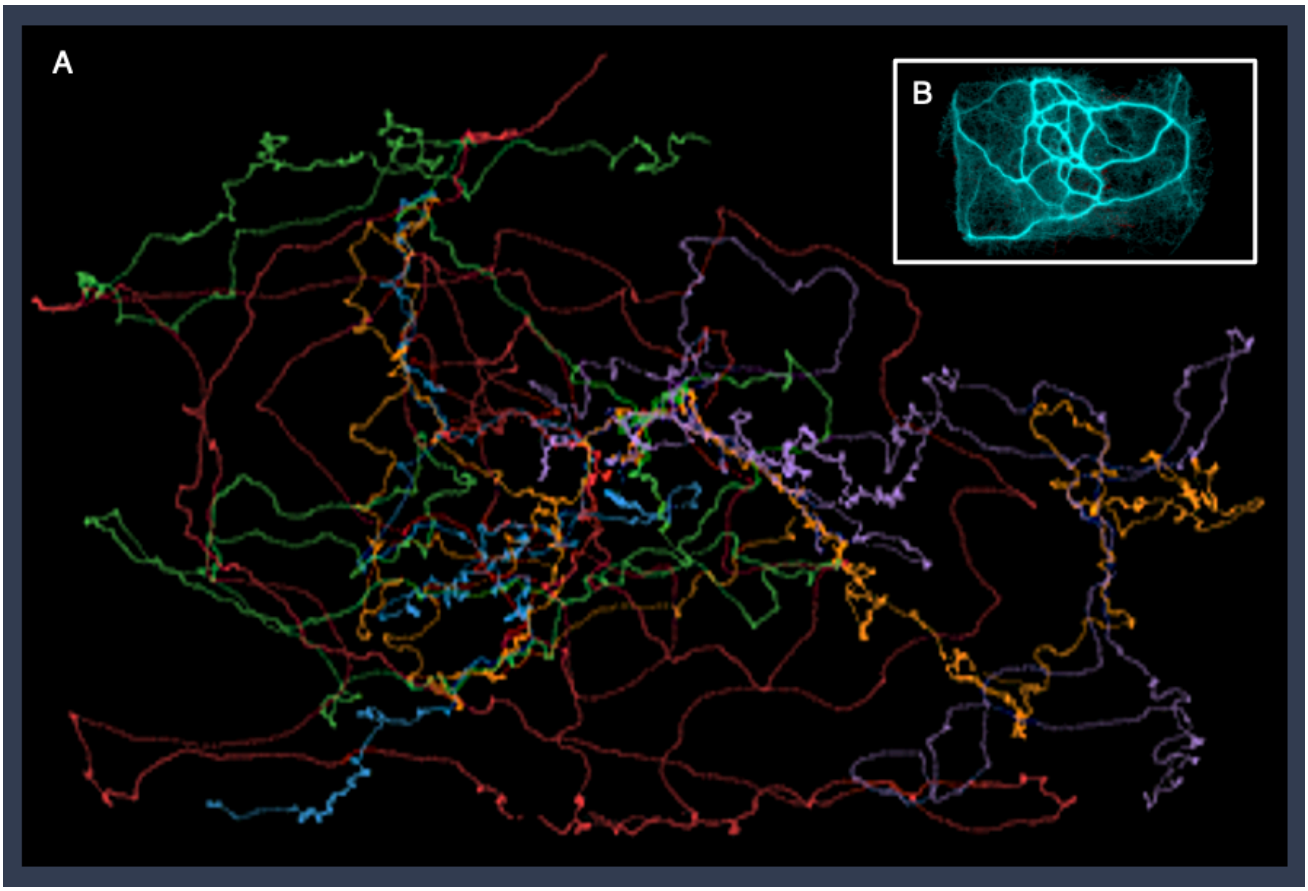

Figure 4 | Individual movement | (A) Trajectories of termite individuals are represented with different colours. Movement metrics (e.g., speed, acceleration, angle) and trail parameters (e.g., tortuosity, mean squared displacement) can be calculated using tracking data. Castes, not represented, but can be filtered from TRex output and plotted if necessary. (B) Whole-colony observation used, as an example, to extract the individual trajectories.

3.2 Collective behavior by soldiers

An interesting behaviour we noticed was that of soldier, which flanked trails formed by workers in some observations (**Fig. 3: C**, highlighted in orange). Eggleton (2010) describes this behaviour as the following: “Trail-guarding is where a trail of foraging workers is defended by actively guarding soldiers […] The most striking example of this is found in *Hospitalitermes*, where workers scrape micro-epiphytes from the bark of standing trees and nasute soldiers stand at the edge of the foraging parties and are highly effective at deterring ants from attacking (Miura and Matsumoto 1998). Such a positioning by soldiers, at the flanks of trails, has been also reported in other species (e.g., Traniello, 1981; Miura and Matsumoto 1998), but not yet mechanistically investigated.

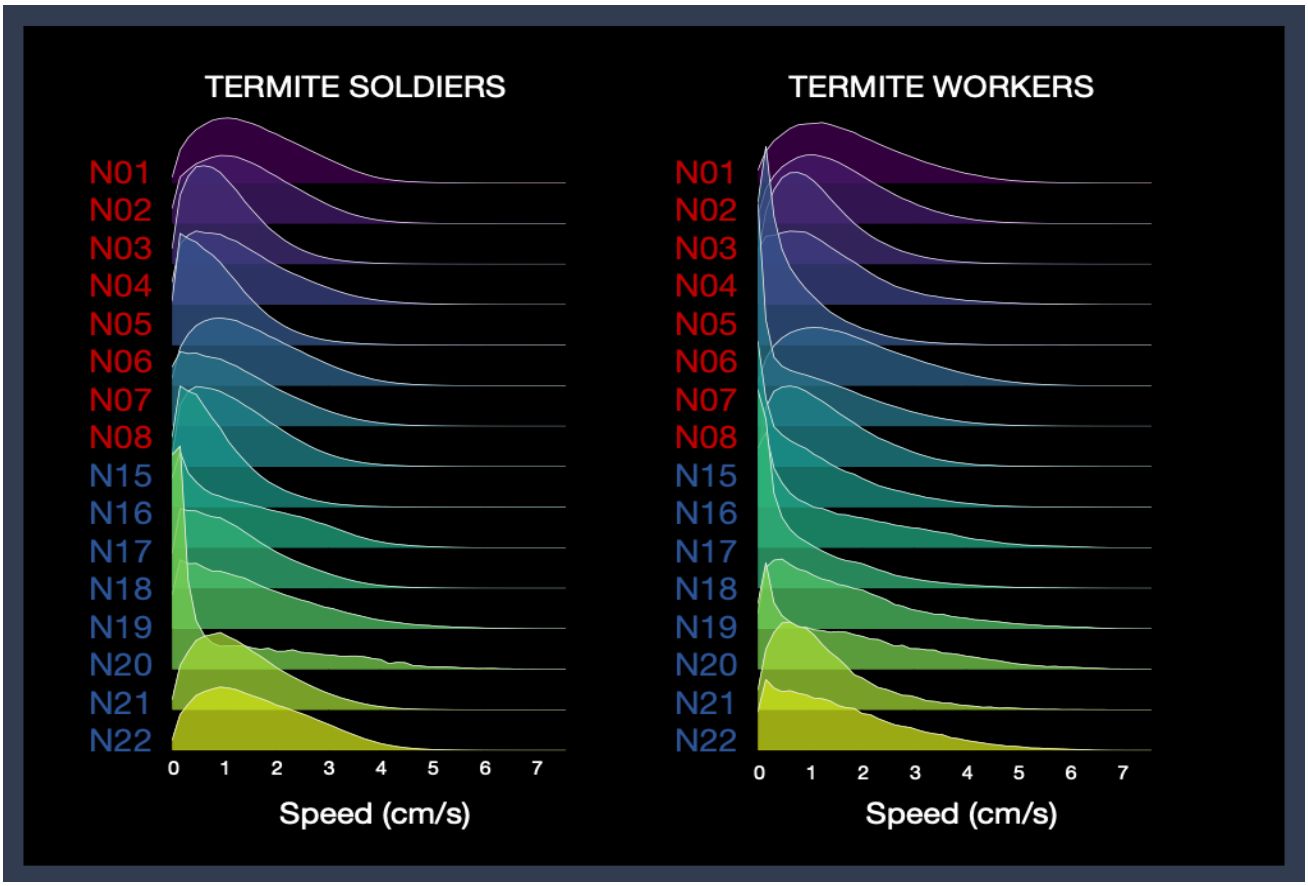

Figure 5 | Speed distribution | Speed profiles per caste (soldiers and workers) were calculated for each whole-colony observation using Kernel Density Estimation (KDE) methods. Curves were generated using the first six minutes of tracking data (approximately 9000 frames). Nests collected in 2019 (from N15 to N22) are highlighted in blue. Nests collected in 2020 (from N01 to N08) are highlighted in red. Transparency and colour-space (viridis) were used to facilitate visualization.

3.3 Speed profiles

Results analysing the onset of our whole-colony observations (i.e., first 9000 frames) show neither consistent differences in speed profiles of *C. cyphergaster* per termite caste (**Fig. 5**), nor significant differences between individual average speeds of termite workers (0.88±0.88cm/s) and termite soldiers (0.95±1.03cm/s). This later result contrasts with previously reported individual average speeds for *C. cyphergaster* (workers: 3.95±0.33mm/s; soldiers: 8.99±0.16mm/s; for details see Cristaldo et al., 2015). Yet, it is important to stress the differences in scope and experimental conditions between that study (an investigation of behaviour in response to alarm conditions) and the present one (an assessment of collective movement and exploration without external stimuli).

3.4 Positive feedback and amplification: faeces and trail pheromone

We detected that paths more frequently used by termites had traces of a dark material secreted by individuals (**Fig. S2**, Supplementary Material). Souto and Kitayama (2000) suggested that, in *C. cyphergaster*, both trail pheromone produced in the sternal gland and faeces are used to maintain trails: pheromones, which are more volatile and decay in relatively shorter period (30-60 min), are used to establish new trails connecting the nest to food sources; faeces, which contain less volatile chemical particles, keep trails for longer periods. In theory, by using faecal droplets to relocate older paths, termite colonies would simultaneously reduce costs with pheromone production and have access to previous foraging locations, functioning as collective memory. When handling captive colonies of termites in laboratory, we noticed such behaviour on several occasions, but because it was not scope of this study, we did not conduct experiments to investigate the pattern specifically. Experiments using colonies over multiple, in which termites have access to trails previously marked by themselves, would be useful to test the above hypothesis and remains as a potential following study. We found no direct correlation between the size of nests (measured in volume) and the proportion of soldiers allocated during the initial exploration.

4 DISCUSSION

Here we provided a protocol to replicate in laboratory spontaneous emergence of self-organized trails in termites, a collective behaviour exhibited by several species in natural environments that remains, to date, relatively less studied. To the best of our knowledge, our method is unique in providing a solution that minimizes the effect of vertical walls, present in more traditional experimental arenas. Physical barriers in arenas can cause disruption of free movement of individuals and, therefore, prevent the observation of certain individual or collective behaviours.

4.1 Comparison with alternative methods

Alternative methods to observe whole colonies of termites have been developed previously, but often lack some features of our protocol, such as a higher degree of freedom in terms of trail formation. With the method we propose, individuals coming from the nest and consequently reaching the observation area, are allowed to take any direction in 360 degrees. Such freedom of choice contributes to generate multiple outcomes in terms trail formation (**Fig. 3**). In contrast, other protocols use more limited structures to connect nest and experimental arena, such as tubes (Howse, 1966; Su et al., 2017), bridges (e.g., Haifig et al., 2015) and tunnels (e.g., Affolter & Leuthold,

2000). Although the use of these narrow structures may facilitate the formation of consistent trajectories and aid the measurement of traffic flow, the number of possible collective outcomes is invariably reduced.

4.2 Advantages of the method

In termites, and other social organisms, it is a common practice to conduct research with assemblages of colonial members (i.e., groups of nestmates) considering them representative parcels of the original colony. Although possible, many times it is unfeasible to investigate tens of thousands of individuals from multiple colonies simultaneously. When conducting research with subgroups, however, caution should be taken when interpreting results from experiments with termite groups and making inferences or drawing conclusions about colony behaviour. One advantage of our protocol is that it allows investigation of whole colonies of eusocial insects, which may be helpful to overcome limitations of smaller setups using colonial subgroups.

By folding the arena surface and creating an infinite outer loop external to the observation area, where individuals can continuously move without immediate obstacles (except for other nestmates), we managed to replicate spontaneous emergence of collective trails. These networks of trails resemble those formed in natural conditions and enable the observation of both individual and collective behaviour of termites. Also, we detected two obvious advantages of having a setup in which the arena remains connected to a natural nest throughout an entire experiment or observation. First, since individuals can access the observation area uninterruptedly (**Fig. 2**), it is possible to make long-term observations of whole colonies, something extremely valuable when investigating behaviours such as foraging, nest defence and the like. Second, because manual transfer of individuals from their nests to containers is not necessary, accidental injuries from handling focal animals prior to experiments (e.g., using entomological forceps) are conveniently ruled out.

When studying cryptic biological systems, limitations regarding behavioural observation and recording may influence the decision to include artificial arenas in the experimental protocol, to circumvent specific issues (e.g., visual occlusion). Yet, there are cases in which the adoption of arenas is a key component of the experimental design, for example, when use of space itself is one of the variables of interest and manipulating the area available to individuals is necessary. Regardless of the motivation, studies with colonial organisms using space-restricted arenas often must deal with the behaviour of groups of individuals, rather than the behavior of whole colonies. With the method we proposed here there is no such issue.

4.3 Laboratory versus field experiments

Although field experiments provide naturalistic conditions and allow the extraction of high-quality behavioural data, it is often nearly impossible to disentangle - at least completely - all the variables that may affect a given behavioural response (Peterson et al., 1982). In the wild, independent variables (e.g., predation, weather condition, microclimate, etc.) and uncontrolled extraneous factors can unexpectedly alter or even disrupt natural collective behaviours being recorded. In this sense, laboratorial setups using controlled arenas can mitigate problems by filtering some of the external factors, allowing researchers to focus specifically on certain variables of interest. This is not to say, however, that field experiments cannot be designed in ways to prevent undesirable external influence. In fact, we emphasise that this work should not be taken as an attempt to invalidate field experiments of any sort, nor it should be used as to make a case for pushing forward studies purely developed in laboratory. When combined into a single empirical framework, field and laboratory experiments should provide useful insights about a particular set of behaviours, as their data are likely to be complementary to each other, enabling cross validation during analyses.

4.4 Importance of findings and final considerations

Our findings also provide empirical evidence that self-organized trails in termites can emergence and dissolve in the absence of external stimuli. In our semi-folded arenas, we provided no food or water sources, yet termites collectively coordinated themselves in all observations. This observation is important, as it presumably supports a hypothesis that collective coordination is not necessarily driven by resource availability and can emerge gradually already in the exploration phase, which takes place way before resource exploitation. Our protocol, originally conceived to replicate spontaneous emergence of collective trails in termites, has the potential to be extended to other studies. These could involve different ecological aspects, not only of termites, but also other group living organisms sharing similar biological traits. An important caveat of our experimental procedure is that, with the observation area connected to whole colonies, the number of individuals in video (i.e., within a frame) can escalate very quicky. Working with so many individuals necessarily require a robust tracking method to extract individual trajectories with confidence. In our case, TRex was a suitable choice due to its fast performance with *C. cyphergaster* videos. Yet, nothing prevents that other tracking solutions can be used. Finally, the method we put forward seems sufficiently practical to foster the discovery of cryptic behavioural dynamics, which ultimately should provide opportunities to elucidate behaviours whose function remain, to date, poorly understood.

Chapter II

From random to coordinated collective motion: assessing the influence of group size and group composition in the movement of termites

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ABSTRACT

Coordination is fundamental for large-scale animal groups on the move. When foraging, termite societies coordinate movement by establishing a series of interconnected pathways, thus facilitating navigation through complex environments. Several studies have elucidated the chemical ecology behind the formation of such trails, but little is known about behavioural mechanisms modulating the patterns. In this research, we developed an empirical framework to investigate the underlying mechanisms of coordinated collective motion in termite societies. Specifically, to test how group structure affects collective patterns, we evaluate both the emergence and maintenance of self-organised trails in different group sizes and caste-ratio configurations. Using high-resolution videos and image-based tracking technologies, we detect trajectories and extract posture data for individuals over time. By performing quantitative analysis of movement, we gather information about the spatiotemporal dynamics, allowing comparisons of performance between groups in different scenarios. Our results provide evidence that the singularity of each termite caste goes beyond their innate morphological differences. The behavioural patterns observed for soldiers and workers indicates that the rules governing the collective motion of each group are unique. This means that workers and soldiers should not be interpreted as equal contributors in shaping collective patterns. Still, although castes operate in different ways, we reveal phase transitions in the behavioural response and an optimal caste-ratio configuration in which consistent coordination is more likely to emerge. We argue that mechanisms of coordinated motion in termites have been shaped under specific selective pressures, not necessarily those acting in Hymenoptera, as often assumed or suggested in the past.

Keywords: Movement ecology, Collective behavior, Trail formation, Self-organization, Termitidae, Nasutitermitinae, *Constrictotermes cyphergaster*

1 INTRODUCTION

Social colonies require both physiological (i.e., individuals) and organismal (i.e., colonies) integrity to persist in the wild. Group living, although generally expected to lower predation risk (Ioannou, 2021), may also put animals in situations where they become vulnerable to either specialized or opportunistic predators. In termites, predation risk is a strong selective pressure driving natural selection. In fact, termites can be complementary diet, if not the main source of nutrition, of a variety of predators (Traniello & Leuthold, 2000), such as ants, beetles, hemipterans, phasmids, spiders, centipedes, phalangids, tubellarians, (Sheppe,1970), not to mention vertebrate groups, such as anteaters (Lubin et al., 1977) and other mammalian predators (Redford, 1987). Coordination seems, therefore, crucial when navigating through complex environments. Understanding mechanisms underlying coordinated motion in termites could provide new insights about the evolution of collective behaviour in complex biological systems.

Research with termites, however, has focused largely on space-restricted experimental setups (e.g., Petri dish) with a reduced number of individuals, rather than observation of whole colonies. Additionally, several studies treat and assess behavioural aspects of termite castes separately (e.g., observing only workers or only soldiers), often neglecting the influence each cast may have on one another. Despite being arguably intrinsic to colonies, coordinated movement in termites remains poorly understood and relatively less studied as compared to other social groups (e.g., eusocial Hymenoptera).

Here, using large-scale semi-folded arenas (Hugo et al, unpublished), high-resolution behavioural recording, and automated tracking, we investigated the spontaneous emergence and maintenance of collective trails in termite colonies. By extracting movement data from whole-colony observations, we tested the hypothesis that variation in group size and group composition are key components of coordinated collective motion in termites. In particular, we predicted that: (i) group size and (i) caste-ratio fluctuations driven by changes in recruitment are correlated with the degree of organisation of collective trails. To the best of our knowledge, our study is the first to provide detailed behavioural estimation per caste of entire termite colonies under controlled conditions. We argue that studies evaluating group or collective behavioural patterns in termites - and other eusocial organisms - should consider the inherent differences, preferences and asymmetries embedded in the behaviour of caste types.

2 MATERIAL & METHODS

2.1 Biological model

Constrictotermes cyphergaster (Silvestri, 1901) [Termitidae: Nasutitermitidae] is a Neotropical termite widespread throughout South America (Krishna et al., 2013) (**Fig. 1**). The species has nocturnal foraging behaviour with trails organized collectively in exposed columns (Moura et al., 2006). In contrast to other termites, *C. cyphergaster* forages mostly in the open and without protection of covered galleries (Mathews, 1977).

2.2 Study site and collection

We collected 15 colonies of the termite *C. cyphergaster* from a Cerrado area in Brazil near the municipality of Divinópolis (20°0820S, 44°5302W), State of Minas Gerais. The area is currently classified as 'equatorial savanna with dry winters' (Ratter et al., 1997, Kottek et al., 2006). Nests collected for this study were distributed sparsely (i.e., never neighbouring one another) within a preserved fragment of approximately 40 hectares. Sampling occurred during the transition from dry to wet season in Brazil (November) in the years of 2019 and 2020. Once collected, all biological material was immediately transported to the laboratory and kept under suitable conditions of temperature and relative humidity for termites (Collins, 1969).

2.3 Preliminary quantitative analysis of movement

Prior to this study, we conducted controlled experiments with *C. cyphergaster* in Petri dishes to generate testable predictions about group size and group composition. The rational for these experiments and preliminary results are presented in the Supplementary Material.

2.4 Experimental design (behavioural recording)

The experimental procedure used to observe coordinated collective motion in this study is described in detail in a companion publication (Hugo et al., in preparation), in which we provide validation for the proposed method using semi-folded arenas.

2.5 Statistical analyses

To investigate, how group size and composition affect coordination in termites, we ran both Linear Models (LM) and Linear Mixed Models (LMM) using R (R Core Team, 2020) and RStudio (RStudio Team, 2019).

In the first part of the analysis, using tracking data from whole-colony observations (n=15 nests), we calculated group size (i.e., number of termites per nest), and caste ratio (i.e., proportion of soldiers per nest). Coordination of termite colonies (i.e., path formation) was estimated with Kernel Density Estimate (KDE) plots with bins set to 100 (using "geom_bin2d" in "ggplot2" R package). We created one KDE plot per nest, extracted 'n-density values' (i.e., density of points per bin, scaled from zero to one) and calculated the sum of density values per plot. Because the variables 'group size' and 'caste ratio' were highly correlated (corr. coefficient = 0.7), we ran two separate LMs: first, we included 'coordination' as response variable with 'group size' as fixed effect; then we included 'coordination' as response variable with 'caste ratio' as fixed effect.

In the second part of the analysis, we explicitly tested whether coordination increases over time and if there is a difference in the increase between different group sizes and caste ratios. To do so, we created one KDE plot for every minute of tracking (therefore, six KDE plots per nest) and calculated sum of density values as described above. We ran two LMMs: one with 'coordination' as response variable, interaction between 'time' and 'group size' as fixed effect and nest ID as random effect; and another with 'coordination' as response variable, interaction between 'time' and 'caste ratio' as fixed effect and nest ID as random effect. Variable 'coordination' was square-root transformed to reach normal distribution. In addition, the variables 'coordination' and 'group size' were rescaled (range: 0 – 10) to improve model performance. LMMs were fitted with a restricted maximum likelihood method using lmerTest (Kuznetsova et al., 2017). Assumptions of normally distributed and homogeneous residuals were fulfilled for all models.

3 RESULTS & DISCUSSION

3.1 Group size and composition

As expected, the results of LMs showed that coordination of termites in the nest or with other words, path formation, increases with group size (**Tab.1A**). In addition, coordination of termites decreased with higher proportion of soldiers in the nest or increased with higher proportion of workers in the nest (**Tab.1B**). In the second part of the analysis, LMMs revealed that coordination in termites increases linearly over time. The increase was more pronounced in nests having larger groups and caste ratio with lower proportion of soldiers (**Tab.2**, **Fig.1**).

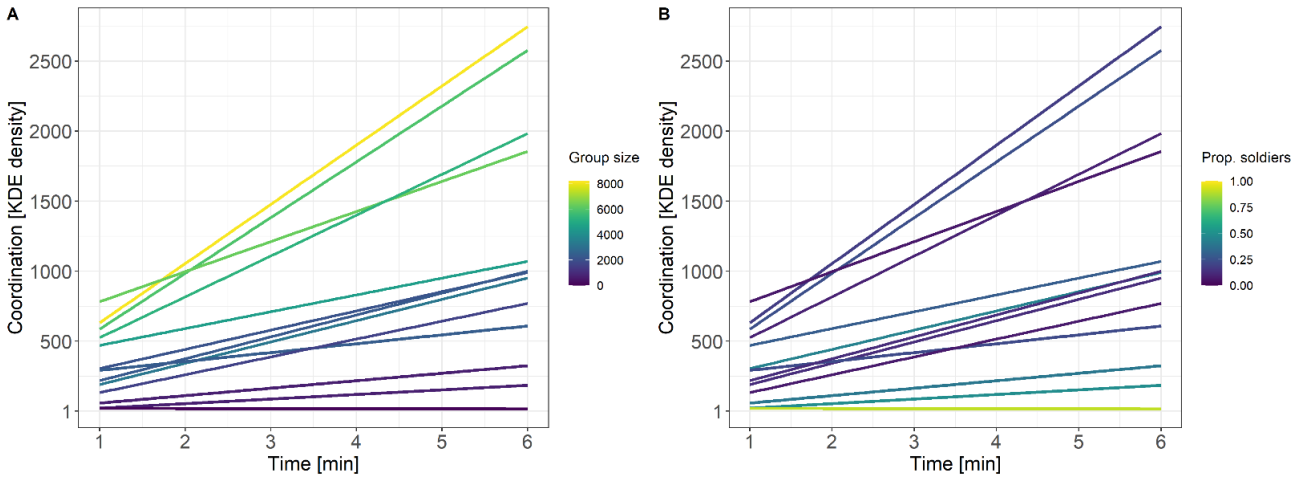

Figure 1 | Coordination across time: coordination in termites increases linearly over time. The increase is more pronounced in nests having (A) larger groups and (B) caste ratio with lower proportion of soldiers. Darker to lighter colours represent a gradient of (A) increasing group size and (B) increasing proportion of soldiers.

3.1 Emergence of collective trails

Results about the emergence of collective trails within the first six minutes of whole-colony observations are summarised in **Figure 2**. Except for two observations from colonies with smaller nest volumes ((i.e., N16HHS2019 and N19HHS2019, with 8.7L and 10.77L, respectively) all colonies exhibited a consistent pattern of coordination within six minutes of exploration, with the formation of paths throughout the arenas' observation area. These two colonies, however, exhibited collective trails later in the time series (**Fig. S1**, Supplementary Material). Contrastingly, in a third observation from a colony also of smaller nest volume, trails were consistently formed (i.e., N22HHS2019 with 9.01, **Fig. 2**).

Table 1 | Linear Models (LM) with: 'coordination' as response variable and (A) 'group size' or (B) 'caste ratio' as fixed effects. Statistical significance is highlighted in bold.

A – Group size

Fixed effects	Estimate	SE	t value	P value
(Intercept)	56.74	80.78	0.7	-
Group size	0.27	0.02	13.73	**< 0.001 *****
N observations	13			

B – Caste ratio

Fixed effects	Estimate	SE	t value	P value
(Intercept)	1426.5	263	maj.42	-
Caste ratio	-1685.7	732.2	-2.3	**< 0.042 ***
N observations	13			

Table 2 | Linear Mixed Models (LMM) with: (A) 'coordination' as response variable, interaction between 'time' and 'group size' as fixed effect and nest ID as random effect; and (B) 'coordination' as response variable, interaction between 'time' and 'caste ratio' as fixed effect and nest ID as random effect.

A - Group size

Fixed effects	Estimate	SE	*df*	t value	P value
(Intercept)	0.35	0.45	23.15	0.79	-
Time	0.31	0.07	63.00	4.23	**< 0.001 *****
Group size	0.41	0.09	23.15	4.61	**< 0.001 *****
Time × Group size	0.08	0.01	63.00	5.61	**< 0.001 *****
Random effects	Variance	SD			
Nest ID	0.55	0.74			
Residual	0.43	0.65			
N observations	78				
N nests	13				

B - Caste ratio

Fixed effects	Estimate	SE	*df*	t value	P value
(Intercept)	2.98	0.79	13.60	3.78	-
Time	0.91	0.07	63.00	12.72	**< 0.004 ****
Caste ratio	-3.46	2.20	13.60	-1.58	0.138
Time × Caste ratio	-0.98	0.20	63.00	-4.92	**< 0.001 *****
Random effects	Variance	SD			
Nest ID	2.80	1.67			
Residual	0.47	0.68			
N observations	78				
N individuals	13				

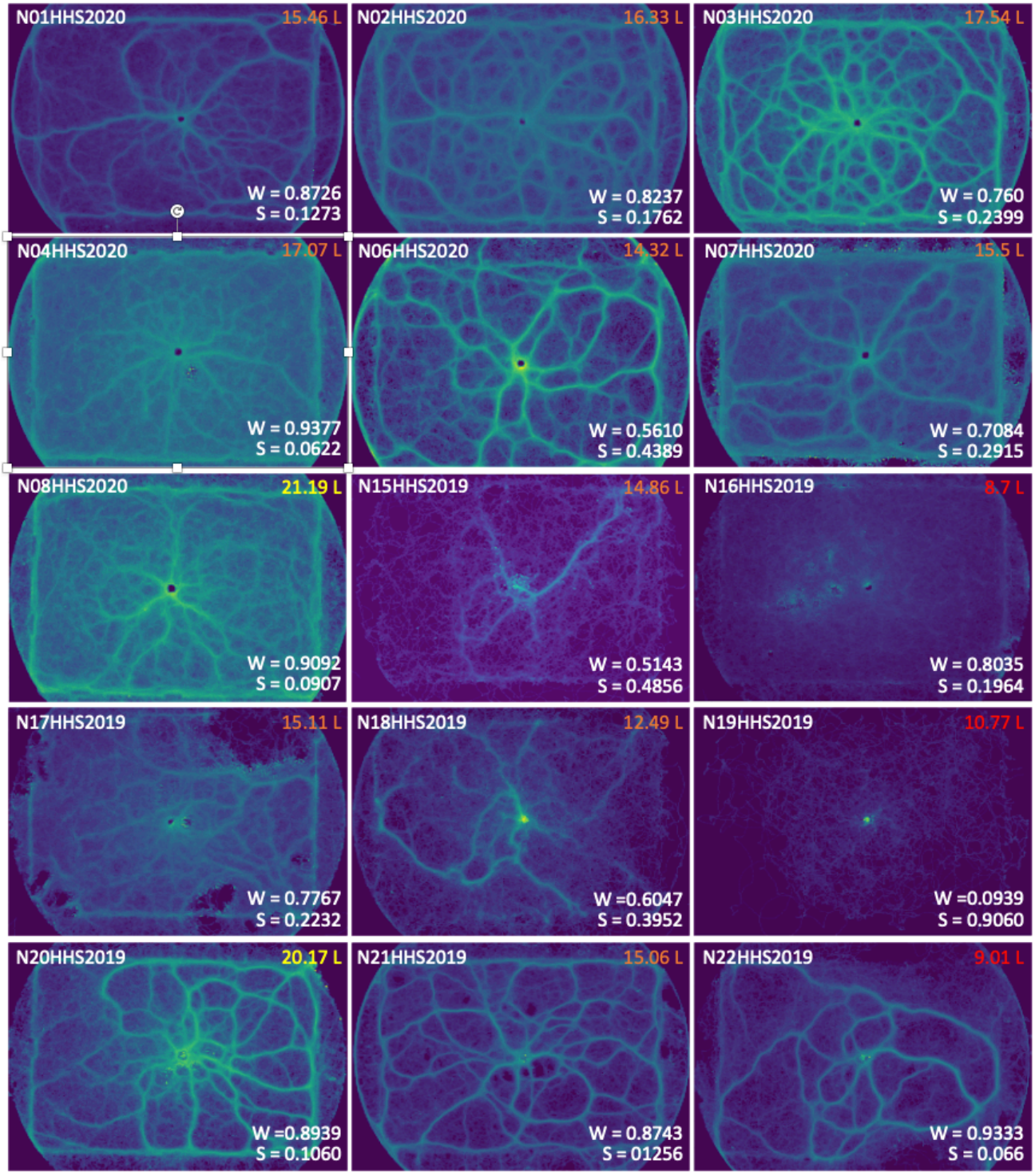

Figure 2 | Emergence of coordinated collective trails: Coordination of termite colonies over six minutes was measured using Kernel Density Estimate (KDE). Coordination was calculated for each whole colony observation (upper-left) using positional data of individuals [x-y coordinates]. KDE plots were produced in Python with normalised data [gamma=0.35, bins=100]. Proportion of workers [w] and soldiers [s] are represented for each whole-colony observation (bottom-right corner); Calculated nest volumes [L] are presented in colours, from red to yellow (upper-right corner).

During foraging, the proportion of soldiers relative to workers varies greatly across termite species. For instance, in *Nasutitermes corniger* (Traniello, 1981; Traniello & Buscher 1985) and *Heterotermes tenuis* (Casarin et al., 2008), only soldiers participate in the beginning of foraging, a strategy plausibly evolved with strong predation as selective pressure (for a list of 'enemies of termites', see Deligne et al., 1981). Other species of the same family, however, show no such patterns and in fact allocate both workers and soldiers in the initial stages of exploration (*Velocitermes heteropterus*: Haifig et al., 2015). An uncommon yet reasonably expected pattern is that of *Syntermes* species, in which soldiers not only arrive late at the foraging location but also retire rather prematurely from it. Although observed nearly a century ago (Snyder 1929), the pattern has not been explicitly investigated yet, perhaps for the difficulty of sampling the species from underground nests (Constantino, 1995). In a separate study investigating behaviour of *Syntermes molestus* (Hugo et al., in preparation), we detected differences between movement patterns of workers and soldiers (unpublished data). Further investigation is nevertheless required to fully understand the behaviour of the castes.

Polyethic recruitment patterns has been suggested to depend primarily on quantitative differences in pheromone production of the soldier and worker castes, rather than qualitative properties of chemicals exhibited by each of them (Traniello & Busher, 1985). Previous observations of *C. cyphergaster* in the field (Moura et al., 2006) describe soldiers as 'the first caste to leave the nest and the last to return'. Our observations (from field and laboratory) seem to be in line with this, although it is worth mentioning that there seems to be a behavioural variation in terms of the initial recruitment of soldiers. In addition, at least two studies conducted independently, with *C. cyphergaster* (Moura et al., 2006) and *Nasutitermes costalis* (Traniello, 1981), report that the recruitment of first termite workers, described by some as 'scouts' (Casarin et al., 2008), occurs only after that emergence of the first soldiers, being delayed by approximately 30 minutes.

Unlike the eusocial Hymenoptera, dynamics of path formation in termites is a topic understudied and quantitative data about the emergence of collective trails are extremely scarce in the literature. Further studies focused on the mechanistic nature of coordinated collective motion in termites should be insightful in understanding the evolution of self-organized patterns of movement.

Chapter III

First evidence of wasp brood development inside active nests of a termite with the description of a previously unknown potter wasp species

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ABSTRACT

Potter wasps (Vespidae: Eumeninae) are known to exhibit not only sophisticated preying strategies but also a remarkable ability to manipulate clay during nest building. Due to a mixture of plasticity in building behaviour and flexibility in substrate preferences during nest-building, the group has been reported nesting in a variety of places, including decaying nests abandoned by termite species. Yet, evidence of wasps nesting inside senescent termite mounds is poorly reported and, to date, accounts confirming their presence inside active colonies of termites are absent. Here, we address a novel intriguing association between two species from the Brazilian Cerrado: a previously unknown potter wasp (nest invader) and a termite species (nest builder). Besides scientifically describing *Montezumia termitophila* sp. nov. (Vespidae: Eumeninae), named after its association with the termite *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae: Nasutitermitinae), we provide preliminary information about the new species' bionomics by including (i) a hypothetical life cycle based on the evidence we collected and (ii) a footage showing the first interaction between a recently eclosed wasp and a group of termites. In doing so, we attempt to provoke relevant discussions in the field and, perhaps, motivate further studies with the group. Finally, we describe a solution to efficiently detect and sample termitophilous species from termite nests, an intrinsic yet challenging task of any studies dealing with such a cryptic biological system.

Keywords: Brood development, *Montezumia*, *Constrictotermes cyphergaster*, Vespidae, Nasutitermitinae, Symbioses.

1 INTRODUCTION

A species' ability to persist locally and over time depends on its capacity to successfully breed and give rise to subsequent generations. This definition seems to hold irrespective of major transitions in evolution (Maynard Smith & Szathmáry, 1995), being observed in solitary, gregarious, semi-social, and eusocial organisms. Among the variety of evolved strategies for developing immatures into adult individuals, nest building has evolved independently across taxa into many forms. The variation in nest architecture includes, but is not limited to, structures built by single individuals on top of pre-existing nests (such as in the species here reported), conglomerates of reproductive cells assembled by semi-social organisms (Bohart & Stange, 1965; West-Eberhard, 2005; Lopes & Noll, 2019), and large-scale nests collectively built by massive eusocial colonies (Seeley & Morse, 1976; Hölldobler & Wilson, 1990; Eggleton, 2010). Yet, despite the variety of strategies, groups taxonomically independent and, therefore, evolving under seemingly unique selective pressures may still coincide in their nesting location. In fact, while several species settle in proximity and coexist harmoniously, in some cases in complete absence of interdependency (e.g., plesiobiosis, Kanizsai et al., 2013), a relatively smaller group is known to "cheat" by deliberately using a physical structure built by another species (Nash & Boomsma, 2008; Uboni et al., 2012). In termites, arguably one of the most successful builders in nature (Wilson, 1971), this pattern has been long and recurrently observed (Kistner, 1969; Mathews, 1977; Kistner, 1979; Collins, 1980; Redford, 1984; Kistner, 1990); nests built by a single builder species, and thus meant to house primarily nestmates, becoming a hub of opportunistic invasive species.

Because nest building is presumably a costly and time-consuming process (Korb & Linsenmair, 1999), one would expect that species other than the builder would attempt to avoid such a commitment and take advantage of pre-existing constructions, either "gently" (e.g., nest sharing) or "by force" (e.g., nest usurpation). Theoretically, while in the former approach invaders must find a way to stably coexist with their hosts, the latter only requires a hostile take-over in which the builder is killed off and replaced by the invader. Although practical, such a binary simplification should be considered carefully; in reality, the outcome of an association between species over time is more likely to transit in a continuum between the extremes. In either case, what seems consistent is the fact that nest invaders are somehow lured by specific benefits associated with the host nest. Indeed, besides providing shelter, nests may be a pool of valuable resources that drive trophic interactions, particularly those exhibiting predatory (Jaffe et al., 1995) or parasitic habits (Yashiro & Matsuura, 2007).

In termites, builder species invariably exhibit mechanisms to prevent nest invasion, such as nestmate recognition (Clement & Bagneres, 2019) and a caste system in which colony members – namely, soldiers – play a defensive role (Prestwich, 1984). At the same time, however, nest invaders have developed sophisticated sensory deception mechanisms to go unnoticed by their hosts (Nash & Boomsma, 2008), such as chemical and morphological mimicry. The existence of such mimicry strategies illustrate how evolution may shape nest invaders into true specialists, experts in deceiving the termite builder (examples in Cunha et al., 2015; Rosa et al., 2018; Pisno et al., 2019; Oliveira et al., 2019). The arms race between builder and nest invader, driven by adaptations and counteradaptations from both sides (Dawkins & Krebs, 1979), is thought to produce cases in which the symbiosis is taken to another level, where one of the species – if not both – ends up relying exclusively on the other for survival. Such a dependency on a host is exhibited by some secondary termite species (e.g., inquilines) and several other insects (e.g., termitophiles), which gain entry to the termite nest when host colonies are still alive. However, because a termite construction is likely to persist in the wild long after its colony dies, another great number of species opportunistically use abandoned nests as shelter (Costa et al., 2001), becoming occasional occupants (Wilson, 1971). Together, these facts seem to indicate that regardless of the status of its colony, a single termite nest will most likely attract a large number of foreign species throughout its time of existence.

Potter wasps (Vespidae: Eumeninae), known for their striking ability to shape clay during nest building, have been reported nesting in a variety of substrates (Cowan, 1991; Garcete-Barrett & Hermes, 2013; Hermes et al., 2015), including decaying nests of termites abandoned by the builder species (Batra, 1979). Curiously, to date, there is no evidence of nesting by wasp species inside active colonies of termites. Such a pattern, upon first glance, could be interpreted as a result of nesting preferences exhibited by the group. However, except for some knowledge of bees as "guests" in termite nests (for examples of species in Apidae see Carrijo et al., 2012), little is known about the remainder clades of Hymenoptera, such as Vespidae, which may ultimately contribute to a misrepresentation of the habits exhibited by the group.

In this study, we describe a new species of potter wasp from the Brazilian Cerrado, *Montezumia termitophila* sp. nov. (Vespidae: Eumeninae) and report the first record of brood development by a wasp species inside active nests of the termite *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae: Nasutitermitinae). In addition to reporting the unusual nesting site of the new wasp species, we discuss relevant yet poorly understood proximate causes of such a development. Additionally, to add notes on the species' biology and potentially stimulate further studies, we propose a life cycle for the new wasp based on the evidence we collected. Finally, we provide a

protocol for detecting and sampling termitophilous species from termite nests, an intrinsic but challenging task that, if poorly conducted, may contribute to misrepresenting the diversity of several cryptic organisms, such as the new wasp we discovered.

2 MATERIAL & METHODS

2.1 Terminology

Conventionally, animals invading nests of social organisms are referred by terms that provide a direct reference to the host group they are found with (e.g., “sphecophiles” with wasps, “melitophiles” with bees, “myrmechophiles” with ants, “termitophiles” with termites; Wilson, 1971). Yet, in Termitology (i.e., study of termites) two terms have been adopted when referring to organisms in association with host termites: “termitophiles” and “termitariophiles” (Kistner, 1969; Kistner, 1979; Kistner, 1990). While the former indicates species with association with the host termite colony, the latter indicates species in association with the physical structure of termite nests (i.e., termitarium), not with colony individuals. Recently, this distinction has become clearer with studies in which the authors provide an explanation for choosing either one of the terms (Carrijo et al., 2012; Cunha et al., 2015; Rosa et al., 2018; Pisno et al., 2019). Here, we explicitly follow Kistner’s (1969) original concept of termitophilous associations which defines “termitophiles” as non-termite species that either (i) live inside galleries of host termite nest or (ii) have obligatory association with the host termite colony. This mention is important to clarify which concept we considered when defining the specific epithet for the new species, choosing “termitophila” instead of “45termitariophila”.

The terminology we considered for referring the new eumenine wasp also deserves comment. As clade of primarily solitary vespids, the eumenines have been traditionally treated as closely related to the social subfamilies within the Vespidae (Pickett & Carpenter, 2010). However, phylogenomic studies (Bank et al., 2017; Piekarski et al., 2018) have challenged the traditional view on the phylogenetic relationships among vespid subfamilies, with the eumenines comprising a paraphyletic assemblage regarding the Polistinae + Vespinae. This is paramount in understanding the evolution of life strategies and nesting behaviors since the different phylogenetic scenarios may lead to diverging conclusions regarding the origin and maintenance/spread of different nesting strategies. Since we are considering the evolution of nesting behaviour herein, we will use the classification of Hermes et al. (2014), which includes the zethines among the Eumeninae, to provide a broader array of nesting strategies displayed by this group of wasps.

2.2 Host termite species

Constrictotermes cyphergaster (Silvestri, 1901) is a Neotropical termite species that builds typical arboreal nests (Vasconcellos et al., 2007, Krishna et al., 2013) and is widespread throughout South America (Mathews, 1977). With nocturnal foraging habits and diet mostly based on lichens (Barbosa-Silva et al., 2019) and tree bark (Moura et al., 2006), the species has drawn some attention due to its close association with nest invaders, such as *Inquilinitermes microcerus* (Silvestri, 1901) and *Inquilinitermes fur* (Silvestri, 1901) (for examples of studies, see Cunha et al., 2003; Cristaldo et al., 2012; Florencio et al., 2013; Cristaldo et al., 2014; Cristaldo et al., 2015; Santos, 2016; Cristaldo et al., 2016; DeSouza et al., 2016 ; Cruz et al., 2018; Rodrigues et al., 2018; Hugo et al., 2020). Because of their limited constructive abilities (Emerson, 1938), these invader termite species are known to rely exclusively on nests of *C. cyphergaster* to establish their colonies, being not found anywhere else to date and, therefore, classified as "obligatory inquilines" (Mathews, 1977).

Mechanisms underlying obligatory termite-termite associations are supposedly specific to each host-inquiline pair. For *I. microcerus*, in particular, colonies are typically found cohabiting nests of *C. cyphergaster* above a certain nest size, supposedly 13 L in termitarium volume according to Cristaldo et al. (2012). The species is also characterized by a reduced number of termite soldiers (Cunha et al., 2003) and an uncommon lack of aggressiveness towards its host termites. In fact, this inquiline species has been reported to adopt evasive behaviors when receiving attacks and make use of fecal pellets to disrupt the host's aggression (Hugo et al., 2020). This non-threatening behaviour contrasts with the typical aggression seen among termite species (Shelton & Grace, 1996). Although known for several organisms, including colonial (Pierce et al., 2002; Lhomme et al., 2012; Nehring et al., 2015), gregarious (Aureli et al., 2002) and semi-social species (Baan et al., 2014; Gobush and Wasser 2009; Thierry et al., 2008), submissive behaviour remains relatively uncommon among termites, given that most species tend to immediately reciprocate any sort of aggression received (Noirot, 1970; Prestwich, 1984; Shellman-Reeve, 1997). Nests of *C. cyphergaster* are known to house, in addition to inquiline termites, termitophilous species such as *Corotoca melantho* (Coleoptera: Staphylinidae), a viviparous rove beetle that shares morphometric (Cunha et al., 2015) and chemical (Rosa et al., 2018) similarity with their host termites. These soft-bodied, physogastric beetles exhibit reduced body proportions (Cunha et al., 2015) and cryptic habits (Pisno et al., 2019), which often turn detection and behavioral observation into challenging tasks. Yet, with adequate methods, relevant biological information about the group has been revealed, such as mechanisms underlying the nest invasion (Oliveira et al., 2019).

Recently, the number of studies investigating species in association with *C. cyphergaster* has increased. **Figure 1** presents an overview of the organisms currently known to invade active nests of *C. cyphergaster*, with their taxonomical information. As shown, a handful of associations have been investigated, being inquiline termites (Blattodea: Termitidae) and termitophilous rove beetles (Coleoptera: Staphylinidae) the groups receiving most attention. In a comprehensive review of groups belonging to Hymenoptera: Apidae (e.g., Apinae, Centridini, Euphorini, Eucerini, Colletinae, Megachilinae), Carrijo et al. (2012) have compiled a number of bee species occurring inside termite nests. In contrast, there is no record to date of wasps being found inside active termite nests.

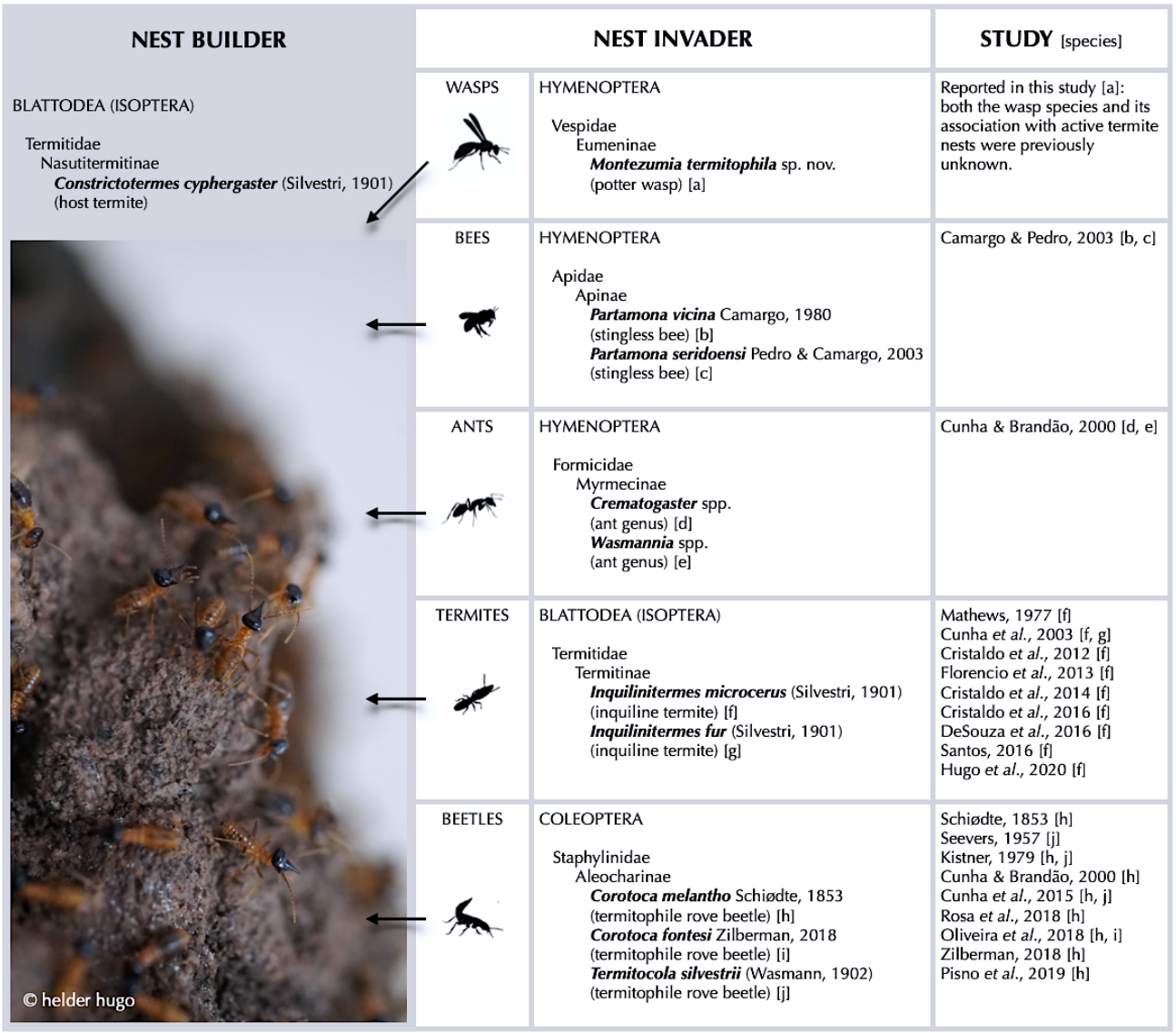

Figure 1 | Organisms currently known to invade nests of the termite *C. cyphergaster*. Nest invaders are represented along with a brief taxonomical classification (from order to genus or species). The references for investigations also contain indications for the species reported (in squared brackets).

2.3 Potter wasps

The nesting behaviors exhibited by potter wasps have been traditionally classified into three types: (i) excavators, where females dig directly into dry substrate and moisten it with regurgitated liquid during excavation; (ii) renters, where females use pre-existing cavities; and (iii) builders, where females collect dry earth and mix it up with regurgitated liquid to make mud cells (Maindron, 1882; Iwata, 1976; Cowan, 1991). However, potter wasp species present plasticity regarding these nesting strategies (Cooper, 1979; Krombein, 1979), and nest architecture may vary as well (e.g., Hermes et al., 2015). Additionally, some species make use of vegetal matter during nest-building, either incorporating leaf particles to provide camouflage to nest (Hermes et al., 2013) or using plant material in cell construction itself as presented by some zethines (Bohart & Stange, 1965; van der Vecht & Fisher, 1972; Claude-Joseph, 1930; van der Vecht, 1981). Females of eumenine wasps usually nest solitarily, but there are accounts reporting the aggregate behaviour of nesting collectively, where several females share one nesting site, but each female tends their own brood (Bohart & Stange, 1965; West-Eberhard, 2005; Lopes & Noll, 2019). In fact, some of these accounts are of particular interest, because they are exhibited by species in the genus *Montezumia* de Saussure. West-Eberhard (2005) observed the nesting behaviour of *M. cortesioides* Willink, 1982, in which several females shared a mud nest with neither division of labor nor hierarchical castes. However, some behaviors may precede the transition from solitary to social life, such as female competition for empty cells, progressive provisioning and some females remaining in the nest for longer periods than others (allowing prey theft). Similar accounts were provided by Lopes & Noll (2019), with less detail, for the species *M. brethesi* Bertoni, 1918.

Associations between potter wasps and termite species have been mentioned a few times in the literature, yet vaguely supported by evidence. Batra (1979) has previously found in India the wasp species *Anterhynchium abdominale* subspecies *bengalense* (de Saussure, 1855) (Vespidae: Eumeninae) in a deteriorating mound of the termite *Odontotermes obesus* (Rambur, 1842). In a short communication, this author describes the positioning and morphology of wasp brood cells along the walls of exposed ventilation shafts, in a severely eroded and queen-less mound. Reports of senescent termite nests without active colonies providing shelter to other species are various (e.g., Collins, 1980; Redford, 1984; Costa et al., 2009). Yet, to date, our study is the first to provide evidence of brood development by a wasp species inside active colonies of a termite species (*C. cyphergaster*).

2.4 Study site, sampling, and nest inspection

We collected 13 *C. cyphergaster* nests from one site in the Brazilian Cerrado (Ratter et al., 1997) near the municipality of Divinópolis (20º 08' 20" S, 44º 53' 02" W), State of Minas Gerais. This area is classified as 'equatorial savanna with dry winters' (Kottek et al., 2006). The 13 termite nests, distributed sparsely in an area of 20 hectares and never neighbouring one another, were collected in March 2018 (between 08:00 and 12:00) and immediately transported to the headquarters of property. Then, they were kept in individual cages for 5 days until nest inspection (**Fig. S2: 5**). These cages, made of cubic wire frames surrounded by a thin tulle fabric, were designed to contain adult wasps after eclosion. As for verifying whether *M. termitophila*'s nesting takes place entirely inside *C. cyphergaster* nests, it would be necessary to (i) obtain empirical evidence of adult wasps eclosing from the inside of termite nests and (ii) document the presence of wasp's offspring in termite nests, by detecting wasp brood cells integrated between termite galleries containing wasp immature stages. Thus, to search for immature stages of wasps (eggs, larvae and pupae) we thoroughly inspected the content of termite nests by peeling off its surface in order to expose subsequent layers of nest galleries (**Fig. S2: 5**). To prevent damaging the biological material we used scoopulas (stainless-steel spatulas of application in chemistry) and collected individuals using entomological tweezers. As opposed to the method of merely breaking *C. cyphergaster* nests into smaller pieces and superficially checking for termitophiles (as conducted in previous studies with the species), the protocol mentioned above allows a more refined inspection with preservation of nest structures. Only by preserving such structures, we were able to later document and elucidate the positioning and architectural features of wasp brood chambers inside the termite nest.

2.5 Recording wasp-termite interaction

To add notes on the nature of the association between *M. termitophila* and *C. cyphergaster*, we observed the interaction between the wasp species and both termite workers and soldiers immediately after its eclosion, to identify possible interspecific aggression. To do so, we recorded a short video (six minutes) of the interaction between a female wasp, later defined as the holotype, and a group of 16 termites in 3:1 worker-to-soldier caste ratio (12 workers + 4 soldiers). The recording arena was set in a glass Petri dish of 60mm diameter lined with paper at the bottom and covered with a transparent lid, and the video was recorded in 4K (50 fps) under visible spectrum of light (with LEDs) using a Sony Alpha 7S camera equipped with a Zeiss Batis 25mm f/2 lens. The room temperature during species manipulation and video recording was set to 25±1°C. To prevent excessive desiccation of termites, individuals were kept inside their nest until the beginning of

recordings. We filmed only the first interaction between *M. termitophila* and *C. cyphergaster*. Because during manipulation three of the four adult wasps found managed to escape, we decided to preserve the remainder female (which was later defined as the holotype) for species identification and not conduct more trials. Although insufficient for statistical analysis, which is beyond the scope of this study, this behavioral record is meant to provide supplementary biological information. As such, it provides a first tentative empirical evidence for a mutual lack of aggression between a recently eclosed *M. termitophila* wasp and its host termites.

3 RESULTS

Our results constitute of two main parts, (i) a taxonomic account and (ii) a commentary on the new species' natural history, both contextualized with the current knowledge on Eumeninae. In addition to formally describing *Montezumia termitophila* sp. nov. (Vespidae: Eumeninae), we present collected evidence that allows us to formulate a new hypothesis, that of adults in this wasp species explicitly use active nests of the termite *Constrictotermes cyphergaster* (Silvestri, 1901) (Termitidae: Nasutitermitinae) for brood development and, therefore, completion of its life cycle.

3.1 Taxonomic account

***Montezumia termitophila* Hermes & Garcete-Barrett, new species (Fig. 2)**

DIAGNOSIS**.** *Montezumia termitophila* **new species** belongs to the *M. infernalis* (Spinola, 1851) species group *sensu* Willink (1982). An emarginated clypeal apex (**Fig. 2.1**), the lack of a keel in the mesepisternum right below the pronotal lobe (**Fig. 2: 3**), the lack of a humeral carina on the pronotum (**Fig. 2: 3**), and the metanotum with transverse toothed crests (**Fig. 2: 4**) are features that place the species within the mentioned group of species. Within the *M. infernalis* group, *M. termitophila* readily runs into couplet 48 of Willink´s (1982) key. Furthermore, the species keyed to in the previous couplets all possess a smooth posterolateral margin in the propodeum when seen from above, which is somewhat angled in *M. termitophila*. Also, species such as *M. nigra* (Zavattari, 1912), *M. ignobiloides* Willink, 1982, *M. grossa* Willink, 1982 (**Fig. 3: 9-12**), *M. morosa* de Saussure, 1852 (**Fig. 3: 13-16**), *M. trinitata* Willink, 1982 and *M. obscura* Zavattari, 1912 are all part of a mimetic complex resembling the eusocial *Polybia ignobilis* (Haliday, 1836). The color pattern also differs between *M. termitophila* and *M. marthae* de Saussure, 1875, the latter presenting the metanotum, propodeum and metasomal tergum I almost entirely yellow. *Montezumia termitophila*, in fact, resembles the

eusocial *Agelaia 51 ngulate* (Fabricius, 1804) and the eumenine *Pachymenes ater* de Saussure, 1852 regarding body color, which is dark brown with yellow marks lacking almost entirely. Finally, *M. termitophila* differs particularly from *M. trinitata* by the lack of an apical lamella on metasomal tergum II (present in *M. trinitata*) and by the brownish body color with golden pilosity especially evident on scutellum, metanotum and propodeum (black ground color and brownish pubescence only slightly evident in *M. trinitata*).

DESCRIPTION. Holotype female. **Color**: Body color dark-brownish with light-brownish markings (**Fig. 2: 1-8**) as follows: stripe on metanotum dorsally adjacent to mesoscutum; pronotal lobes entirely; marks on upper portion of mesepistermum; tegulae entirely; spots on lateral portions of scutellum; basal stripe on metanotum; lateral surface of mid and hind coxae; all tarsi entirely. Yellow thin stripes on anterior surface of fore tibiae. Brownish to ferruginous stripes on lateral portions of metasomal tergum I, becoming light yellowish towards the apex of the tergum.

STRUCTURE: Body length from head to apex of T1 approximately 15 mm; forewing length 16 mm. Clypeus wider than long, apex emarginate with teeth evident; inter-antennal longitudinal carina present, with transversal arc-like ramifications at mid antennal sockets; cephalic foveae slightly evident, surrounding area little differentiated; occipital carina well developed and uniform along entire length; pronotal foveae conspicuous; humeral region somewhat angled near pronotal carina, but not forming a ridge or lamella; pronotal carina well developed and uniform along entire length; mesepisternal carina present and reaching the dorsal sulcus; mesepisternum without keel right below the pronotal lobe; notaulices on mesoscutum slightly indicated, parapsidal lines present; sulcus between mesoscutum and scutellum ridged and deeper medially; metanotum with transverse toothed crests, posterolateral surfaces of propodeum somewhat angled dorsally; medium sulcus of posterior surface of propodeum deep on upper portion; median carina of posterior surface of propodeum present on lower portion; T1 longer than wide, with a shallow pre-apical fossa; S1 somewhat triangular, twice as wide as long; T2 without apical lamella.

SCULPTURE: Punctures dense throughout entire head and mesosoma, contiguous. Clypeus striatopunctate towards apex. Frons slightly striate between antennal sockets and mid ocellus. Posterior surface of propodeum weakly striate medially. Punctures smaller on metasoma, sparser on T1. **Pilosity**: Golden erect pilosity on most of head and mesosoma, especially dense on scutellum, metanotum and propodeum. Golden erect pilosity on

metasoma restricted to sterna and sides of T1; remainder of the metasoma with scattered short and very sparse pilosity.

MALE: males were observed and photographed emerging from termite nests, but collection of voucher specimens for identification was unsuccessful (documentation provided in the Appendix).

ETYMOLOGY: The specific epithet is allusive to the fact that *M. termitophila* resides within nests of *C. cyphergaster* termites throughout its brood development, with no signs of conflict between both species.

TYPE MATERIAL: Holotype female at *Coleção Entomológica da Universidade Federal de Lavras*, Minas Gerais, Brazil (CEUFLA).

TYPE LOCALITY: Specimen collected on March 25th, 2018, by one of the authors (HH) from a Brazilian Cerrado site near the municipality of Divinópolis (20º 08' 20" S, 44º 53' 02" W), State of Minas Gerais. The Holotype female was found originally in a brood cell inside an arboreal nest of the termite *C. cyphergaster* still in larval stage (pupa shown on **Fig. 4**). The brood cell was carefully removed from the termite nest, conditioned in a separate container, and kept under controlled temperature and humidity until the wasp's eclosion.

ADDITIONAL MATERIAL EXAMINED: closely related species to *M. termitophila* were examined and compared to enhance the hypothesis of the latter as new to science. One paratype female of *M. grossa* and the lectotype female of *M. morosa* were examined. The first was collected in British Guiana in 1937 and identified by the late Abraham Willink and is currently housed at the Natural History Museum in London (NHM). The second specimen was collected in Rio de Janeiro (Brazil) and bears no indication of collecting date. It was also identified by Abraham Willink and is housed at the *Muséum National d'Histoire Nautelle* in Paris (MNHN). Also, one of the authors (MGH) has recently published a phylogenetic investigation on the genus *Montezumia* (Hermes & Carpenter, 2012), which encompassed the examination of several species within the taxon, therefore reassuring that *M. termitophila* is in fact a species previously unknown.

Figure 2. *Montezumia termitophila* new species, Holotype female. (1) Head in frontal view, scale = 2 mm; (2) Pronotum and mesoscutum in dorsal view, scale = 1 mm; (3) Pronotum and mesepisternum in lateral view, scale = 2 mm; (4) Scutellum and metanotum in dorsal view, scale = 1 mm; (5) Posterior surface of propodeum, scale = 1 mm; (6) Metasomal sternum I, scale = 1 mm; (7) Metasomal terga in dorsal view, scale = 2 mm; (8) Habitus, scale = 5 mm.

Figure 3. Two species closely related to *M. termitophila*. (9-12) *Montezumia grossa*, Paratype female. (9). Head and mesosoma in lateral view; (10) Head in frontal view; (11) Scutellum, metanotum and posterior surface of propodeum in dorsal view; (12) Metasomal terga in dorsal view. (13-16) *Montezumia morosa*, Lectotype female. (13) Metasomal terga in dorsal view; (14) Head and mesosoma in lateral view; (15) Head in frontal view; (16) Scutellum, metanotum and posterior surface of propodeum in dorsal view. [Scale variable].

3.2 Biological traits of *M. termitophila*

3.2.1 Morphology, positioning and content of brood cells

The inspection of 13 colonies of *C. cyphergaster* yielded detection of 23 wasp brood cells (Online Supplementary material, **Table S1**). Given that we identified no other wasp species in the material collected, and because all brood cells found had same structure and proportions (**Fig. 4**, Brood development), we registered all of them as belonging to *M. termitophila*. Of these, most were inactive and lacked immatures (n=19), and only a few (n=4) were active, containing either a larva (n=1) or pupae (n=3). During nest inspection, the three pupae developed into adults and emerged from their cell within two days of the detection date, but these escaped when being photographed for documentation (**Fig. 2**, individuals in 1, 2 and 4). Of these, individual 4 was confirmed as a male for exhibiting a hook-shaped last flagellomere in the antennae apex, individuals 1 and 3 were confirmed as females. The sex determination of individual 2 was not possible due to the poor resolution of image.

The brood cells found were positioned mostly on the nest's surface (Appendix, **Fig. S1**), not deeper than two-to-three layers of nest gallery, counting from the most external layer. In one of the termite nests containing wasp's brood cell, vegetal matter was found attached nearby a cell opening (Appendix, **Fig. S2**: nest in 5), but without behavioral observation we are not in the position to tell whether this was placed by *M. termitophila* or not. Regarding the internal morphology of *M. termitophila* brood cells, they were mostly cylindrical and incorporated in the substrate, tightly attached to gallery walls (see example in **Fig. 4**; Brood development). None of the brood cells was interconnected to termite galleries by openings. Yet, they were all located near sections densely populated by termites of the worker caste (albeit soldiers were also present). Inside the brood cells, we detected plastered mud (i.e., mixture of saliva and soil) over the internal walls.

As for that their content, none of the wasp brood cells, including active and inactive ones, were found to contain remnants of prey. Without nest building observation, it is impossible to associate *M. termitophila* to a mass or progressive provisioning behavior, especially considering that some eumenines can be solitary and make small nests with a few cells but have, at the same time, progressive provisioning (e.g., some species of the genus *Synagris*).

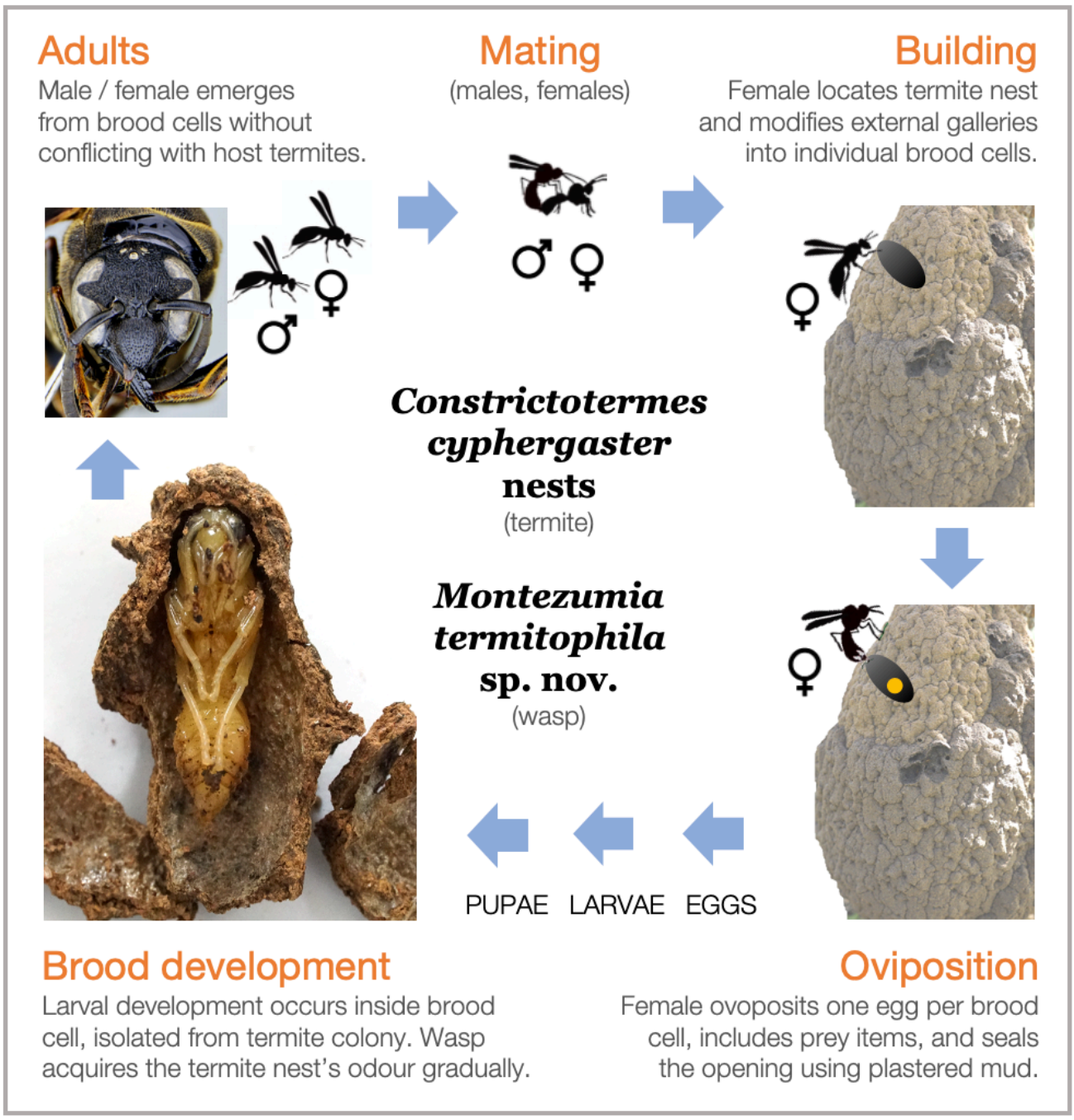

Figure 4. Life cycle proposed for *M. termitophila* Hermes & Garcete-Barrett new species. Behaviors that were not explicitly observed in this study were based on the information available for other eumenines. Illustrative elements are simplified and not necessarily presented in proportional scale.

3.2.2 Proposed life cycle

Based on the evidence we collected from 23 *M. termitophila*'s brood cells found inside 13 nests of *C. cyphergaster*, we hypothesize the life cycle of the new potter wasp species, shown in **Figure 4**. In this study, we did not observe all behaviors mentioned in the illustration. For mating, nest building and oviposition, specifically, we based our assumptions on the available literature described for the remainder species of Eumeninae (see "Potter wasps" in Material & Methods). Alternatively, *M. termitophila* may also nest in substrates other than a termite nest (e.g., in the ground, on top of rocks), as observed in other potter wasps. Yet, nesting inside termite nests and nesting on top of other structures are not mutually exclusive behaviors. Thus, a third hypothesis would be that the new wasp species could actually do both, having flexible nesting preferences.

4 DISCUSSION

In this report, for *M. termitophila*, we provided (i) the species description, (ii) a proposed life cycle based on the documentation of active wasp brood cells within galleries of the termite nest and (iii) a first tentative evidence for a lack of conflict between adult wasps and termites. By itself, the wasp's strategy characterized by the modification of termite nest's galleries into brood cells, constitutes a novel and uncommon form of nesting among potter wasps. While this behaviour is seemingly a variant between the renter and digger types previously described (Maindron, 1882; Iwata, 1976), such a plasticity is in line with descriptions of other species belonging to Eumeninae (e.g., Cooper, 1979; Krombein, 1979; Hermes et al., 2015). To the best of our knowledge, this study is also the first to report wasp brood development occurring inside active termite colonies.

A fundamental question to be addressed about the emergence and stability of the association reported here is which underlying mechanisms allow *M. termitophila* wasps to develop offspring amid active colonies of a termite, in this case, *C. cyphergaster*. We suspect that the exposition of wasps still in its early-developing stages to the termite nest's substrate must have a major role in the evolution of such a nesting behaviour. It has been generally hypothesized that once inside termite nests, termitophile species adopt chemical strategies to evade host detection, such as "chemical insignificance" (low concentration of recognition cues) or "chemical mimicry" (similarity in cuticular profiles) (Uboni et al., 2012). These mechanisms are known to impair inter-specific detection in social insects (Rosa et al., 2018). With olfactory and tactile cues being arguably the basis of termite's intra- and inter-specific recognition (Clement & Bagneres, 2019), colonies may eventually become more susceptible to nest invasion if members are unable to tell the difference between nest invaders and

nestmates. Although more evidence is required to classify *M. termitophila* as a termitophile or termitariophile species, a chemical strategy for host deception would be possible regardless of the type of association between wasp and termites.

The knowledge about the biology of potter wasps is still incipient in many aspects, yet it seems straightforward to link the variety of nesting strategies observed within the group to unique selective pressures acting upon the different species. Accordingly, we suspect that *M. termitophila*'s strategy for brood development is likely to be favoured by a combination of two principal components: (i) plasticity in building behaviour and (ii) flexibility in substrate preferences for nesting. Hence, the emergence of such a nesting behaviour may be plausibly a variation derived from primitive forms, in which the wasp builds a similar structure elsewhere, and not on top of active termite colonies. It remains unclear whether such a development would be incidental or not, but in either case, the strategy may have been favoured by selection, providing it confers adaptive value to the species. The wasp's nesting behaviour may be also related to microclimate conditions or protection from predators and parasites, although with the current knowledge it is only possible to speculate in this regard. It seems worth mentioning that the way how *M. termitophila* uses *C. cyphergaster* nests somewhat resembles a host-commensal relationship, which goes in line with descriptions of other known nest intruders of this specific host termite species (**Fig. 1**). Even though these species may be taxonomically independent, in most cases, they locate and use a suitable host nest to secure their offspring's development (for examples, see **Fig. 1**). A key point to be stressed here is that nest invaders known to complete their life cycle inside *C. cyphergaster* nests, seem to do so without impacting the colony, neither negatively nor positively.

Because this study is the first to record this specific wasp-termite association, drawing conclusions about associated costs and/or benefits is premature. That being said, we have reasons to suspect that *M. termitophila* does not negatively impact colonies of *C. cyphergaster*. For instance, we observed no evidence of conflict between both species when placing a recently eclosed wasp with a group of host termites (**Video S1**, Online Supplementary Material). Further inspection with more sampling will be necessary to confirm this observation and to test whether *M. termitophila* wasps never prey on termites. On the other hand, from a termite's perspective, it could be informative to develop a chemical analysis to investigate the both the larvae and the plastered mud we detected lining the internal walls of brood cells. Such a test would be particularly useful to confirm whether *M. termitophila* larvae have evolved chemicals to deter the termites and so avoid being detected inside the nest. In this same system (*C. cyphergaster*), host deterrence has been previously hypothesized based on the observation of inquiline termites (*I. microcerus*) that employ fecal pellets in response

to the host termite's aggression (Hugo et al., 2020). We emphasize that none of the wasp brood cells we found (active or not) contained remnants of prey material. The absence of such remnants not only makes it difficult to fully confirm or deny *C. cyphergaster* termites as prey, but also prevents us from making further inferences about the wasp's preying strategies other than those already documented in the literature. Preying on termites would be a very unusual behaviour for an eumenine wasp, since all currently known species hunt for caterpillars, beetles and sometimes sawfly larvae. Although it remains open to investigation how exactly *M. termitophila* relates to the remainder described eumenines, we hypothesize that, as occurring in the other species (Maindron, 1882; Krombein, 1979), nectar must be a main source of nourishment for the adults. At the same time, it seems important to reiterate that we cannot rule out yet the possibility of termites as prey for nest provisioning. However, even in that case, with eumenine wasps exhibiting solitary habits and its larvae not demanding a great amount of prey to be fully nourished, it is unlikely that termite colonies would be negatively impacted to a substantial degree. If shown to be true, this set of traits should put *M. termitophila* in a position close to previously described termitophiles, as a species that opportunistically benefit from a termite nest (e.g., being sheltered from predators) without causing substantial disruption or inflicting significant costs to the termite colony. Moreover, it remains to be further elucidated whether *M. termitophila* can nest in the ground or inside colonies of other termites, such as the remainder species belonging to Nasutitermitinae. In species of this subfamily, nests are not identical (Noirot & Darlington, 2000; Krishna et al., 2013) but still present architecture structurally comparable to *C. cyphergaster*'s. Finally, further research focusing on understanding the mechanisms allowing the association between *M. termitophila* wasps and *C. cyphergaster* termites should provide insights into the selective pressures that have shaped the evolution of such an uncommon nesting behaviour.

ETHICS STATEMENT

This study was conducted in compliance with relevant regulations governing animal research in Brazil, which includes (1) permits from the Brazilian Institute for the Environment and Renewable Natural Resources, (2) permission from landowners to conduct fieldwork on their property and (3) tacit approval from the Brazilian Federal Government implied by employing authors to conduct scientific research. The termite species sampled is native from Brazil and currently is not listed as threatened or protected. No genetic information was accessed in this study.

DATA ACCESSIBILITY STATEMENT

We declare that all data supporting the conclusions of this study are available within the article and/or its supplementary information provided (Dryad, Dataset, https://doi.org/10.5061/dryad.05qfttf1b). Non-digital data supporting the study (e.g., the holotype female used for species description, types used for species comparison) are deposited at Coleção Entomológica da Universidade Federal de Lavras, Minas Gerais, Brazil (CEUFLA), the Natural History Museum, London, England (NHM) and at the *Muséum National d'Histoire Naturelle*, Paris, France (MNHN). Upon reasonable request, further information related to this study can be requested from the corresponding author.

NOMENCLATURAL ACTS

The electronic edition of this article complies with the amended International Code of Zoological Nomenclature (ICZN) and, therefore, the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in the online registration system for the ICZN, the ZooBank (LSID: zoobank.org:pub:58378E8D-B5D3-48A9-9F53-9B455717D875). The ZooBank LSIDs (Life Science Identifiers) can be resolved by appending the LSID to the prefix http://zoobank.org/ and the associated information viewed through any standard web browser.

CONFLICT OF INTEREST STATEMENT

None declared.

Chapter IV

Effects of caste dimorphism on movement patterns of termite species

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ABSTRACT

This study explored the effect of caste dimorphism on the movement patterns of termites by quantifying individual motion of caste types. Using high-resolution videos of behaviour and an image-based tracking, we extracted individual movement trajectories of workers and soldiers of the termite *Syntermes molestus*. Specifically, we tested two mutually exclusive hypothesis concerning the movement of caste types. First, we tested the hypothesis that (i) dimensional morphological differences exhibited by the soldier caste (e.g., increased body size) directly influence their movement patterns (which we refer as 'proportional movement hypothesis'). Second, we tested the alternative hypothesis that (ii) despite anatomical differences between workers and soldiers, movement is uniform across these caste types and therefore disproportionate to the observable body size variation (which we refer as 'disproportionate movement hypothesis'). Multivariate statistical analyses revealed significant differences among castes: overall, soldiers were more active and faster than workers. Investigation of Mean-Squared Displacement and Mean-Squared Relative Distance, which we employed to assess exploration, were less sensitive to caste-specific types: not only soldiers but also workers were super-diffusive, a result in nonetheless in line with previous investigations. Higher values in movement metrics of soldiers reflect their augmented morphometric differentiation, which seem to support our proportionate movement hypothesis. The evolution of morphological specialization is often coupled with physiological developments. As such, the interplay between morphology, physiology and movement remains to be further explored. This study provides a deeper understanding of morphological traits derived from caste dimorphism, as an integrative part of behavioural adaptations in the movement of highly social individuals.

Keywords: Movement ecology, Caste dimorphism, Termitidae, Syntermitinae, *Syntermes molestus*

1 INTRODUCTION

A hallmark in the evolution of eusocial insects' is the development of well-defined division of labour among castes (Duarte et al., 2011). In termites, caste dimorphism stems not only from behavioural traits and social interactions but also from morphological differences exhibited by individuals (Snyder, 1926; Roisin, 2000), which enables a group of colonial members – namely, a caste - to perform essential tasks within the colony. This study investigates the influence of morphological aspects derived from caste dimorphism in the movement patterns of termites. Inspired by theoretical concepts of movement ecology, our framework integrates methods and techniques from the fields of Biology and Physics to better understand how the shape and form of evolutionarily adaptive traits may affect the way individuals move. To this end, we observe and measure the behaviour of *Syntermes molestus* (Burmeister, 1934) a Neotropical termite well known for its accentuated caste dimorphism, in which soldiers are not only morphologically distinct but also larger than workers.

1.1 Colony defence as an integrative system

The group of eusocial insects is ecologically and evolutionarily very successful (Wilson, 1971), which is partially due to their evolved abilities to coordinate and defend the integrity of the colony. As in other eusocial groups, colony defence in termites is an elaborated system involving interconnected abiotic and biotic elements (Prestwich, 1984; Scholtz et al., 2008). The abiotic component, represented by a variety of physical protective structures (the nests) is produced by the colony, and linked to the evolution of self-organisation (Camazine et al., 2020; Duarte et al., 2011) and building behaviour (Noirot & Darlington, 2000). To some extent, nests of termites (Noirot, 1970) are comparable to those observed in as ants (Hölldobler & Wilson, 1990) and bees (Seeley & Morse, 1976). Although Hymenoptera and Isoptera (Dictyoptera:Blattodea) evolved independently (Wilson, 1971), eusocial nests of both groups are built collectively by nestmates. Ultimately, they all confer an adaptive value to the colony. Among the advantages of living inside nests, protection against predation, homeostasis, and fortification, are said to be of foremost importance for colony survival (Eggleton, 2010). Another obvious benefit provided by nests, that is particularly intrinsic to termites, is the protection against desiccation (Danks, 2002), since the group is known to be far less resistant than ants (Bujan et al., 2016) or bees (Radchenko & Pesenko, 1994) in this aspect. The task of nest building, however, is invariably costly to eusocial colonies, not to mention time-consuming (Danks, 2002; Ireland & Garnier, 2018). It seems therefore natural that social species would evolve mechanisms and strategies to protect such an 'investment', while defending themselves. This protective role is played by the biotic component of colony defence and includes morphologically

adapted phenotypes linked to the evolution of a specialized defensive caste: the soldiers (Roisin, 2000). In this point, specifically, termites seem to be unique in relation to other eusocial insects (Wilson, 1971). Since they are hemimetabolous - as opposed to hymenopterans - termite soldiers can be actively involved in colony defence well before attaining maturity. Another fundamental difference is that, to the best of our knowledge, in all known termite species, soldiers necessarily pass through a pre-soldier instar and, therefore, cannot be considered as a subcaste of workers, which is the case of some Hymenoptera (Noirot & Pasteels, 1987; Hölldobler & Wilson, 1990).

1.2 The soldier caste in termites

In termites, active defence is arguably not a primary function of workers, although in some species the caste may provide relevant support to more specialised soldiers: workers can engage in protective actions by physically biting opponents targeted by soldiers that do not have functional mandibles (Hugo et al., 2020); in more dramatic cases, they may even commit suicide, as last defensive attempt (Šobotník et al., 2012). Nevertheless, for being primarily involved with other colonial tasks, such as construction, feeding and tending (Eggleton, 2010), workers are less frequently associated with active defence (but see Thorne, 1982, Binder, 1988). The caste of soldiers, on the other hand, have the sole purpose of protecting the colony. Empirical evidence suggests that, as a rule, soldiers will defend no matter, and for that, they could be arguably considered as the truly altruistic caste. Given their substantial contribution to colony defence, the importance of specialized soldier types to colony integrity in termites seems therefore undeniable. Generally, apart from some 'soldier-less' (e.g., *Anoplotermes*; Snyder, 1926; Apicotermitinae; Bourguignon et al., 2016) and 'nest-less' species (e.g., *Inquilinitermes*; Emerson, 1938), most termites have evolved morphologically adapted soldier types (Prestwich, 1984) and abilities to build their own nests (Noirot, 1970). Not surprisingly, for exhibiting colony defence in integrative parts (as highlighted above), termites are ideally suited to address questions about the causes and consequences of specialized defensive traits in complex societies.

Evolutionarily speaking, differentiation of colony members into a defensive soldier caste is an adaptive trait widespread in termites (Prestwich, 1984). Unlike other eusocial insects (e.g., bees, ants, aphids, thrips), the soldier caste in termites is monophyletic and because it evolved prior to a true worker caste (sterile helping caste), it is also an ancestral trait (Noirot & Pasteels, 1987). In terms of morphology, termite soldiers exhibit a staggering variety (Prestwich, 1984; Scholtz et al., 2008): from tiny individuals so-called 'minor soldiers' to massive mandibulate soldier types, as in *Macrotermes bellicosus* (i.e., the largest known termite, with soldiers measuring about 36 mm). As

mentioned previously, soldiers are lacking in only a few genera of Termitidae (Eggleton, 2010), which reinforces the idea that their absence is derived (Roisin, 2000). In the group of species that currently exhibit soldier caste, defensive weaponry typically becomes more evident after individuals pass through the transitional pre-soldier stage; from this point on, soldiers are said to be fully capable of deterring threats (Prestwich, 1984). In several termite species, however, little is known about the underlying mechanisms dictating the behaviour of soldiers. Although is well known that all termite soldiers exhibit heavily sclerotized head capsule with specialized defensive features, other types of morphometric variation (e.g., increased body size) remain relatively less studied (but see 'unidirectional body size reduction': Mizumoto & Bourguignon, 2021). Consequently, reports on individual and collective behaviour exhibited by proportionally larger soldier individuals are scarce in the literature. Knowing the implications of evolved morphological adaptations of soldiers would pave the way for a better understanding of their ecological significance.

1.3 Rationale, hypotheses, and predictions

In this study, using *S. molestus* as a model system, we ask the question of whether the remarkably impressive evolution of shapes and forms in the soldier caste of termites has significant effects on movement, one of the most intrinsic behavioural aspects of living organisms. Upon reaching alarm state, soldiers of this species are characterised for executing fast abrupt movements during defence. Here we ask how the soldier caste operates in non-threatening scenario (controlled experimental arenas), establishing a parallel with the worker caste, of relatively smaller proportions. Our hypotheses lie within the framework of movement ecology and, as a conservative approach, we explore the simplest variables that might be capable of explaining the differences in motion patterns: individual movement parameters (displacement, speed) and exploration indexes (mean squared displacement and mean squared relative distance). Specifically, we tested the hypothesis that, the absence of threats, (i) dimensional morphological differences gradually shaped by evolution and exhibited by termite soldiers (e.g., increased body parts, elongated joints, disproportionate head) influence directly on the movement patterns of the soldier caste. To support such a hypothesis (hereafter, proportional movement hypothesis), it is necessary to observe a consistent proportional increment on movement metrics of soldiers, as compared to workers. Increased speed and exploration indexes observed among soldiers would indicate that the caste is able to move around and explore more quickly than the caste of workers. Alternatively, it is equally possible that (ii) despite anatomical differences between soldier and worker castes, when individuals do not experience threats, movement is uniform across caste types and, therefore, disproportionate to the observable body size variation (hereafter, disproportionate movement hypothesis). To support

this idea, and to reject our proportional movement hypothesis, it would enough to observe no significant differences between the descriptive movement measurements of termite soldiers and workers (i.e., larger vs smaller caste type). If proven true, such a lack of variation would provide empirical evidence that caste dimorphism has little to no influence on how caste types move, specifically when termite soldiers and termite workers are not under threatening situations or have reached an alarm state.

2 MATERIAL & METHODS

2.1 Biological species

Syntermes molestus exhibit nocturnal foraging (Martius & Weller, 1998) with activity periods generally occurring from dusk to dawn (Wheeler, 1936). In the open, the presence of soldiers (larger individuals) is very important, as it provides protection to groups of foragers composed exclusively by the worker caste (smaller individuals). Because the recruitment of soldiers occurs gradually, however, defensive lines take some time to be consolidated at the foraging sites. It has been observed that, for unknown reasons, soldiers retire from defensive lines prematurely, moving back to nest before the very end of foraging events (Mill, 1984; H. Hugo pers. obs.). With less soldiers nearby, workers are presumably more vulnerable to predation. In fact, it is plausible that such activity patterns by soldiers would be linked to the evolution of at least one specialized predator of *Syntermes* species, *Neoponera commutate*. This large raiding ant, found in South America (Mackay & Mackay, 2010), is known to prey almost exclusively on *Syntermes* (Mill, 1982), but more intensively when termite colonies lower their defences at the foraging sites (Mill, 1984). This indicates that, areas less densely populated by soldiers, or in periods when their proportion drops substantially, may be taken as a 'window of opportunity' by species that feed heavily on the termites. Together, these facts highlight the importance of having soldier individuals when foraging in the open.

When in groups, positioning of defensive elements across the space is crucial to determine whether coordinated defensive strategies will be successful or not (Prestwich, 1984). Like many of species of Termitidae, *Syntermes* soldiers do not have functional mandibles to collect resources (Roisin, 2000) and therefore contribute little to foraging; they also need to be fed by workers (Snyder, 1926), and spend a considerable amount of energy engaging on intense conflicts during defence (Deligne et al., 1981; Shelton & Grace, 1996). For colonies, therefore, having soldiers represents a sizable demand in terms of resources. As a result, it is likely that many species evolved in ways to keep the proportion of soldiers at a low level (Haverty & Howard, 1981). In obligatory inquiline termite species

(e.g., *Inquilinitermes microcerus*), soldiers can be extremely rare (Cunha et al., 2003); they have been shown, nonetheless, to be essential to their colony, as an ultimate defence when inquilines are detected by the host termite and inevitably attacked (Hugo et al., 2020). Soldiers of *Syntermes* are equally important when termite species compete for resources. For instance, *S. molestus* and *S. spinosus* are typically seen coexisting in stable assemblages, yet their foraging dynamics is supposedly affected by defensive strategies of each species (Martius & Weller, 1998).

2.2 Study site and sampling

We collected individuals of the termite *Syntermes molestus* (Termitidae: Syntermitinae) from seven natural colonies located in a Cerrado area near the municipality of Peçanha (18° 35' 40.0" S 42° 37' 35.0" W), State of Minas Gerais, Brazil (**Fig. 1A, 1D**). The Brazilian Cerrado (Ratter et al., 1997), categorised as 'equatorial savannas with dry winters' (Kottek et al., 2006), is undoubtedly the richest biome in terms of generic and specific diversity of the subfamily Syntermitinae (Rocha et al., 2017). Species of the genus *Syntermes* Holmgren 1909 built subterranean nests and have diet based on leaf- or grass-litter (Constantino, 1995). With relatively large size, conspicuous epigeal nests of *Syntermes* are of easy detection in the field. Yet, it is often hard to sample individuals due to their evasive behaviour when disturbed. Upon exposure, workers - and surprisingly often, soldiers – of the species tend to evade towards the nest core (H. Hugo, pers. obs.), which is often a few meters underground the soil surface (Noirot, 1970). Some nests of *Syntermes* are completely underground and without the epigeal portion (up to 3 m bellow surface: Constantino, 1995), which can make sampling in the field even harder. This could perhaps explain why the genus is less frequently studied and reported, as compared to other termites.

Sampling occurred in April of 2018, in the dry season, within a preserved fragment of approximately 50 hectares (**Fig. 1E**). Nests sampled (n=7) were never neighbouring one another and, overall, distributed sparsely through the area. All biological material collected for behavioural observation (e.g., termites and portions of their original nests to preserve individuals) was immediately transported to the recording room and kept under suitable conditions of temperature and relative humidity for termites (Collins, 1969) until the beginning of behavioural videorecording (**Fig. 1G, 1H**). After behavioural observation, focal individuals were properly labelled with taxonomic information, fixed with alcohol in glass containers and deposited in entomological collection as voucher specimens (**Fig. 1F**; Lab of Termitology, Department of Entomology, UFV).

2.3 Behavioural recording and tracking

To observe and measure the movement of soldiers and workers of the termite *S. molestus*, we recorded experiments using high-resolution cameras filming under visible spectrum of light. To create images adequate for posterior tracking, we recorded videos of arenas in 4K, using a Sony FDR-AX100 camera along with an evenly distributed illumination, which was provided by a source with three LED-lights (**Fig. 1G**). Each experimental arena used to record termite movement was set in a sterilised glass Petri dish (diameter = 15.5 cm) lined with paper (Whatman®, filter paper, Grade 1) and covered with a transparent lid (**Fig. 1H**), as it has been done previously in studies with other termites (e.g., *Constrictotermes cyphergaster*; Hugo et al., 2020). Before each trial, termites composing an experimental group were submitted to an acclimation period of 10 minutes. Until the beginning of recordings, individuals were kept in artificial nests (created with a moisturised portion of soil material from the original nests), to prevent excessive desiccation of termites (**Video S1**, Supplementary material). To further prevent water loss by individuals, (i) behavioural recordings were limited to 10 minutes, (ii) videos were filmed always between 18:00 and 19:00 and (iii) the room temperature during species manipulation and behaviour recording was set to 25 ± 2°C.

Once all behavioural observations were recorded, tracking data of termites were acquired from high-resolution videos (full frame size: 3840×2160 pixels) using an open-source, image-based tracking software (Ribeiro et al., 2019). Instead of a fully automated approach, tracking was supervised with individual identities being manually corrected via software, whenever necessary (e.g., when workers were partially occluded by soldiers). Movement data were obtained from high-resolution videos recorded at 25 frames per second. The positional information of individuals was appended in pixel coordinates and later converted to centimetres (130 pixels = 1 cm), during calculations and statistical analyses (see Supplementary material).

2.4 Experimental groups

Since termites are highly social (Shellman-Reeve, 1997), it seems more appropriate to measure their movement not only in isolation (e.g., when individuals are alone) but also in social contexts (e.g., when individual are in groups). For this reason, we observed termites of *S. molestus* individually (1 worker; 1 soldier) and in groups of 3 termites (3 workers; 2 workers with 1 soldier), 6 termites (6 workers; 4 workers with 2 soldiers), 12 termites (12 workers: 8 workers with 4 soldiers), and 24 termites (24 workers; 18 workers with 6 soldiers). Thus, each group size defined (i.e., 3, 6, 12 and 24 individuals) had an arrange composed only by workers, and another one with the addition of soldiers

in a 2:1 [worker-to-soldier] caste ratio. Since we performed three replicates for each arrange and never used the same individual twice in trials, we observed the behaviour of 276 termites from 7 different colonies. In the field, sampling of soldiers from natural nests was difficult - due to the evasive behaviour we mentioned previously – and, for this reason, groups composed only by multiple soldiers were not recorded. Nevertheless, we have no reasons to suspect that this affected our analyses negatively. In natural colonies of *Syntermes*, soldiers are greatly outnumbered by workers (Constantino, 1995) and our experimental procedure was designed to represent such a contrast.

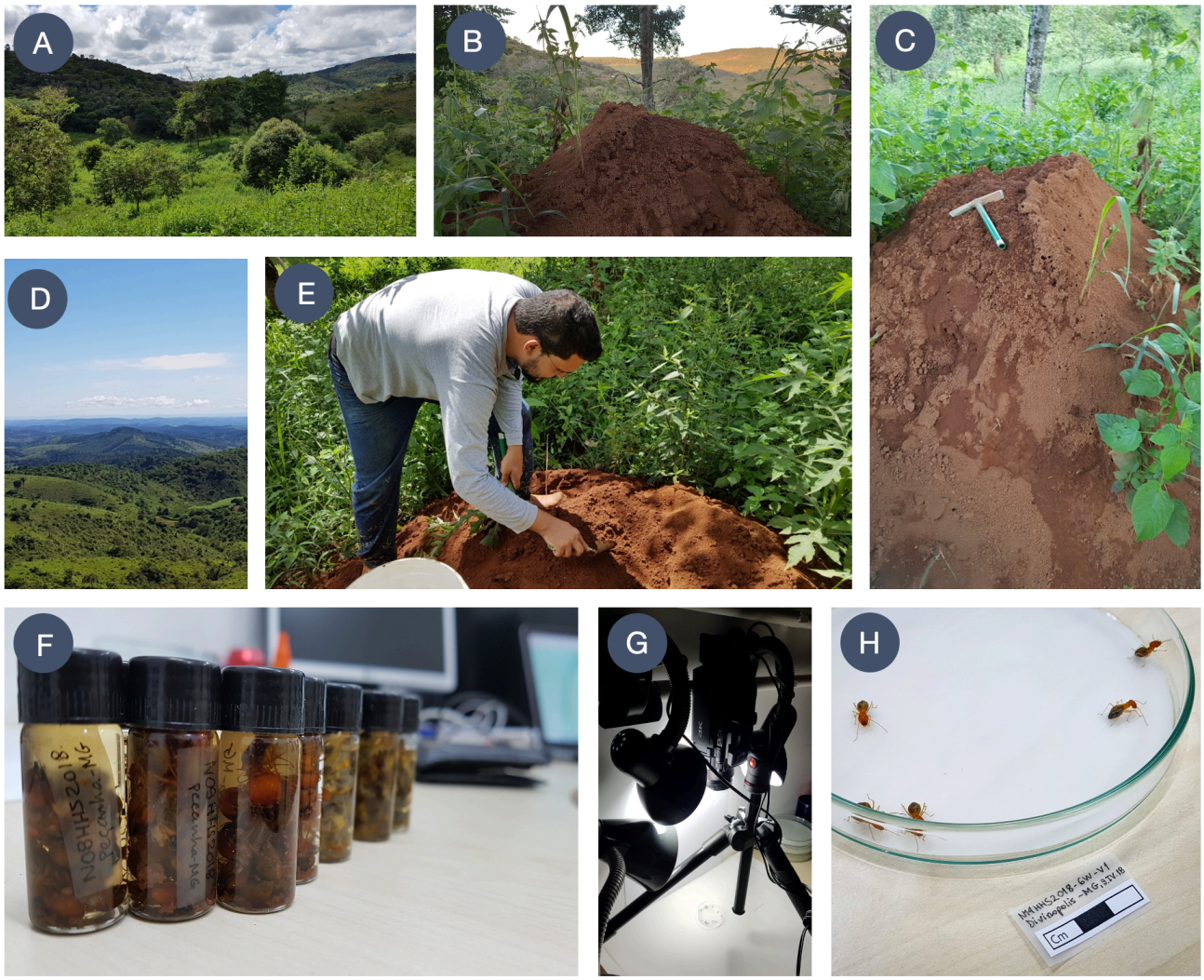

Figure 1 | Study site, sampling, and experimental procedure: Study site in the Brazilian Cerrado [Peçanha, Minas Gerais, Brazil] (A, D); Epigeal nests of *Syntermes molestus* (B, C); Sampling of termite individuals from natural colonies (E); Voucher specimens of *S. molestus* prepared for inclusion in entomological collection [Department of Entomology, Lab of Termitology, UFV, Brazil] (F); Recording setup (G); Example of experimental arena [with termite workers] and graphic scale used for calibration and unit conversion [pixel-to-cm] (H).

2.5 Validation of experimental arena

As in previous studies investigating movement of termites in controlled conditions (Miramontes et al., 2014; Paiva et al., 2021; Hugo et al., in prep.), no obstacle or food was present in the arenas to allow evaluation of free walking behaviour. Also, we used circular containers (i.e., Petri dishes) because, for reasons not fully understood, termites tend to be attracted by acute-angled geometries (Lee et al., 2008) and this would affect free movement of individuals. To verify how the size of containers affect movement metrics, prior to experiments, we inspected the behaviour of isolated termite workers in Petri dishes of different radius (unpublished): in *Cornitermes cumulans*, the main impact of the size of the arena was, as would be expected for such an arena size, at the saturation point of the mean squared displacement (MSD) measure. In Petri dishes with diameters between 5-20 cm, the turning angle distribution was centred in zero, and a value slightly different from zero was observed only in arenas with less than 4.5 cm diameter. Therefore, to avoid interferences on turning angles and have individuals' distributions centred in zero (**Fig. 2A, 2C**), we used Petri dishes with diameter of 15.5 cm.

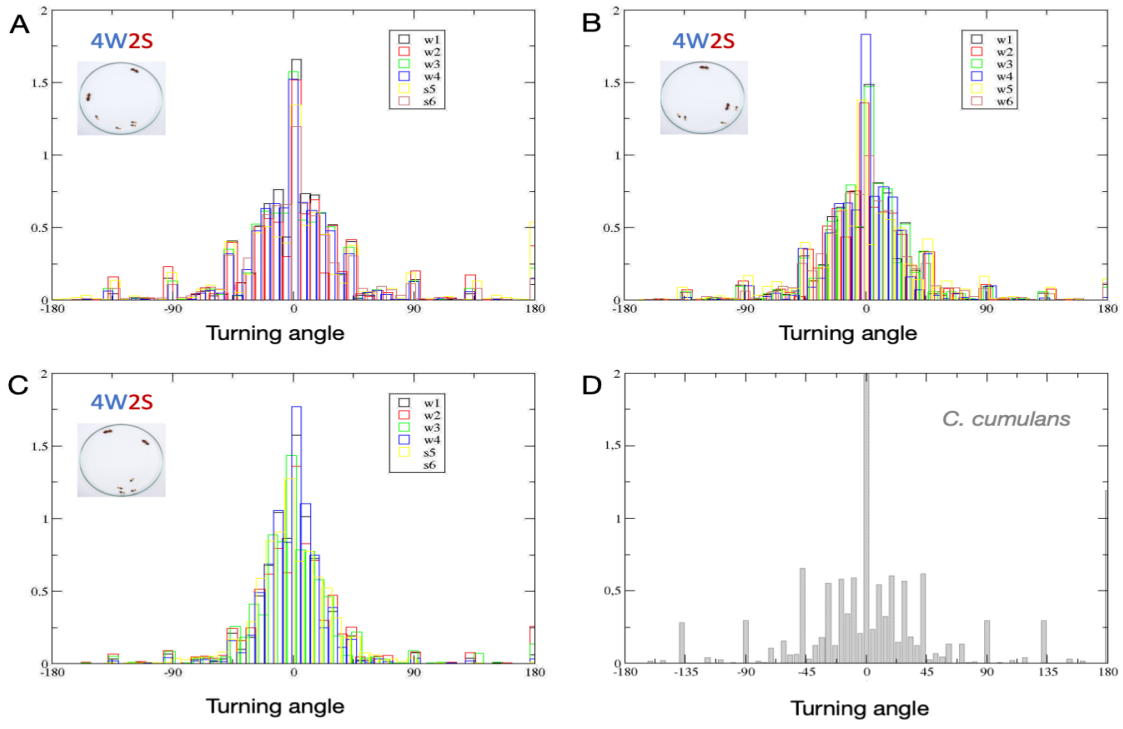

Figure 2 | Turning angles: Representative example of turning angle distribution observed in experimental arenas (15.5 cm diameter) containing four workers and two soldiers of *S. molestus* [4W2S] (A-C). Distributions were plotted with transparency and overlapping each other, showing that in different replicates (A, B, and C), the turning angle of both workers and soldiers is centred in zero; For comparison purposes, a turning angle distribution (also centred in zero) of an isolated worker of *Cornitermes cumulans* was also plotted (D).

2.6 Identifying trajectory patterns with summary plots

Trajectories were compared across groups of different sizes (i.e., number of individuals in arena) and caste compositions (i.e., soldier-to-worker ratio in arena). For each termite individual observed, we produced summary plots containing the following graphic elements: (i) a trajectory plot (x-y coordinates) showing the path walked by the termite with temporal information [represented by the colormap 'viridis']; (ii) a histogram of calculated values of speed [cm/s] a with a fitted parameter (Poisson distribution): (iii) a heatmap of normalised speed [cm/s] over time with speed values [represented by the colormap 'viridis']; and (iv) a plot showing displacement [cm] over time, with the calculated mean value of displacement [represented by a solid horizontal line]. To facilitate identification of castes throughout summary plots, we used a colour code consistent with other figures in this study: soldiers are represented in red, and workers are represented in blue. All functions and calculations necessary to generate summary plots were developed in Python, using custom modules and common libraries and are available in the online Supplementary Material.

2.7 Measuring exploration

The Mean-Squared Displacement (MSD) analysis is a tool to identify super-diffusive aspects of an organism's movement. Within the framework of anomalous diffusion theory (Klafter et al., 1987), the scaling exponent of the MSD is related mathematically to a Lévy probability distribution of the lengths of the steps. Lévy walks are random walks composed of clusters of multiple short steps interspersed with more rarely occurring longer steps. Previous work with *Cornitermes cumulans* used this framework to show that isolated termite displacement, in space and time, presents self-similar patterns highly consistent with super-diffusive Lévy-like movement (Miramontes et al., 2014). Recently, it has been demonstrated that the value of the scaling exponent of the power-law describing the Lévy walk of an individual is collectively modified as the density of animals in the group changes (Paiva et al., 2021). Our preliminary analysis indicated that confined workers and soldiers of *Syntermes* perform super-diffusive movements. Going beyond to just replicate the results observed with MSD in *C. cumulans*, here we also investigate the Mean-Squared Relative Distance (MSRD). It has been theoretically shown (Ueda, 2019) in directed polymer in random media one of the simplest models of disordered systems in statistical mechanics, that the MSD of one polymer end exhibits super-diffusive behavior, while the MSRD of two polymer ends exhibits sub-diffusive behavior. Inspired by this work, we investigated pairwise mean-squared relative distance between termites in circular arenas.

2.8 Statistical analyses

Statistical analyses were conducted in R (R Core Team, 2020) with Rstudio (RStudio Team, 2019) using Linear Mixed Models (LMM). Models were fitted with a restricted maximum likelihood method using lmerTest (Kuznetsova et al., 2017). Assumptions of normally distributed and homogeneous residuals were fulfilled. To investigate whether movement metrics differ between caste types (i.e., soldier vs. worker), first, we included 'displacement' as response variable in models, with 'caste type' as fixed effect and 'termite identity' as random effect. Individual displacement [cm] was measured for each termite as the Euclidean distance between the position in two consecutive frames, which was used as a proxy for movement activity. Second, to test whether speed differs between caste types, we fitted 'speed' as response variable, with 'caste type' as fixed effect and 'termite identity' as random effect. Individual speed [cm/s] was calculated as the distance covered by each termite individual within 25 frames [one second]. Both displacement and speed were square-root transformed to reach normal distribution.

3 RESULTS

Our results are composed of following parts: (i) trajectory patterns, including descriptive measurements of worker and soldier individuals; (ii) displacement and speed profiles, showing both raw data and results of statistical tests conducted with multivariate analysis; and (iii) explorations indices, describing the motion pattern detected. Additionally, in our Supplementary Material, we included videos of *S. molestus* and extended analysis containing summary plots and representative examples of MSRD calculations.

3.1 Trajectory patterns

Analyses of trajectory patterns indicated that, in experimental arenas, both workers and soldiers of *S. molestus* exhibit individual differences in terms of trajectory, speed profiles and displacement patterns. Regarding activity, a higher proportion of workers (19.3%, 5 out of 43 individuals) showed lower levels of mobility. In contrast, a lower proportion of soldiers (11.6%, 45 out of 233 individuals), showed lower levels of mobility. We also noticed that, when isolated, workers and soldiers often showed seemingly contrasting trajectory patterns (**Fig. 3A, 3B**), but when composing a group, both castes followed similar trajectories (**Fig. 3C, 3D**), potentially in response to pheromone trails.

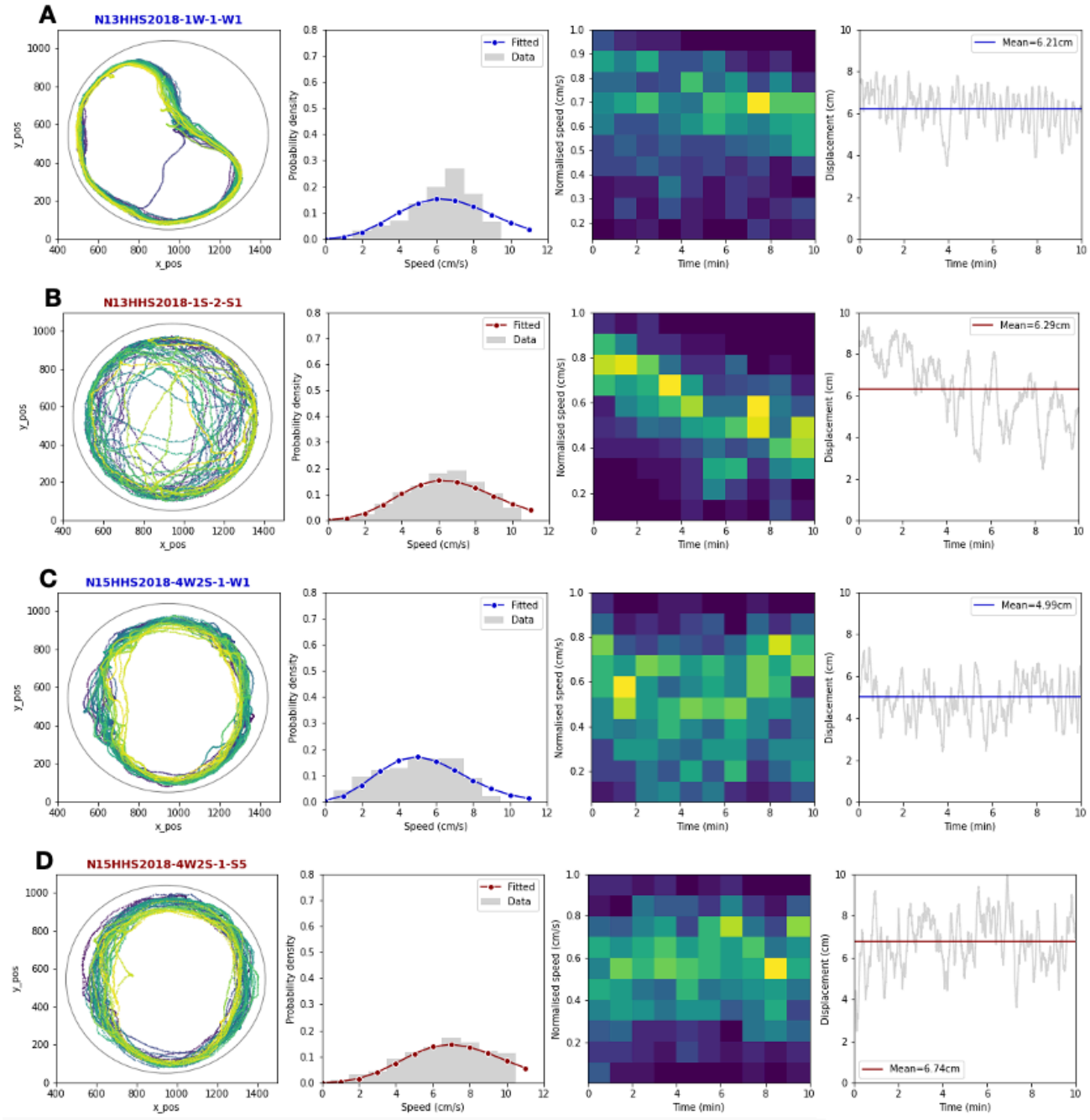

Figure 3 | Trajectory patterns and movement metrics: Representative examples of trajectory patterns of soldiers (isolated: B; in groups: D) and workers (isolated: A; in groups: C) of *S. molestus* in experimental arenas (15.5 cm diameter). Movement metrics (i.e., histogram of speed, normalised speed over time, and displacement over time) were plotted next to individual trajectories to facilitate visual analyses of patterns. Note that: when isolated, worker (A) and soldier (B) show contrasting trajectories; but when in groups, worker (C) and soldier (D) consistently follow similar trajectories. Soldiers are represented in red and workers in blue. The complete set of summary plots with all termites observed is available in the Supplementary Material.

3.2 Displacement and speed profiles

Multivariate statistical analyses (LMM) with displacement revealed that the caste of soldiers (mean ± SE: 0.02 ± 0.00 cm) was significantly more active than the caste of workers (mean ± SE: 0.01 ± 0.00 cm), overall showing larger values of displacement (**Tab.1**, **Fig. 4-B**). In terms of speed, we found that soldiers (mean ± SE: 0.02 ± 0.00 cm/s) were also significantly faster that workers (mean ± SE: 0.01±0.00 cm/s), exhibiting higher speeds (**Tab. 1**, **Fig. 4-D**). To assess individual movement patterns per caste, we additionally visualized both cumulative displacement and cumulative speed over time. Cumulative values of displacement and speed were calculated using the base R function "*cumsum*". Cumulative displacement increased linearly over time for most individuals, with soldiers reaching larger values of displacement (max ~800 cm) and more variation in displacement values, compared to those of workers (**Fig. 4-A**). Cumulative values of speed increased asymptotically to a limit (max ~5 cm/s), with soldiers reaching higher speeds and showing more variation in speed values compared to those of workers (**Fig. 4-C**).

Table 1 | Linear Mixed Models (LMM) with: 'sqrt displacement' (A) and 'sqrt speed' (B) as response variables; 'caste' as fixed effects; and 'termite identity' as a random effect. Statistical significance is highlighted in bold.

A - SQRT DISPLACEMENT (cm)

Fixed effects	Estimate	SE	*df*	t value	P value
(Intercept)	0.12	0.01	274.13	20.80	-
Caste	-0.03	0.01	274.13	-4.11	**< 0.001 *****
Random effects	Variance	SD			
Termite identity	0.00	0.04			
Residual	0.00	0.06			
N observations	4223828				
N individuals	276				

B - SQRT SPEED (cm/s)

Fixed effects	Estimate	SE	*df*	t value	P value
(Intercept)	0.15	0.00	253.79	41.05	-
Caste	-0.01	0.00	254.80	-2.91	**< 0.004 ****
Random effects	Variance	SD			
Termite identity	0.00	0.02			
Residual	0.00	0.05			
N observations	14927				
N individuals	276				

3.3 Exploration indexes

Investigation with Mean-Squared Relative Distance (MSRD) confirmed preliminary results obtained previously using Mean-Squared Displacement (MSD) methods: both workers and soldiers of *Syntermes* in confinement perform super-diffusive movements (**Fig. S2**, Supplementary Material). Although this result is in line with patterns identified in other termite species (e.g., *C. cumulans*), it is apparent that these methods were less sensitive to differences in the size of individuals and, to our experimental procedure specifically, less effective in telling caste-specific differences apart.

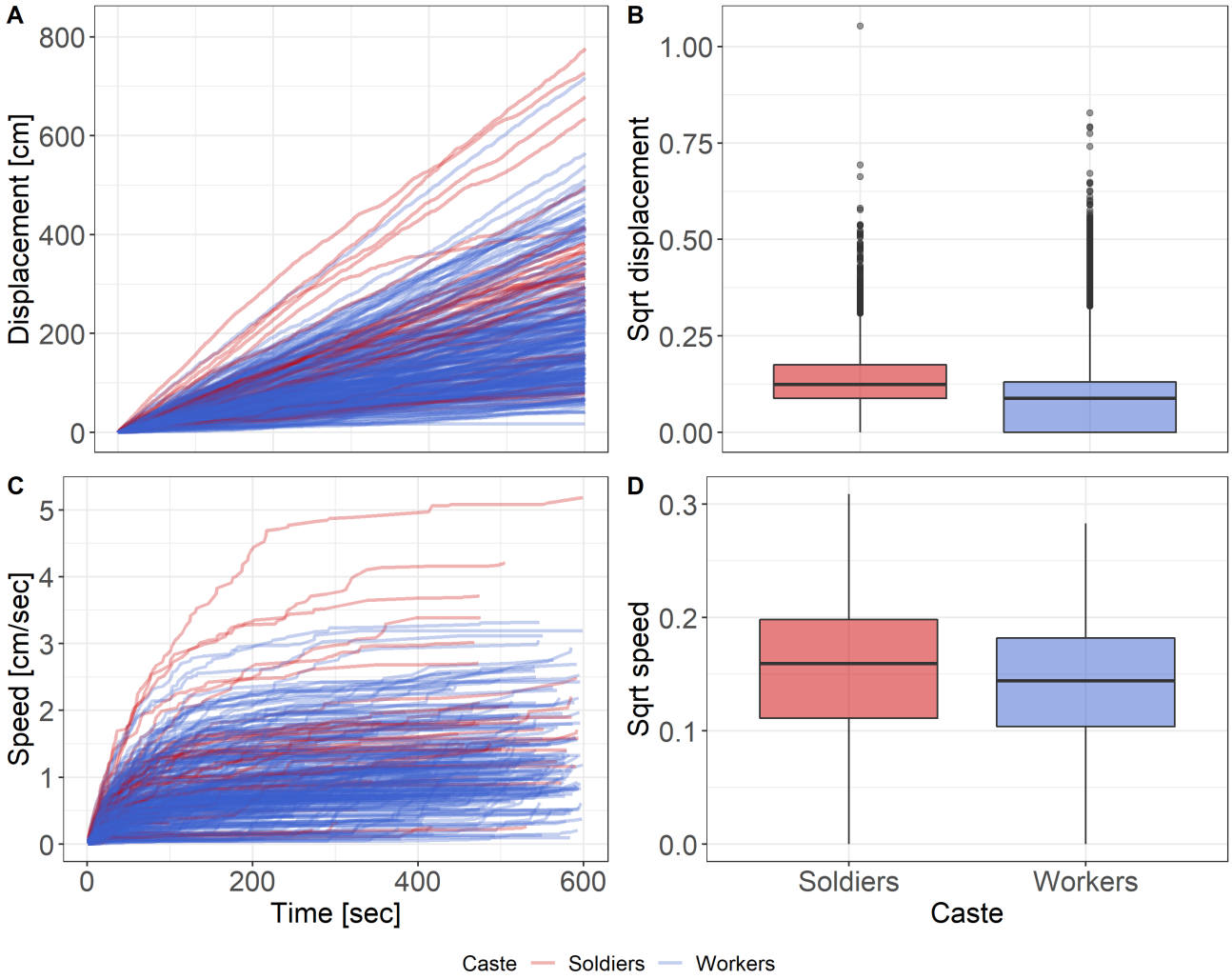

Figure 4 | Displacement and speed patterns per caste type: Cumulative values of displacement (A) and speed (C) increase linearly and asymptotically over time, respectively. Overall, termite soldier show both larger displacements (B) and higher speeds (D), compared to termite workers. The soldier caste is represented in red and the worker caste in represented blue.

4. DISCUSSION

The knowledge of *Syntermes* is still incipient in many aspects (but see Emerson, 1945; Baker et al., 1981; Mill, 1984; Constantino 1995; Martius, 1998; Martius & Weller, 1998), and because our findings are the first accounts of the behavioural ecology of *S. molestus*, it would be unwise to draw elaborate conclusions about the functioning and evolution of movement patterns observed. That being said, as it is, our results seem to support our proportional movement hypothesis, since we did observe a consistent proportional increment in the movement metrics of soldiers, as compared to those of workers (**Fig. 3**, **Fig. 4**). This suggests that, as opposed to a single algorithm of motion operating in both castes (i.e., disproportionate movement hypothesis), morphological differences exhibited by the soldier caste allow soldier individuals to move differently, in a way that reflects their evolutionarily adaptive traits.

4.1 Movement trends

We found that, overall, soldiers moved faster than workers and with longer discrete displacements. This is not to say, however, that workers were somehow limited in their movement abilities. In fact, a few worker individuals showed a cumulative displacement rather comparable to those observed among soldiers (**Fig. 4A**). As a trend, however, the total distance walked by workers in experimental arenas was never higher than those observed among soldiers. Also, the proportion of individuals that exhibited lower mobility was higher in workers (19.3%), compared to soldiers (11.6%). Moreover, the fact that the cumulative displacement increased linearly in almost all termites (**Fig. 4-A**) suggests that, by default, workers and soldiers in experimental arenas walked at a relatively constant pace. This trend can be also observed, in higher detail, with the summary plots we produced for individual termites (Supplementary Material).

As for the asymptotic trend observed in the cumulative speed of individuals, we suspect that it has to do with the experimental conditions provided: within the finite space of our circular arenas (15.5 cm diameter), individual speeds can only increase up to a certain point. Additionally, the fact that roughly around the 300 seconds mark speed curves of individuals tended to asymptote in a linear pattern (**Fig. 4-C**), indicates that, under the conditions provided, termites were able to reach their maximum speed. We also interpret this result as an indication that we successfully prevented external factors that could lead termites to increase their speed instantly (e.g., triggered by alarm signals).

4.2 Super-diffusive motion

An intriguing result in our findings was that super-diffusive movement was the default mode of walking in both caste types. This evidence, we believe, is not merely correlative: while previous empirical observation was done with individuals of virtually identical proportions (single caste of same size: Paiva et al., 2021; Miramontes et al., 2014), in this study observed individuals with contrasting morphometric features (two castes of different sizes: smaller vs. larger). Yet, super-diffusive motion was detected in both systems and, particularly in this work, for both castes using two different methodologies (MSD and MSRD). It should give pause for thought that these conflicting results, while challenging our predictions, could be possibly taken as a starting point to further explore this issue: for instance, by focusing in addressing raising questions, such as: why do we see similarities in such different systems? A better understanding of motion patterns should provide useful insights about the evolution of movement in group-living organisms.

4.3. Interactive aspects of movement

One aspect that was not in the scope of this work but certainly deserves attention in a follow-up examination, is the interplay between movement and interactions between individuals. When in groups, antennation and grooming are behaviours frequently performed by termites, with workers engaging in interindividual interactions for extended periods of time (Shellman-Reeve, 1997, Eggleton, 2010). We suspect that it would be possible to link some of the patterns we observed in this study with fluctuations in the interaction rates, within and between castes. This intuition comes from insights we had when investigating collective behaviour in another study, with *C. cyphergaster* (Hugo et al., in preparation). In that species, the proportion of soldiers relative to workers seem to influence the formation of collective trails, thus affecting the movement of individuals (unpublished data). Interestingly, with *S. molestus*, we noticed that in the groups with the highest number of soldiers (18W6S), workers showed lower values of both speed and displacement. Curiously, the same was not observed for the soldiers composing those groups, which moved similarly to soldiers composing groups with other caste ratios. One way to possibly explain this difference is that, with more soldiers in arena there is less space available for workers. In confined spaces, such as our arenas, larger dimensional proportions of soldiers may have somehow affected the way workers moved. For soldiers' displacement there was no such issue: being larger, they could simply walk over smaller workers (something that is observable in our videos). This explanation would not require elaborate mechanisms to make sense: in theory, it would be enough to just confirm the assumption that workers - being blind – tend to 'bump' into larger individuals more frequently. Because contact

in termites is often followed by antennations, this could affect movement if it happens frequently. With our current data we cannot assure that this was the case, however, since it is also possible that other factors (e.g., chemical signals) may have regulated the motion of workers. In some termite species, for example, aggregation pheromone has been shown to have both attractant and arrestant effect over workers (Mitaka et al., 2020), which tends to slow down nearby individuals. It is possible that *S. molestus* evolved a similar mechanism, perhaps using a different substance in a similar fashion. Either way, to rigorously assess the effects of chemical communication on the movement of termites, further studies with proper methods and controls are required.

4.4 Final considerations

It is worth mentioning that, since we intentionally provided both (i) acclimation period and (ii) a stimuli-free environment to individuals under observation, our results are only valid in the absence of threats. Despite being extremely relevant and currently understudied in the species we investigated, behavioural patterns associated with alarm communication and defensive actions (e.g., response to alarm signalling) were out of the scope of this study. In fact, in our trials we observe no defensive behaviour whatsoever. As a result, we cannot make inferences about strictly defensive traits of soldiers, nor confirm whether movement metrics of termite individuals were influenced by alarm pheromones.

The influence of accentuated caste dimorphism on the behaviour of termite species is a particularly important but poorly understood topic. Studies focusing on behavioural descriptions of species that have evolved accentuated morphological differentiation can provide a means to investigate the interplay between morphology and behaviour, as well as to better understand the proximate and ultimate causes of caste dimorphism in eusocial societies.

ETHICS STATEMENT

This study was conducted in compliance with relevant regulations governing animal research in Brazil, which includes (1) permits from the Brazilian Institute for the Environment and Renewable Natural Resources, (2) permission from landowners to conduct fieldwork on their property and (3) tacit approval from the Brazilian Federal Government implied by employing authors to conduct scientific research. The termite species sampled is native from Brazil and currently is not listed as threatened or protected. No genetic information was accessed in this study.

General Discussion

The goal of this thesis was to elucidate mechanisms of individual and collective behaviour in termite species. As such, in **chapter I**, I reported a variety of collective outputs for different whole-colony observations using a controlled experimental setup. Field experiments have been - and still are - intrinsic to addressing most fundamental questions in biology. Yet, environmental complexity often translates to an inherent inability of methods to filter out completely major external factors affecting a given behavioural response. Because termites have cryptic habits and are geographically distributed in environments where biological diversity is high ('termite belt', **Fig. 1**), studies in the field with natural colonies must account for several variables that affect behaviour. The results presented in chapter I - and later extended in chapter II - contribute to validate the use of semi-folded arenas as a controlled device to study colony behaviour in termites. As mentioned in the chapter, I am certain that with a few modifications it is possible to apply this method to several other group-living species, not necessarily eusocial.

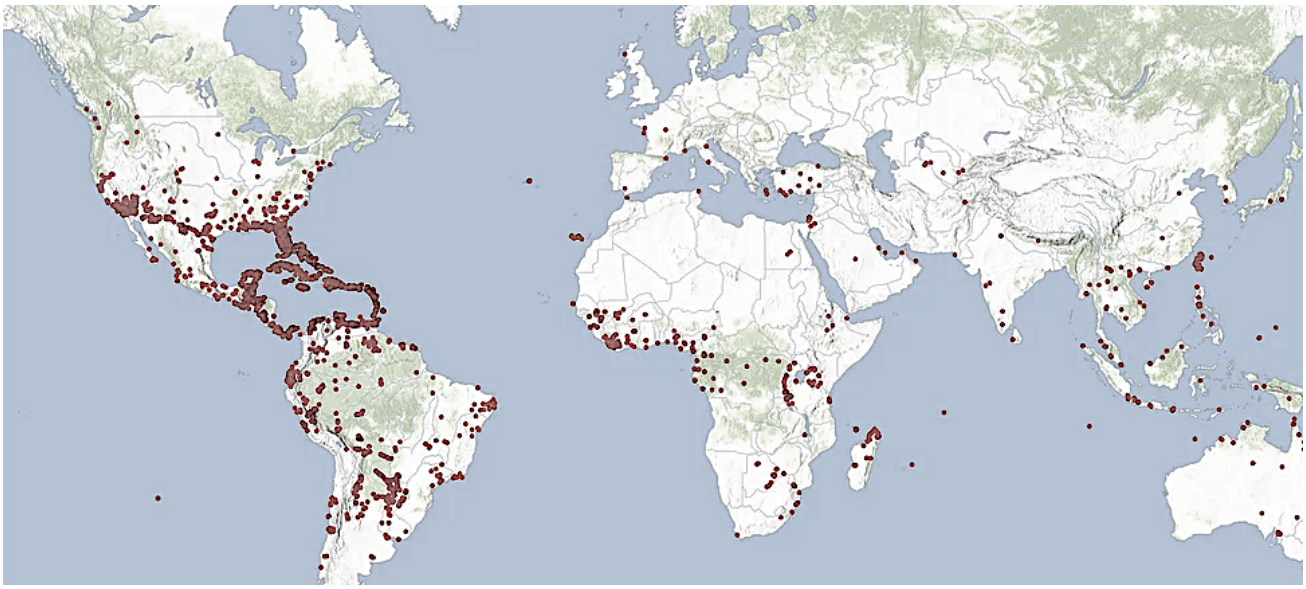

Figure 1. | Termite worldwide distribution: red points represent locations where the occurrence of termite species scientifically registered; Dashed lines highlight the so called 'termite belt' a section containing areas that presumably provide more suitable conditions for termite's persistence. (Image modified from termitediversity.org; ©Rudolf H. Scheffrahn)

The method I proposed with my co-authors turned out to be very efficient in its purpose and, as such, was adopted in **chapter II**, a companion study that looked at the effects of group size and group composition in the coordination of termites. The two studies, currently in preparation for submission seem promising in their results and instigated several questions that could be potentially further explored using the same high-resolution dataset.

In **chapter III**, my co-authors and I managed to describe a previously unknown potter wasp species. This work was a result of my persistence in trying to know what type of secondary species were inhabiting nests I was using when conducting a separate study, about nonaggressive behaviour in inquiline termites (i.e., Hugo et al., 2020). As I described in the resulting publication of chapter III it was necessary to come up with proper methods to collect both adult wasp individuals and larval stages. One possible follow-up study would be about the behavioral ecology of M. termitophila. Whether its nesting with C cyphergaster was occasional or not, it would be insightful to know which mechanisms allowed a wasp to nest amid a termite colony full of soldier individuals. A set of empirical evidence was collected and reported in this paper, but the question of whether the wasp *Montezumia termitophila* (termitophile) have evolved specific behavioural mechanisms to brood development inside nests of the termite *Constrictotermes cyphergaster* (host) remains to be further investigated.

Finally, in **chapter IV**, conducted with S. molestus suggests that there are consequences of the morphological specialization in the movement rule of soldiers. We did not observe differences in terms of exploration indices (i.e., MSD and MSRD), but further investigation with perhaps more data could be useful to tell whether this was a limitation of our dataset or if in fact both castes perform super-diffusive movements.

Conclusions

As we move forward and gain greater understanding of the organising principles that govern the interactions of the individual component parts of a collective system, I believe, we should continue to be able to identify elements in behavior that transcend the limits of our traditional and systematic perception of biology.

Acknowledgements

Thesis

Several people have aided me in the production of this thesis. First, I would like to thank Iain Couzin, for his friendship, patience, and guidance during my PhD. Under his supervision, it was a pleasure to be part of such an innovative and exciting environment as the Department of Collective Behavior. I am deeply grateful to all members of my thesis advisory committee for their substantial support. I am also sincerely grateful to my colleagues who directly contributed to my doctoral projects and who have shared authorship with me in the publications resulting from my research. I would like to first thank Dago, Katja and Mäggi from the MPIAB for supporting me out in so many ways and with many troubles. I also extend my thanks to Daniel, Michael, Marthe, and all scientific and administrative staff at the University of Konstanz. I would like to thank Francisca and Frau Matzner for their patience and for helping me with the process of submitting my thesis. I also am thankful for the logistical and financial support I received from several research entities: the Deutscher Akademischer Austauchdienst (DAAD); the International Max Planck Research School for Organismal Biology (IMPRS); the Department of Collective Behaviour of the Max Planck Institute of Animal Behavior; the Department of Biology of the University of Konstanz; and the Department of Entomology of the Universidade Federal de Viçosa. I also thank the marvelous group of termites and all individuals I sacrificed along the quest for knowledge. I always treat them respectfully while doing research, as any other living being. My biggest thanks go to my beloved family and dearest friends. In special, I am deeply grateful to my wife Teja, my partner in crime and an infinite source of love and happiness, who supported me tremendously during the most difficult times of my PhD. Thanks also to Billy J. McClarence - best doggo ever - for his loyalty and for helping me to go through tough stressful days. Finally, huge thanks to my mom Kátia, my dad Edson, my sister Lívia and my brother Julio, for being there for me no matter what happens.

Chapter I

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Chapter II

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Chapter III

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Chapter IV

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Author contribution

Chapter I

Conceived and designed the study HH, IDC; Developed fieldwork, behavioural recording, data collection: HH; Acquired tracking data: HH, TW; Conducted KDE analysis: HH; Prepared documentation, images, videos, and supporting information included in appendix: HH; Wrote the manuscript: HH; Commented and improved the manuscript prior to submission: IDC, TW.

Chapter II

Conceived and designed the study HH, IDC; Investigation: HH, TC; Developed fieldwork, behavioural recording, and data collection: HH; Conducted statistical analysis: TC, HH; Prepared documentation, images, videos, and supporting information included in appendix: HH; TC; Funding and logistical support: IDC, OS; Wrote the manuscript: HH; Commented and improved the manuscript prior to submission: IDC.

Chapter III

Conceived and designed the study: HH & MGH. Conducted fieldwork, behavioral recording and identified morphotypes: HH. Validated morphotypes and described the new species: MGH & BRGB. Prepared documentation, images, videos, and supporting information included in appendix: HH & MGH. Drafted the manuscript: HH. Commented and improved the manuscript prior to submission: MGH, BRGB & IDC.

Chapter III

Conceived and designed the study HH, IDC; Investigation: HH, TC, LRP, IDC; Developed fieldwork, behavioural recording, and data collection: HH. Conducted statistical analysis: TC, HH; Conducted MSD and MSRD analysis: LRP; Prepared documentation, images, videos, and supporting information included in appendix: HH, TC; Wrote the manuscript: HH; Commented and improved the manuscript prior to submission: IDC, TC, LRP.

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PUBLISHED PAPERS

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Hugo, H, Walter, T, Couzin, ID (in prep.) Using semi-folded arenas to replicate spontaneous emergence of collective trails in whole colonies of termite species

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Walter, T, **Hugo, H**, Couzin, ID (in prep.) Identifying the spatiotemporal locations of anomalies in collective behavior using simplified representations of trajectory data

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Using semi-folded arenas to replicate spontaneous emergence of collective trails in whole colonies of termite species

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1. Nests of *Constrictotermes cyphergaster* (Termitidae: Nasutitermitidae)

Figure S1 | Arboreal nests of *C. cyphergaster*: Representative examples of nests collected. In this species tend to be attached to tree trunks by a small section, a few insertions with a picola hammer in the part of the nest connected to the tree are enough to detach the whole nest without causing much damage.

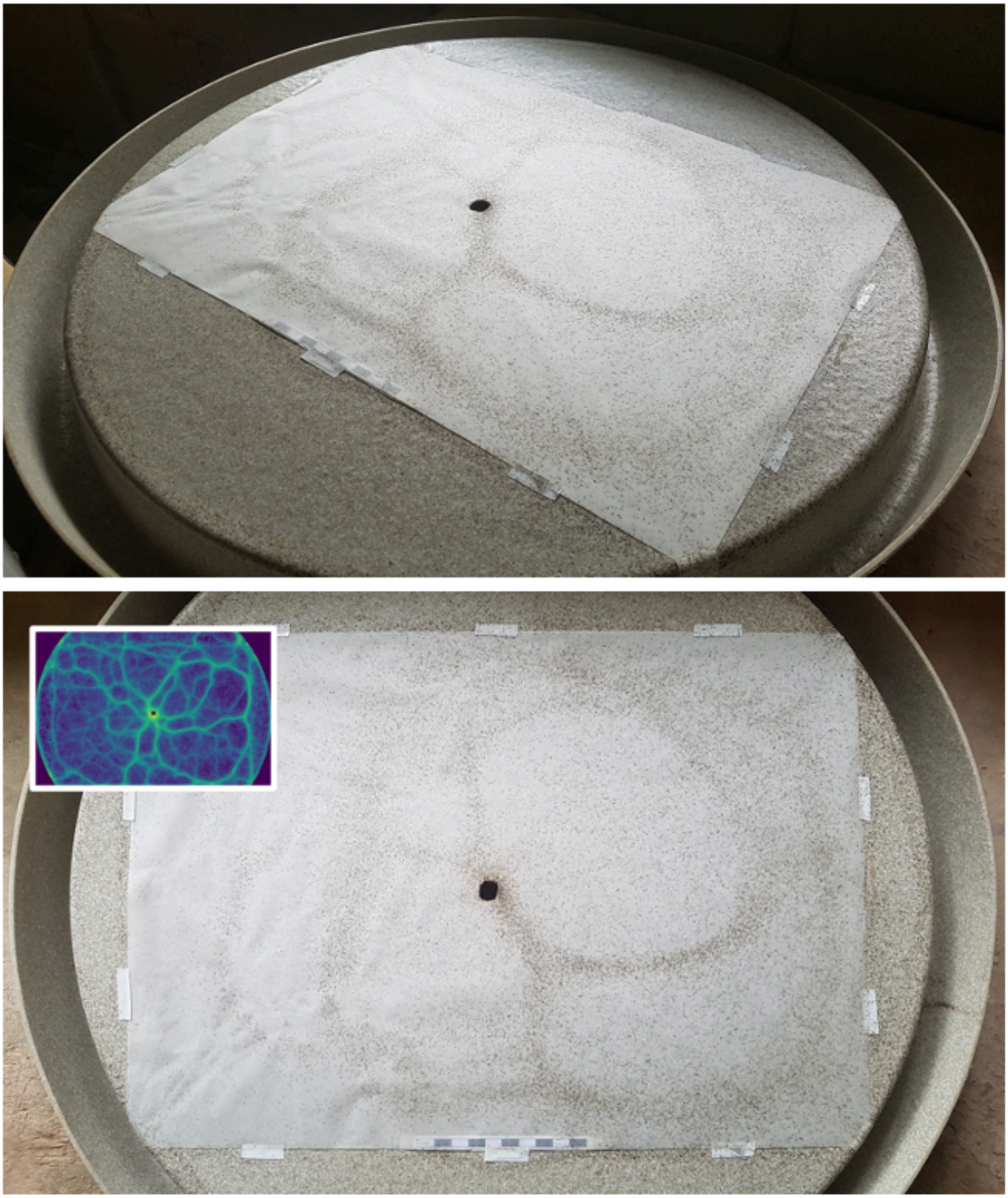

Figure S2 | Semi-folded arena after behavioural recording: At the end of each whole-colony observation, we detected that paths more frequently used by termites had traces of a dark material secreted by individuals, supposedly a mixture of trail-pheromone and faeces, as suggested by Souto & Kitayama (2000).

From random to coordinated collective motion: assessing the influence of group size and group composition in the movement of termites

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1. Whole colony observations

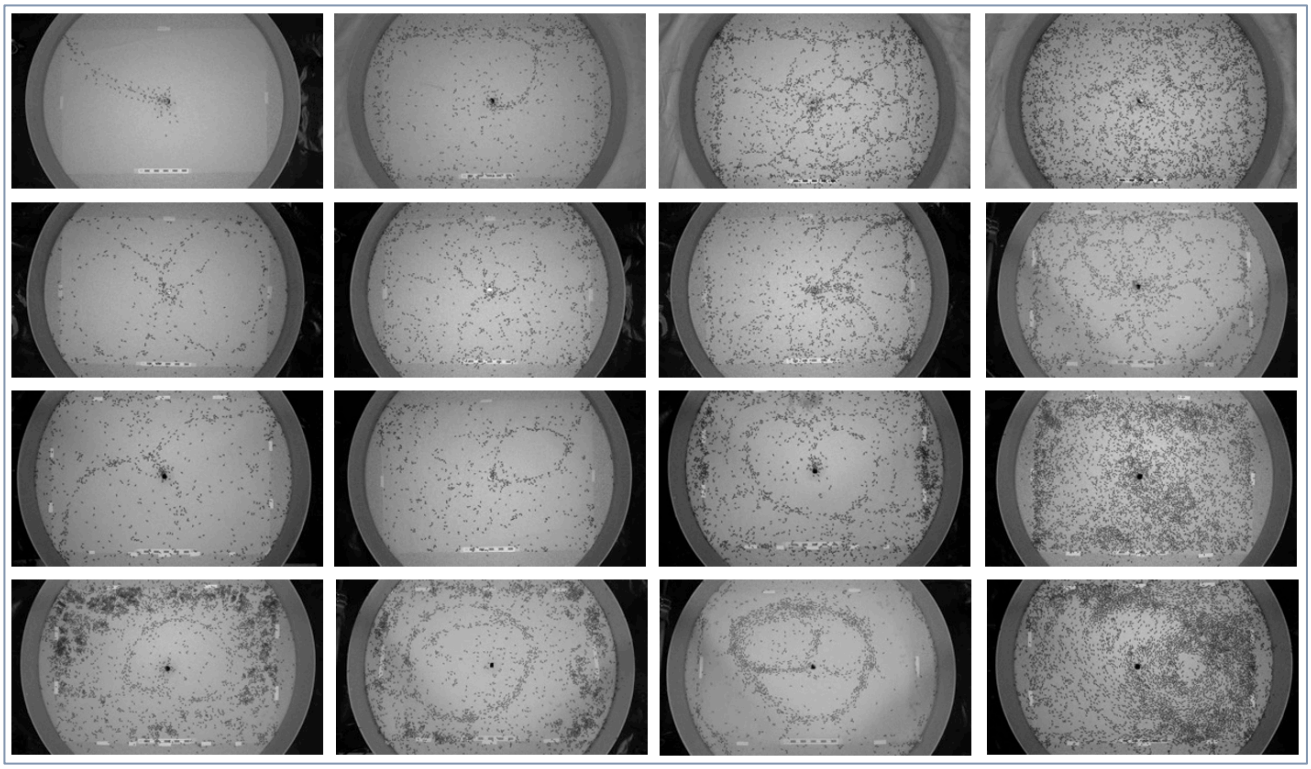
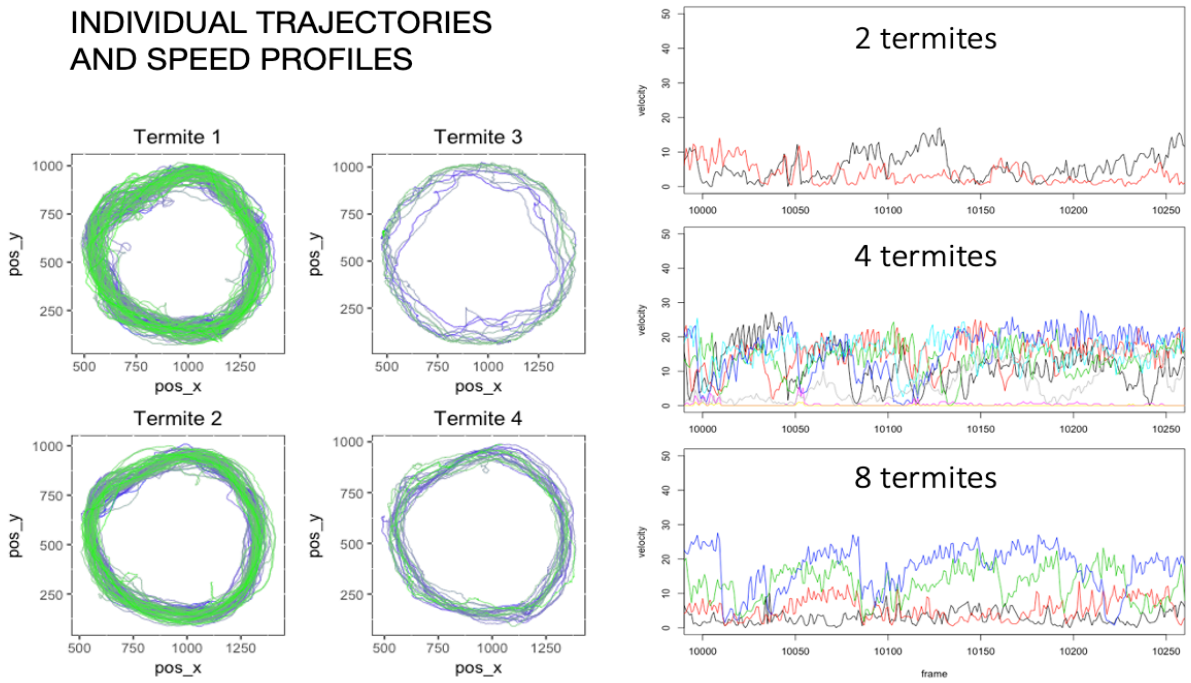
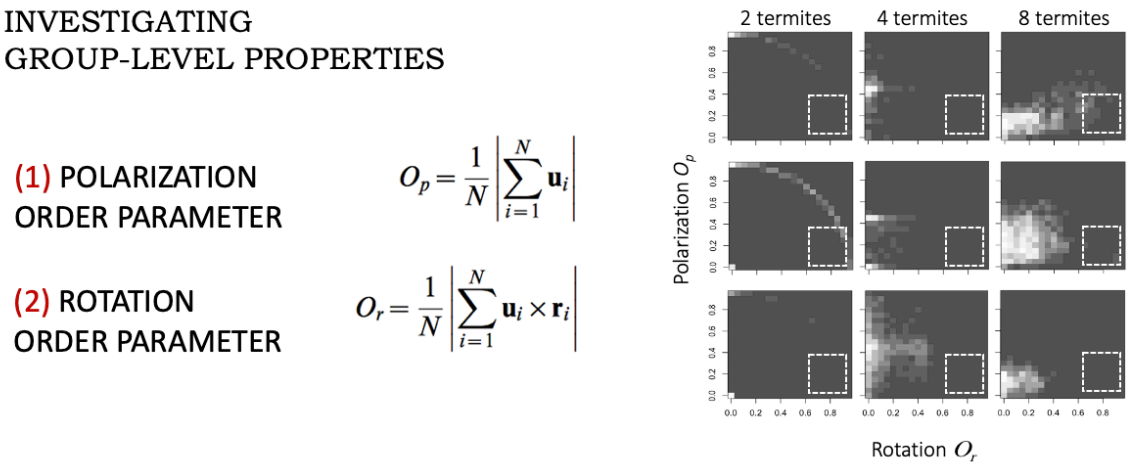


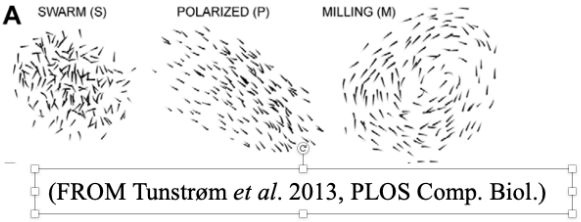
Figure S1 | Whole-colony observations: Snapshots of behavioural observations recorded using semi-folded arenas. Each exploration activity was recorded using a unique termite colony and consisted of a video with 900000 frames on average.

2. Preliminary quantitative analysis of movement with *Constrictotermes cyphergaster*

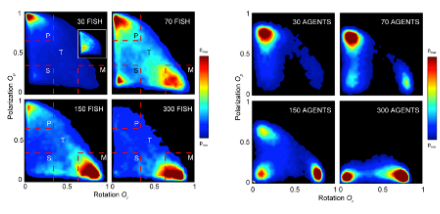
First we plotted trajectories and speed to identify patterns; then we investigated order parameters (polarization and rotation); Because rotation and polarization results could be ambiguous (sum of values 1 and -1) we calculated instantaneous alignment (used as a proxy for coordination)







(FROM Tunstrøm *et al.* 2013, PLOS Comp. Biol.)



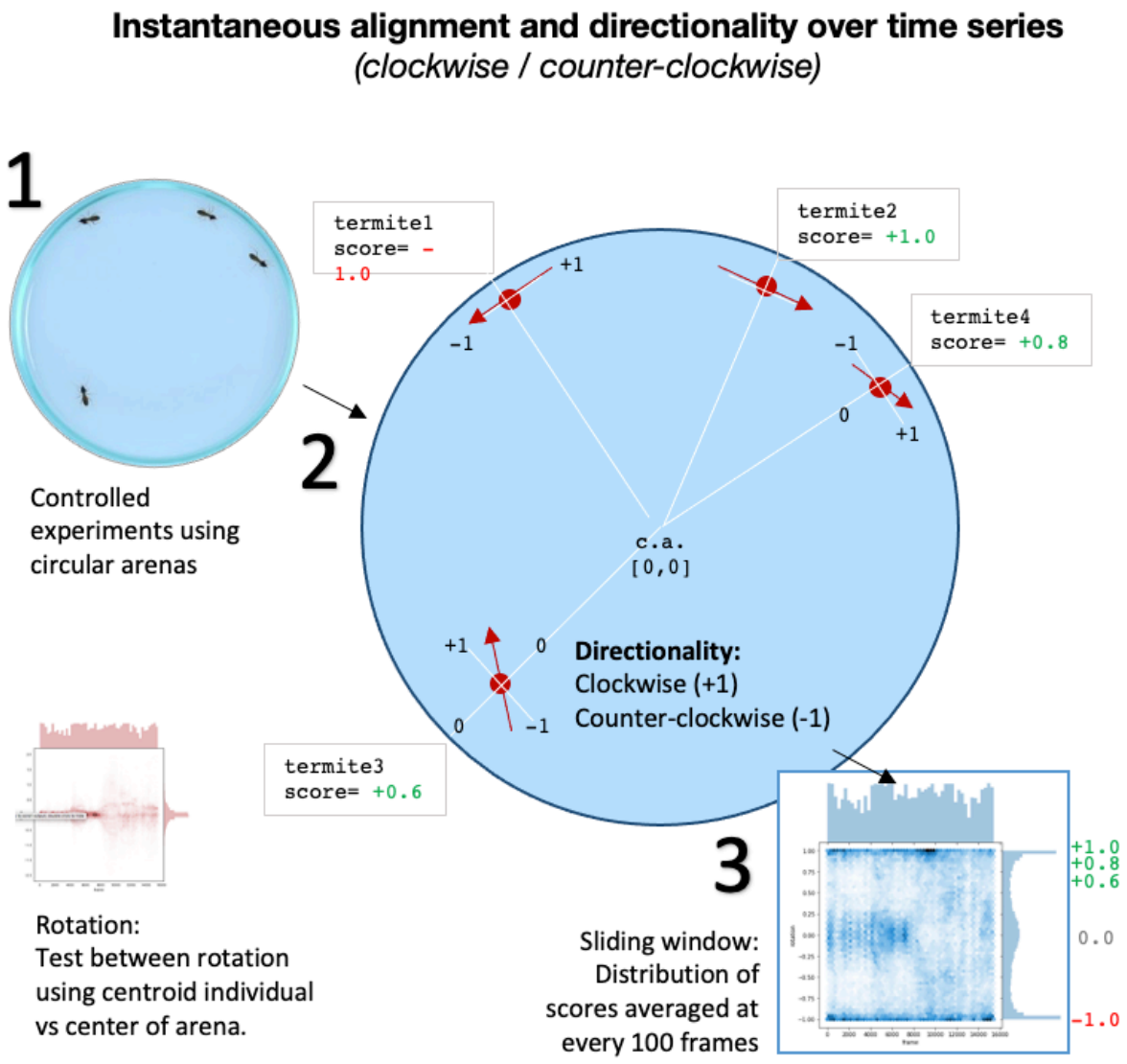
Movement analysis

Context

When moving from place to place, termites naturally form self-organized lanes. In experimental conditions, this behaviour can be replicated in loops (‘milling behaviour’). Differently from ants, however, termites can escape from such a behaviour, which indicates that chemical signal (pheromones) may not be the only driver in place.

Main question

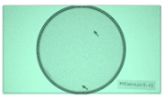
How coordinated, collective motion in termite groups is affected by its structure. To address this question, we manipulate (i) group density (area occupied by all individuals divided by area available) and (ii) group composition (worker-soldier caste ratio), and measure individual and group-level properties.

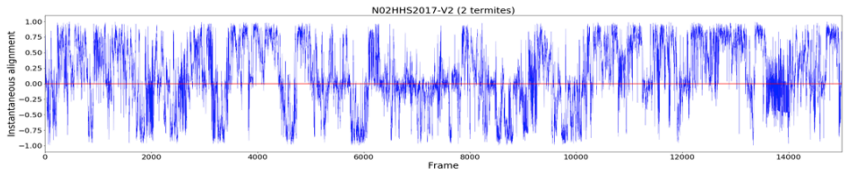


CALCULATION INSTANTANEOUS ALIGNMENT, AS PROXY OF COORDINATION

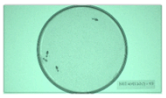
$$\Phi^t = \frac{2}{m\pi}\sum_{i=1}^{m}\chi i^t$$

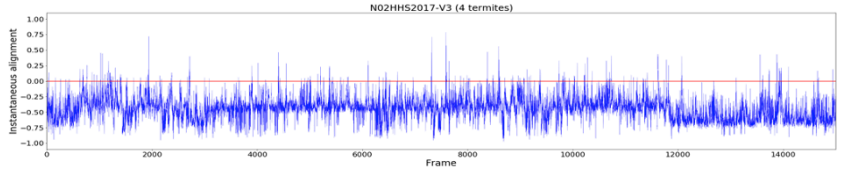
2 termites



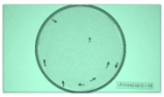


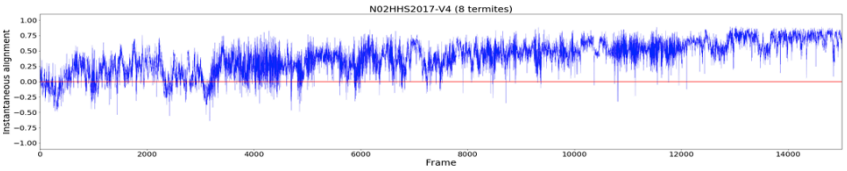
4 termites





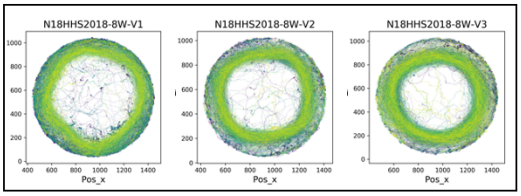
8 termites

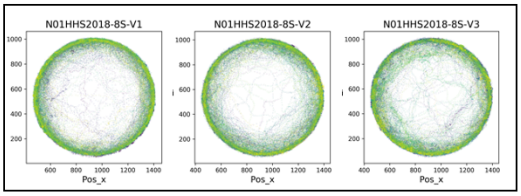


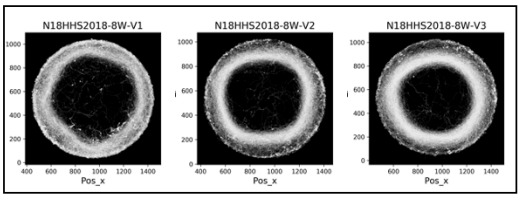


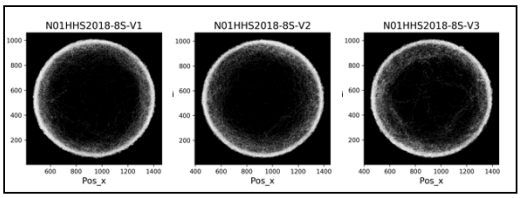
WORKERS

SOLDIERS

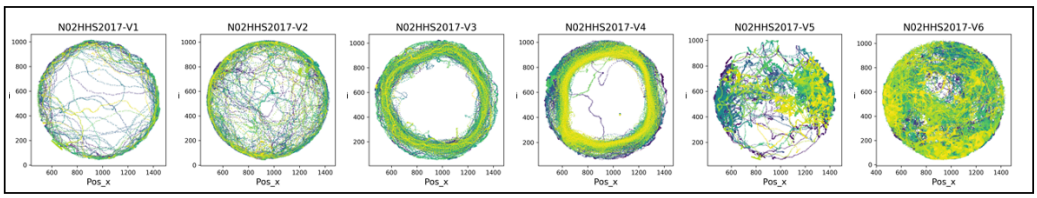


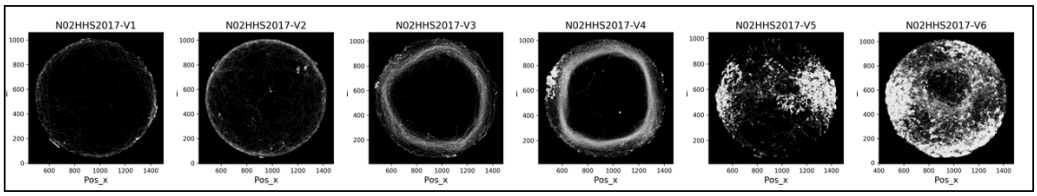




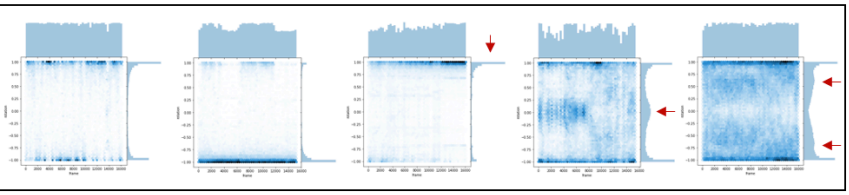


GROUP SIZE = C. cyphergaster (1, 2, 4, 8, 16, 32 termites)





Instantaneous alignment is calculated for a minimum of 2 individuals



First evidence of wasp brood development inside active nests of a termite with the description of a previously unknown potter wasp species

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Video S1. Interaction between female *Montezumia termitophila* and workers and soldiers of *Constrictotermes cyphergaster*; observation Available in DRYAD: https://doi.org/10.5061/dryad.05qfttf1b

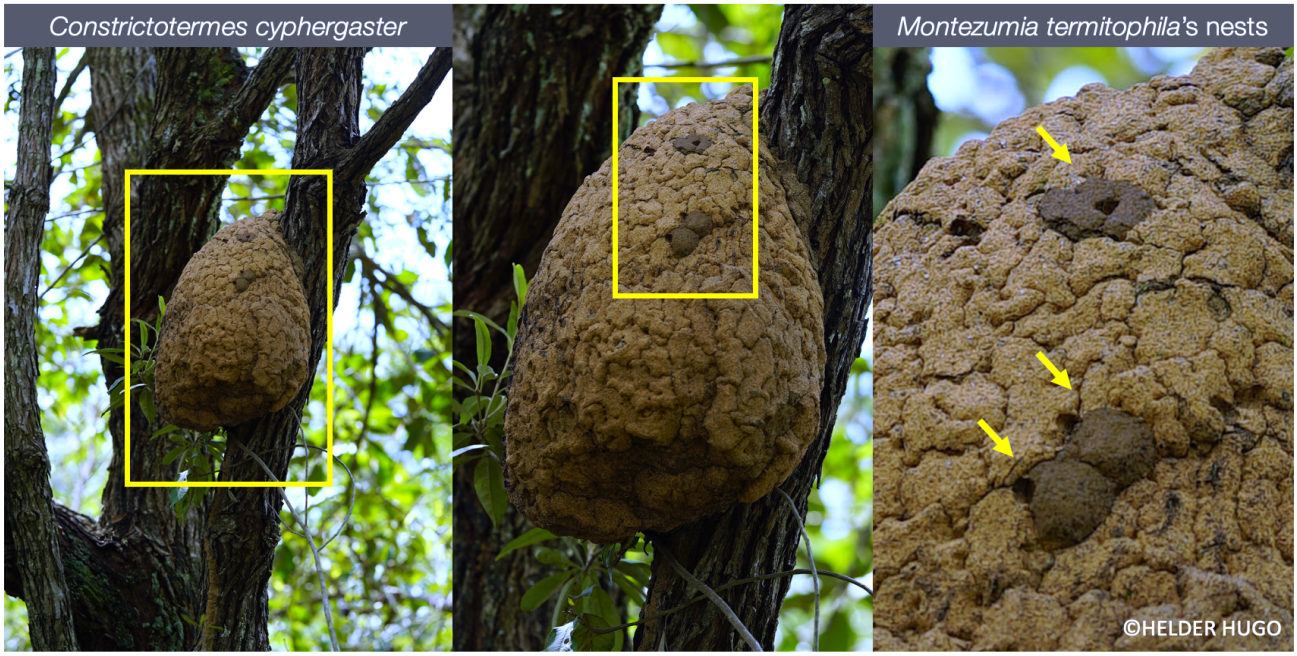

Figure S1 | Representative example of arboreal nests of *C. cyphergaster*. Markings in yellow highlights three *M. termitophila*'s brood cells present on the nest surface.

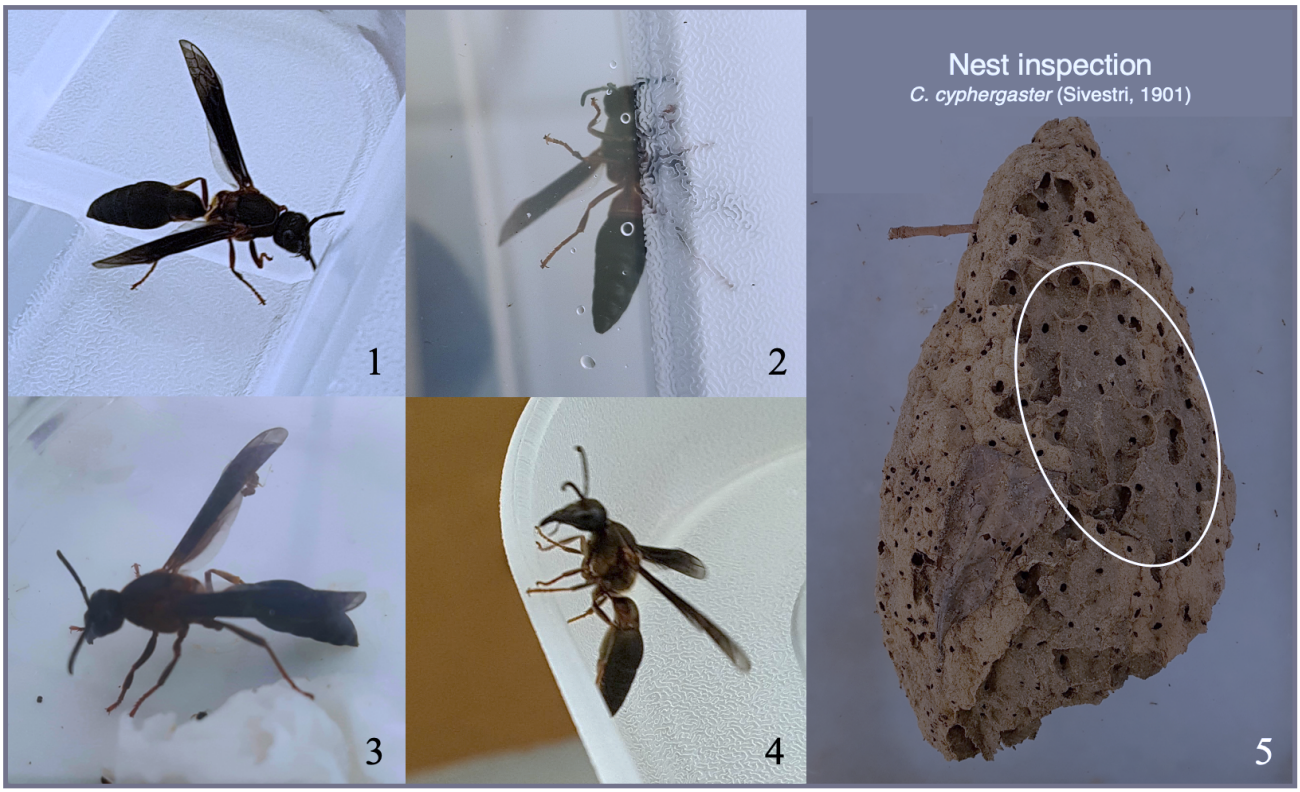

Figure S2 | Adults of *M. termitophila* emerged from brood cells found in *C. cyphergaster* nests. (1) Female; (2) Sex not confirmed; (3) Female holotype; (4) Male; (5) Example of termite nest having its galleries exposed layer by layer during nest inspection. The section already exposed using scoopulas is highlighted.

Effects of caste dimorphism on movement patterns of termite species

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1. Predation as selective pressure in *Syntermes* spp.

Figure S1 | *Syntermes* spp. versus specialist predators: Predation of soldier, Credits: © andriusp (A). *Pachycondyla* spp. during raid (B, C) Credits: © Piotr Naskrecki. Available in: mindenpictures.com

2. List of termites that showed lower levels of mobility in experimental arenas

Soldier caste = 5/43 (11.6%)
Termite IDs = 18W6S-1-S19, 18W6S-1-S24, 18W6S-2-S22, 18W6S-2-S23, 18W6S-3-S20

Worker caste = 45/233 (19.3%)
Termite IDs = 24W-1-W12, 24W-2-W4, 24W-2-W7, 24W-2-W8, 24W-2-W9, 24W-2-W10, 24W-2-W13, 24W-2-W14, 24W-2-W15, 24W-2-W16, 24W-2-W18, 24W-3-W5, 24W-3-W7, 24W-3-W8, 24W-3-W21, 6W-2-W5, 6W-3-W3*, 6W-3-W5, 18W6S-1-W1, 18W6S-1-W2, 18W6S-1-W3, 18W6S-1-W4, 18W6S-1-W5, 18W6S-1-W7, 18W6S-1-W8, 18W6S-1-W14, 18W6S-1-W15, 18W6S-1-W16, 18W6S-1-W18, 18W6S-2-W1, 18W6S-2-W2, 18W6S-2-W3, 18W6S-2-W4, 18W6S-2-W5, 18W6S-2-W6, 18W6S-2-W7, 18W6S-2-W8, 18W6S-2-W11, 18W6S-2-W15, 18W6S-2-W17, 18W6S-3-W1, 18W6S-3-W3, 18W6S-3-W4*, 18W6S-3-W9*, 12W-1-W5.

***Idle individuals =** 18W6S-3-W4*, 18W6S-3-W9*, 6W-3-W3*

3. Super-diffusive patterns of *Syntermes molestus*

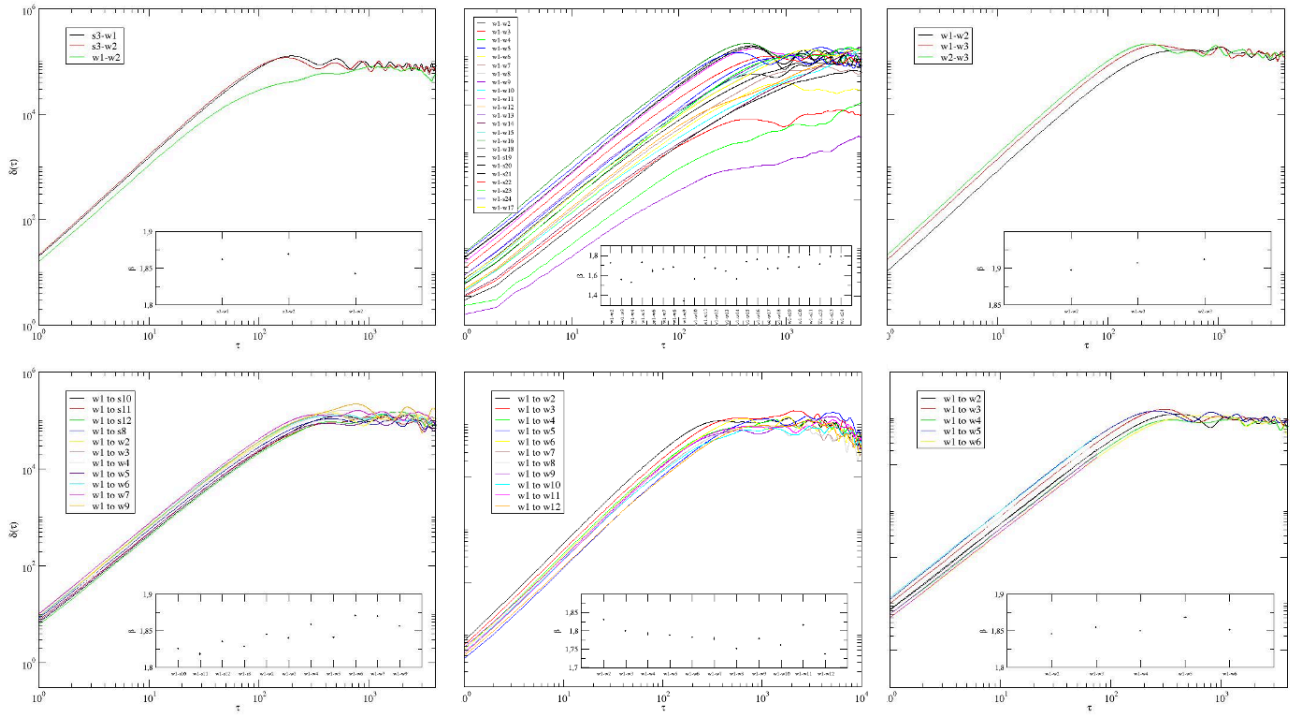


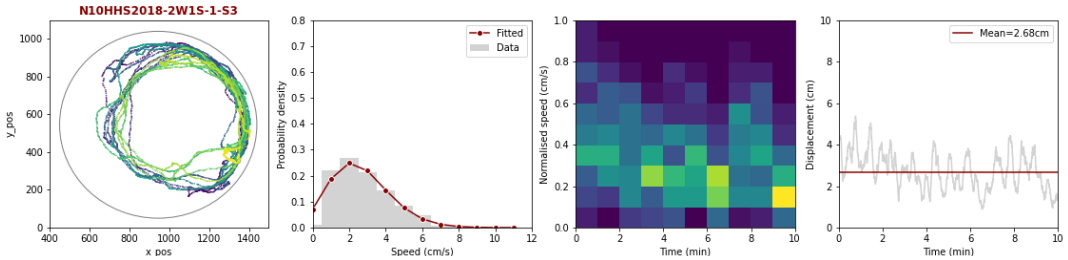
Figure S2 | Mean-Squared Relative Distance: Representative examples of MSRD calculated for groups of termites, showing that both soldiers and workers of S. molestus performed super-diffusive movement in arenas.

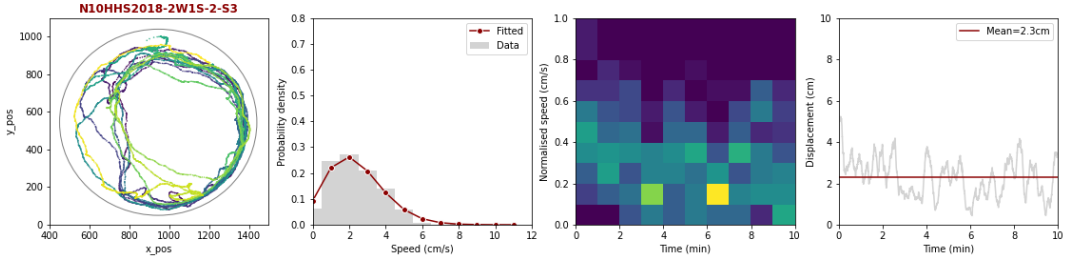
4. Summary plots

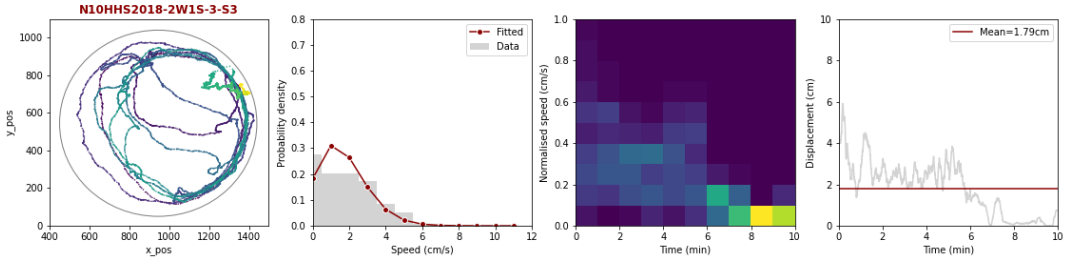
Summary plots for each termite individuals contain the following graphic elements:

- a trajectory plot (x-y coordinates) showing the path walked by the termite with temporal information represented in ‘viridis’;
- a histogram of calculated values of speed [cm/s] a with a fitted parameter (Poisson distribution):
- a heatmap of normalised speed [cm/s] over time with speed values represented in ‘viridis’;
- a plot showing displacement [cm] over time, with the calculated mean value of displacement represented by a solid horizontal line.

To facilitate identification of castes throughout summary plots, we used a colour code consistent with other figures in this study: soldiers are represented in red, and workers are represented in blue. All functions and calculations necessary to generate summary plots were developed in Python, using custom modules and common libraries.


N10HHS2018-2W1S-1-S3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.68cm


N10HHS2018-2W1S-2-S3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.3cm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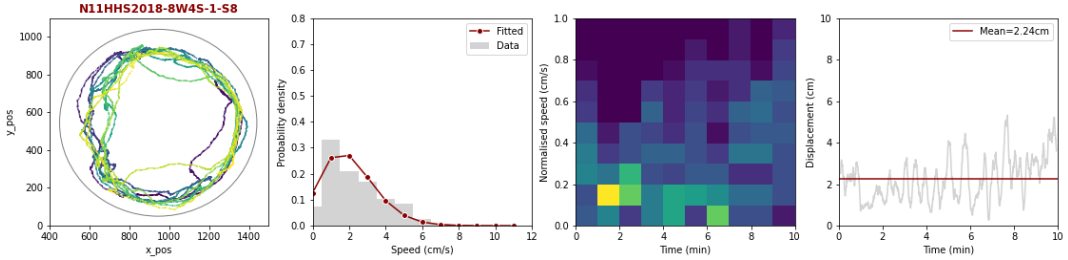
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.79cm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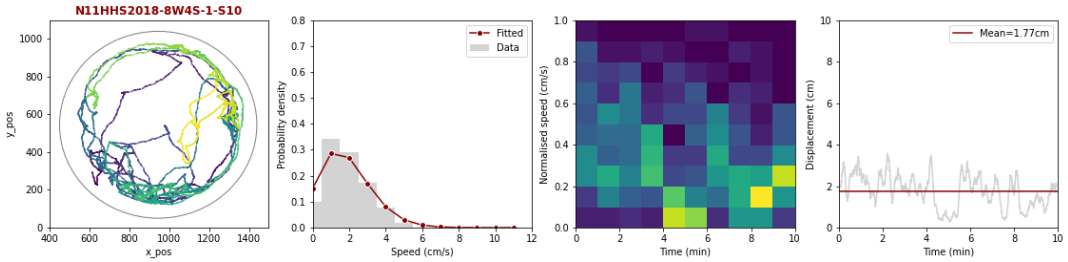
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.24cm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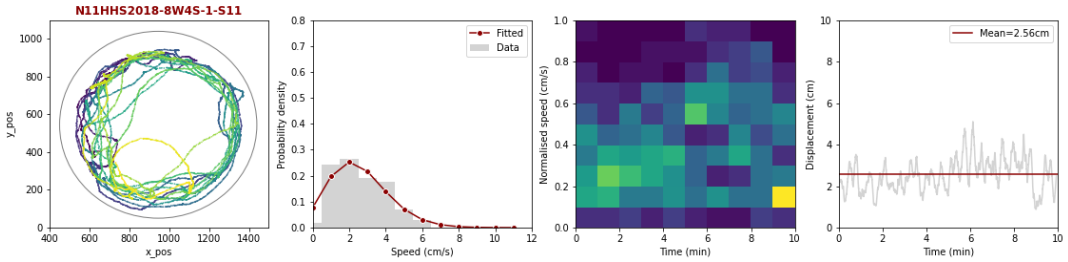
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.77cm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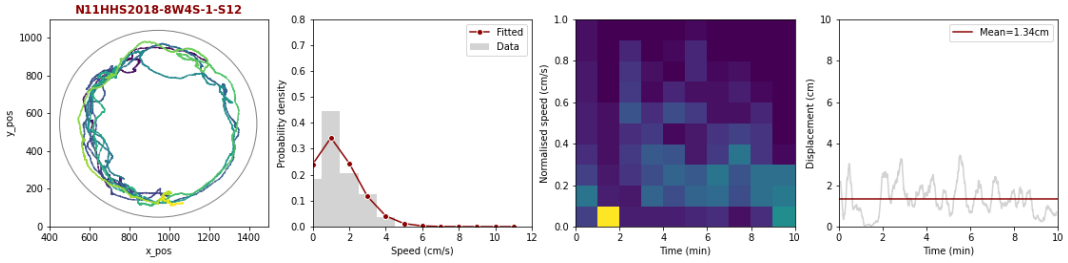
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.56cm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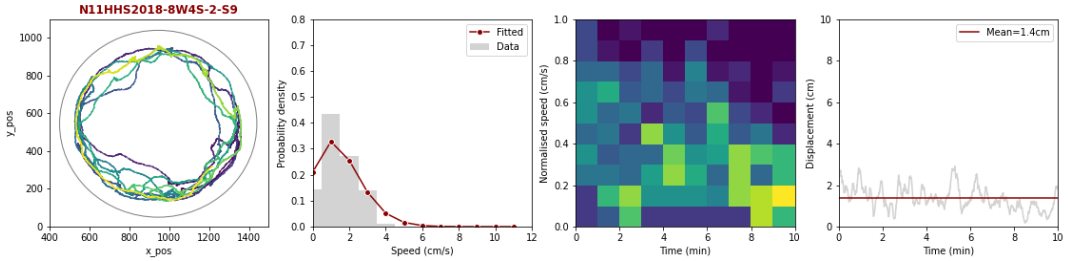
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.34cm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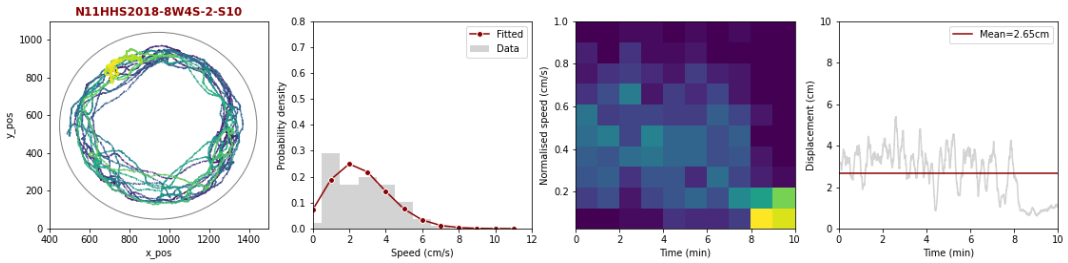
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.4cm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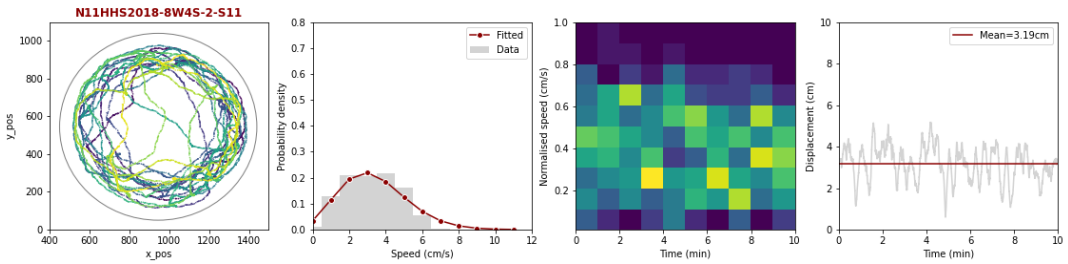
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.65cm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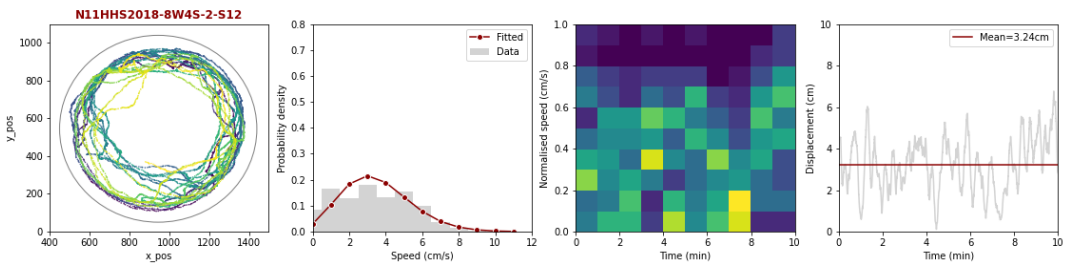
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=3.19cm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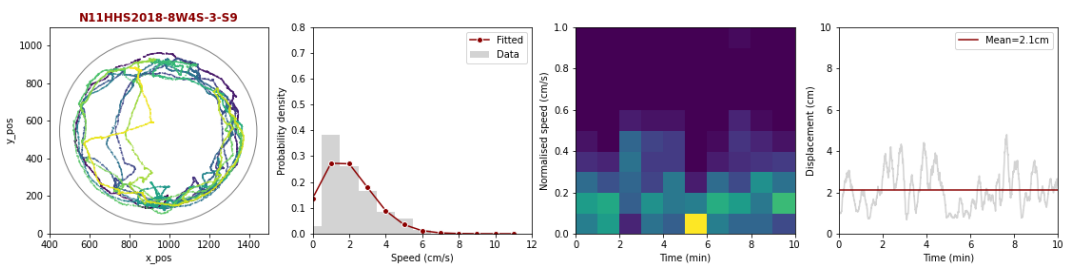
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=3.24cm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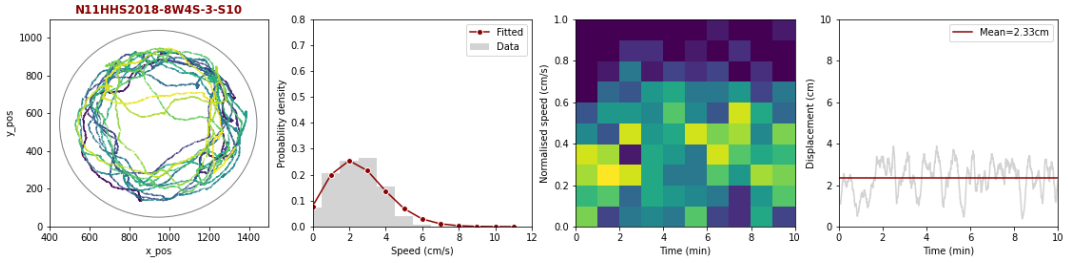
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.1cm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x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
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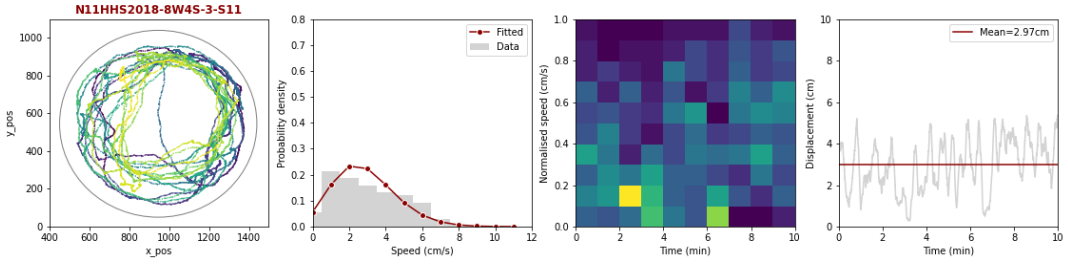
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x_pos
Probability density
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Fitted
Data
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Time (min)
Displacement (cm)
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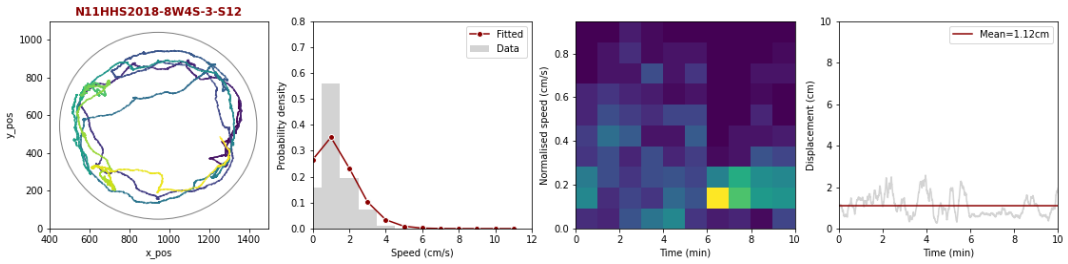
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x_pos
Probability density
Speed (cm/s)
Fitted
Data
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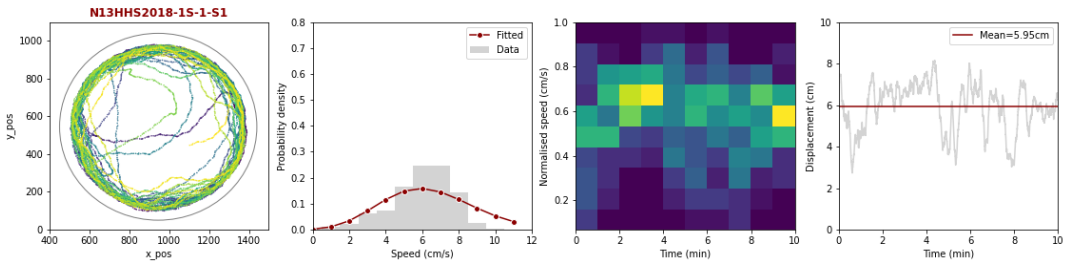
Time (min)
Displacement (cm)
Mean=1.12cm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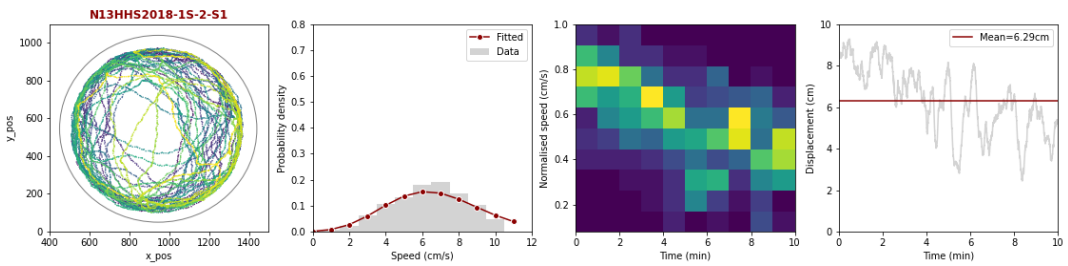
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=5.95cm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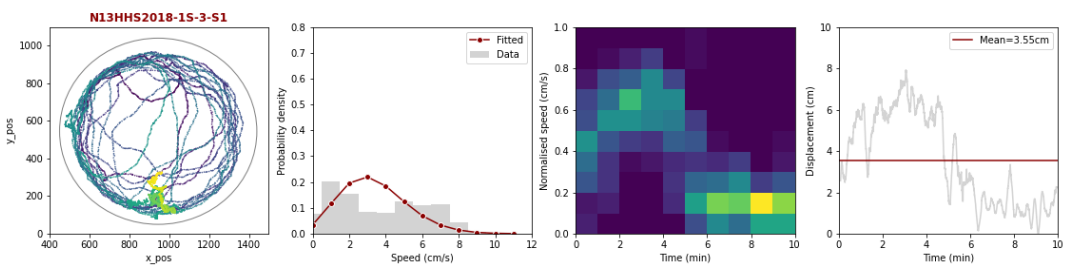
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=6.29cm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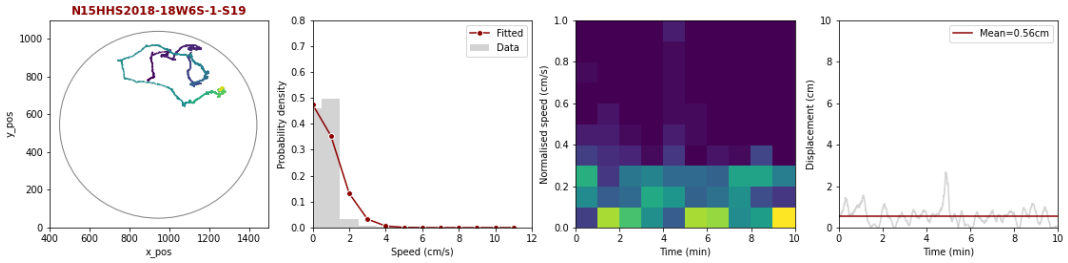
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=3.55cm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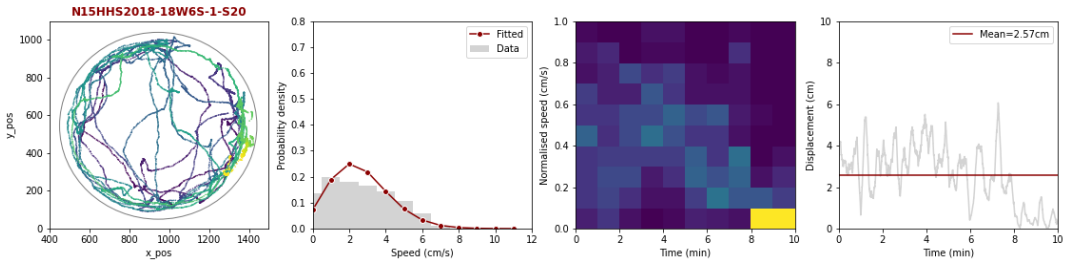
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.56cm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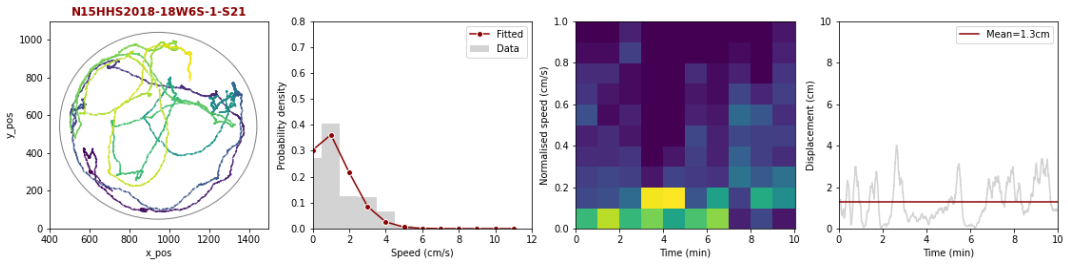
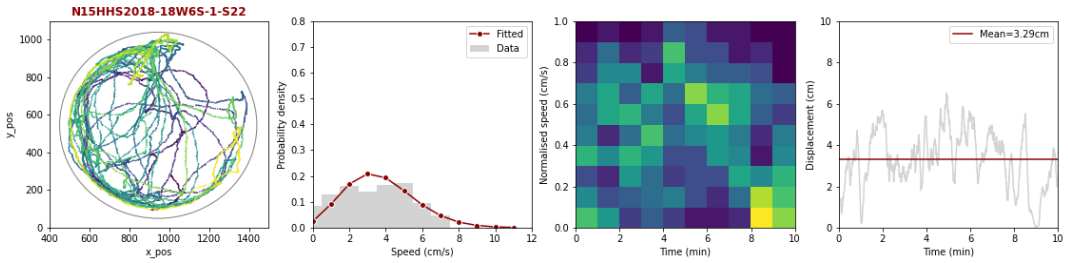
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.57cm


N15HHS2018-18W6S-1-S21
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.3cm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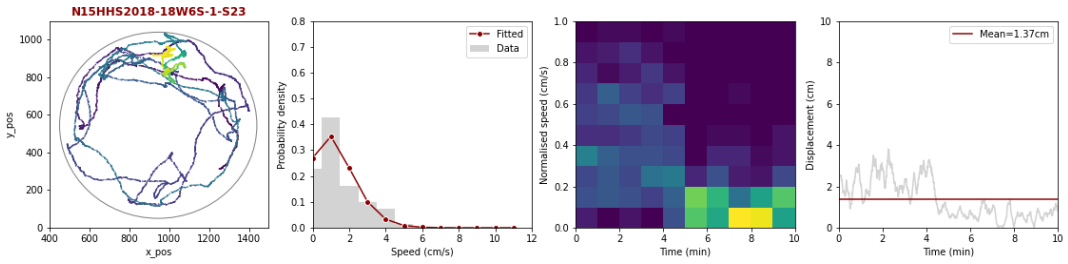
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=3.29cm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Probability density
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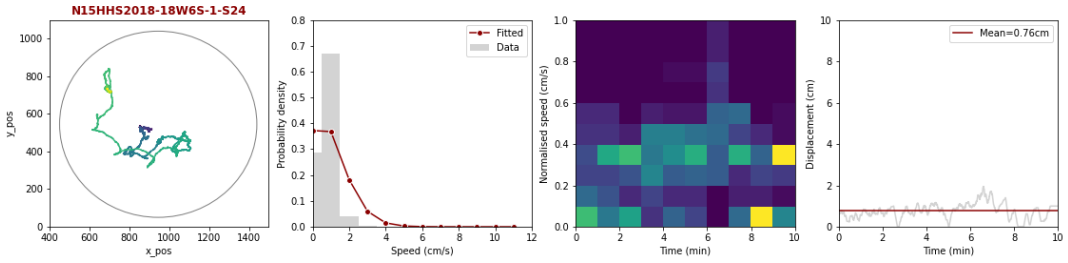
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.37cm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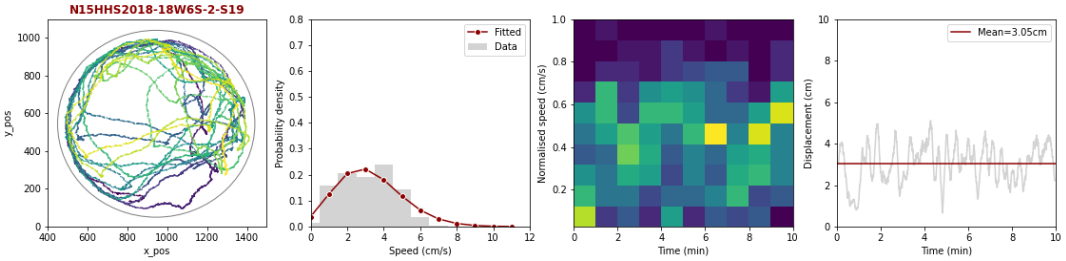
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.76cm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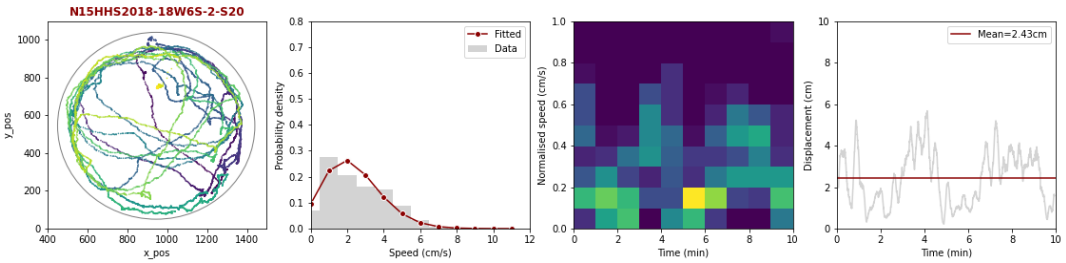
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.05cm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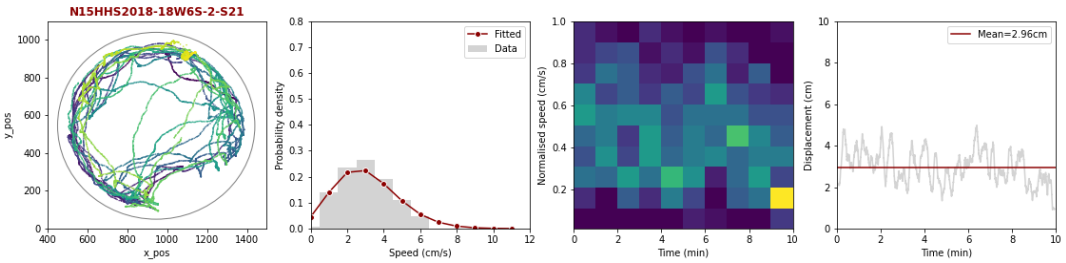
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.43cm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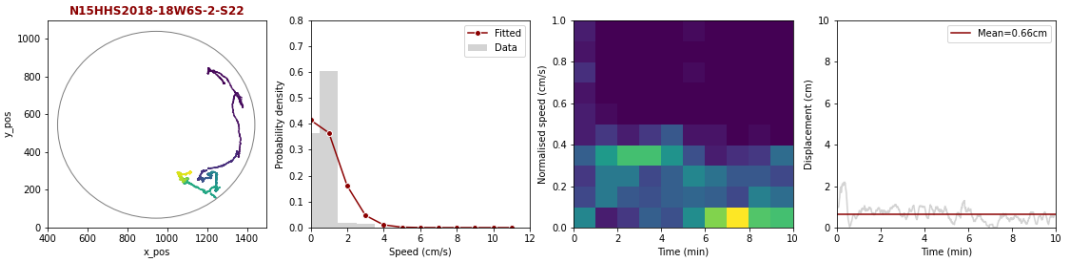
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.96cm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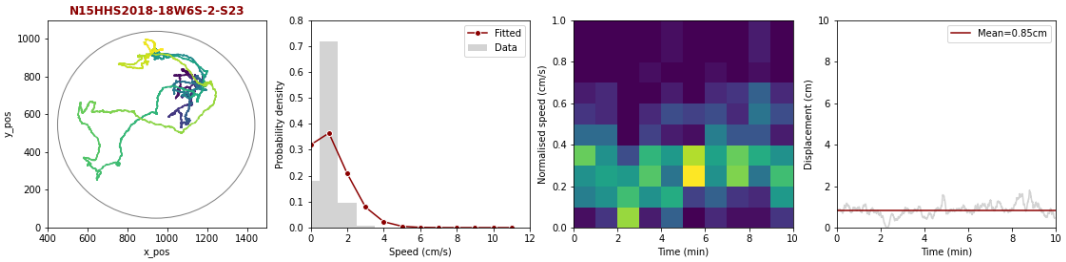
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.66cm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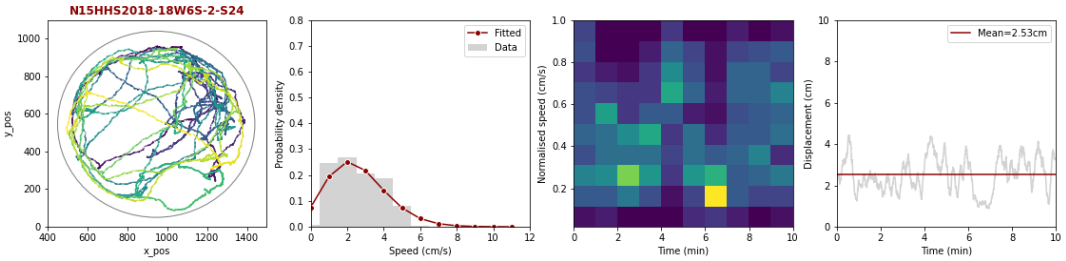
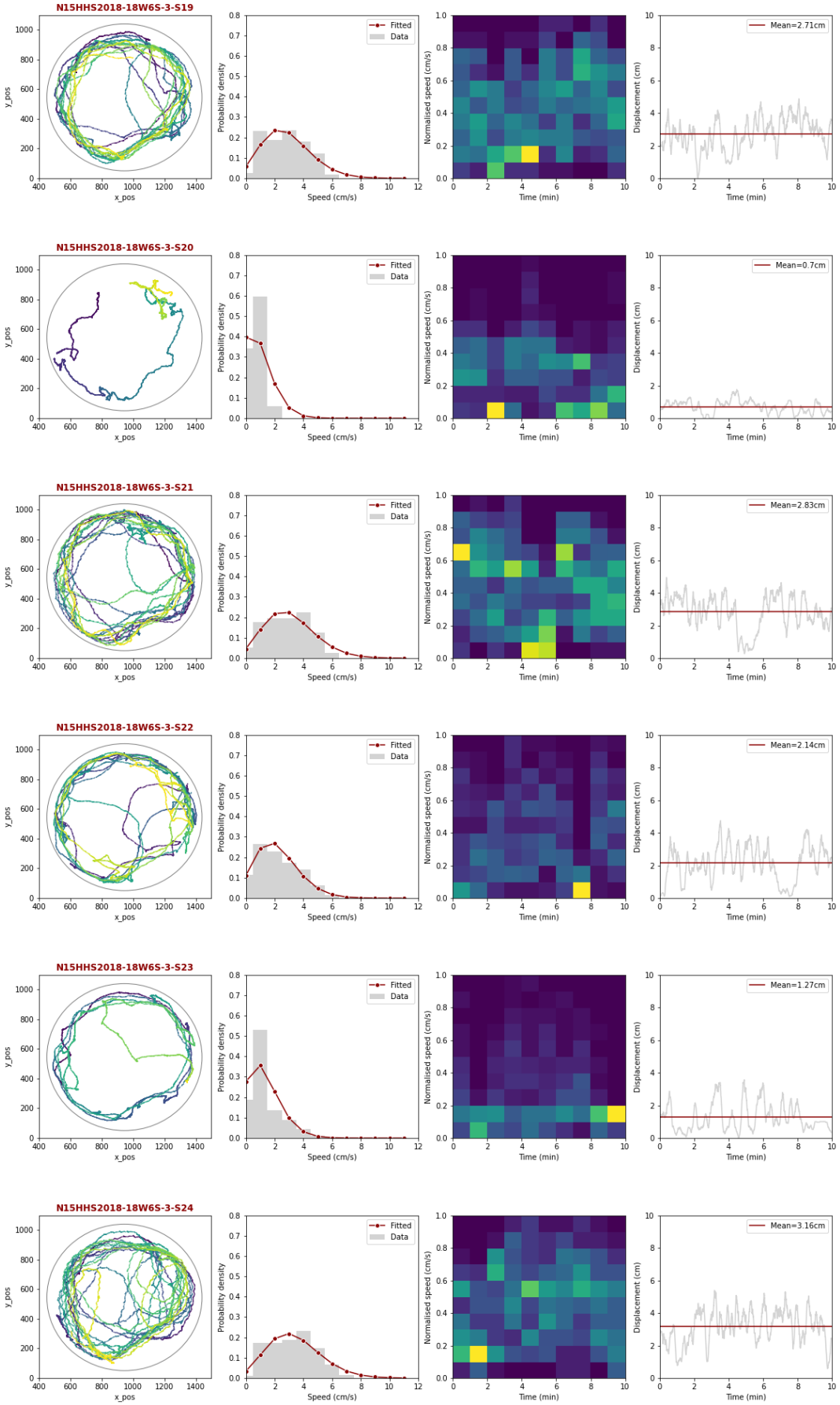
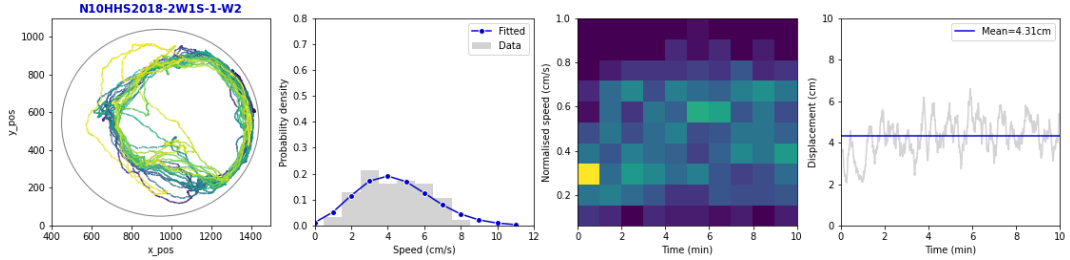
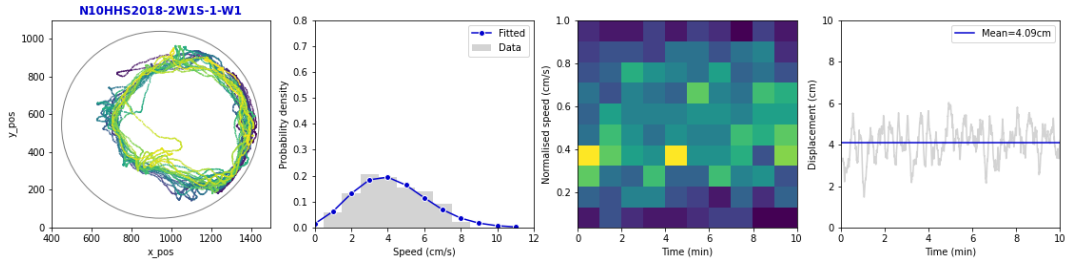
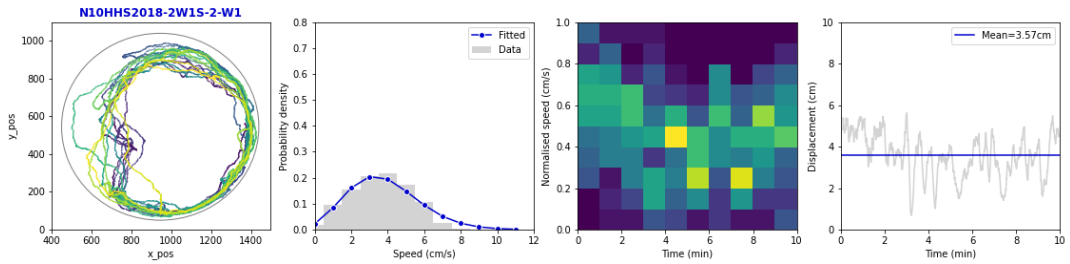
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.85cm

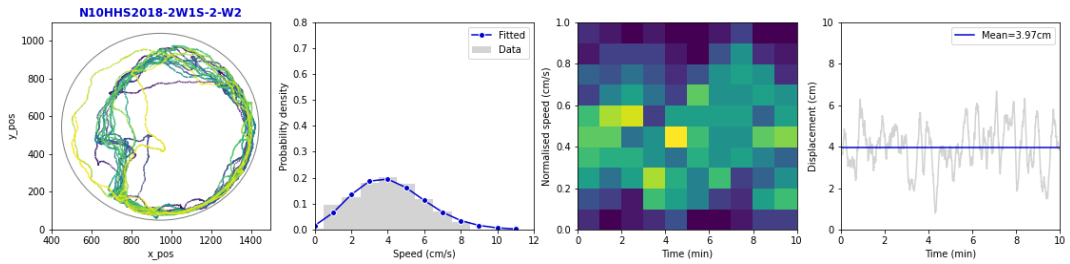

N15HHS2018-18W6S-2-S24
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.53cm

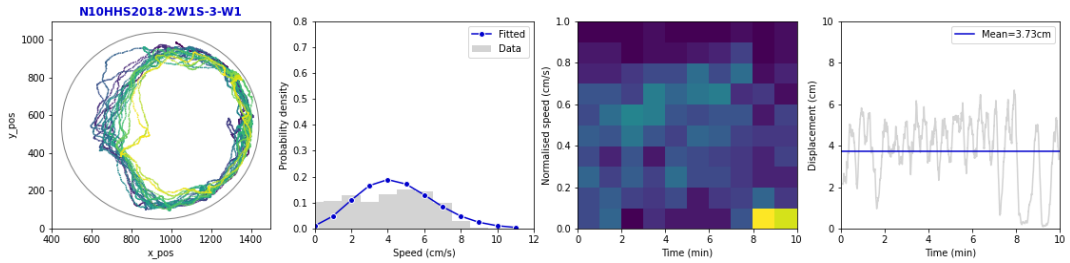

N15HHS2018-18W6S-3-S19
Fitted
Data
Mean=2.71cm
N15HHS2018-18W6S-3-S20
Fitted
Data
Mean=0.7cm
N15HHS2018-18W6S-3-S21
Fitted
Data
Mean=2.83cm
N15HHS2018-18W6S-3-S22
Fitted
Data
Mean=2.14cm
N15HHS2018-18W6S-3-S23
Fitted
Data
Mean=1.27cm
N15HHS2018-18W6S-3-S24
Fitted
Data
Mean=3.16cm
x_pos
y_pos
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Displacement (cm)

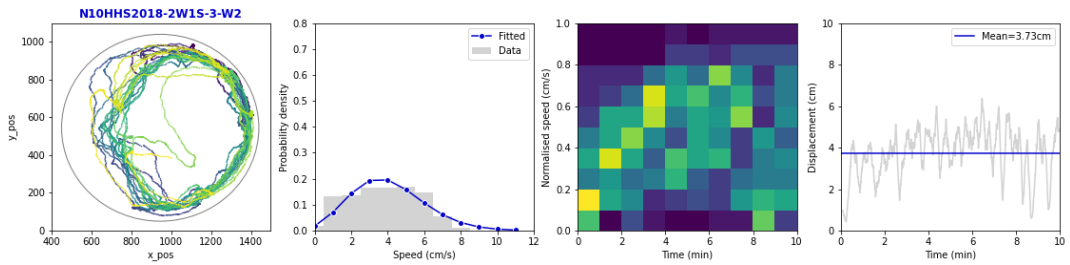

N10HHS2018-2W1S-1-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=4.31cm
Displacement (cm)
Time (min)

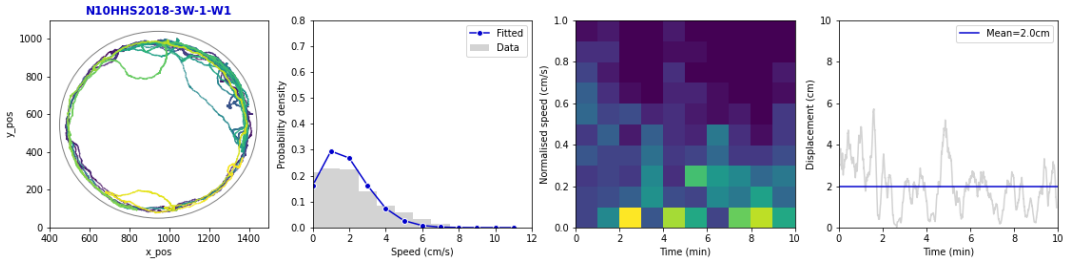

N10HHS2018-2W1S-1-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=4.09cm
Displacement (cm)
Time (min)

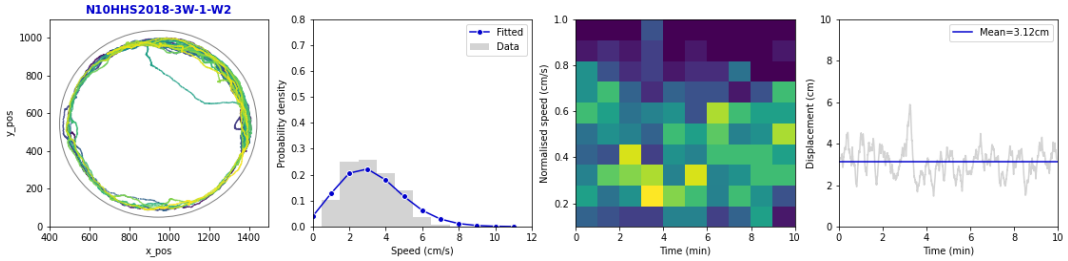

N10HHS2018-2W1S-2-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.57cm
Displacement (cm)
Time (min)

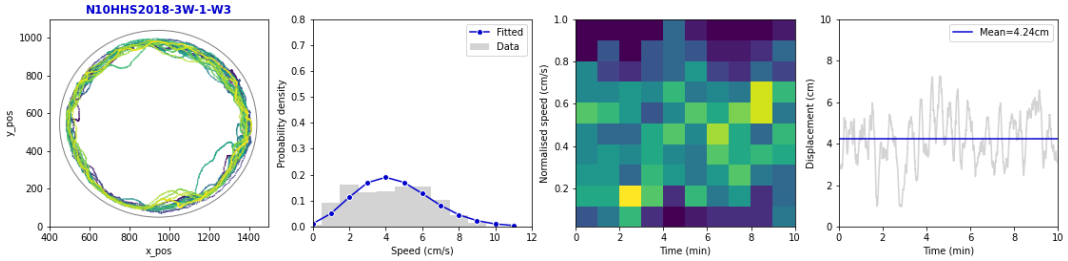

N10HHS2018-2W1S-2-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.97cm
Displacement (cm)
Time (min)

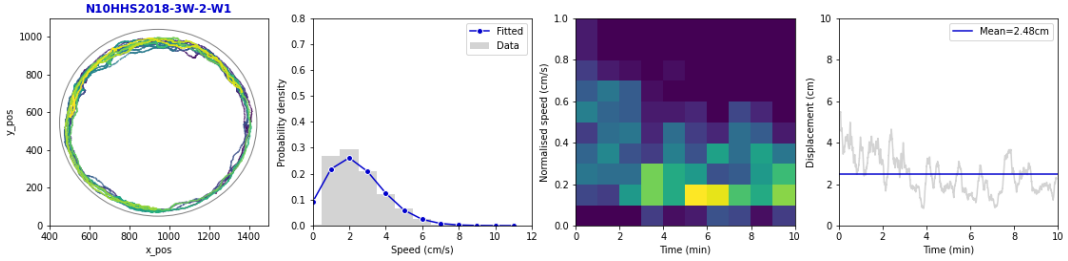

N10HHS2018-2W1S-3-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.73cm
Displacement (cm)
Time (min)

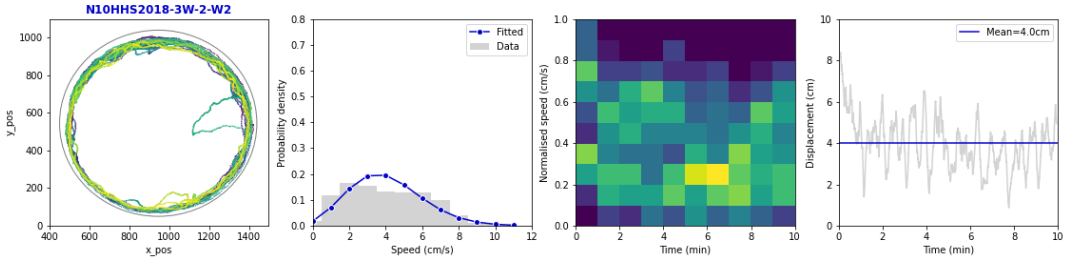

N10HHS2018-2W1S-3-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.73cm
Displacement (cm)
Time (min)

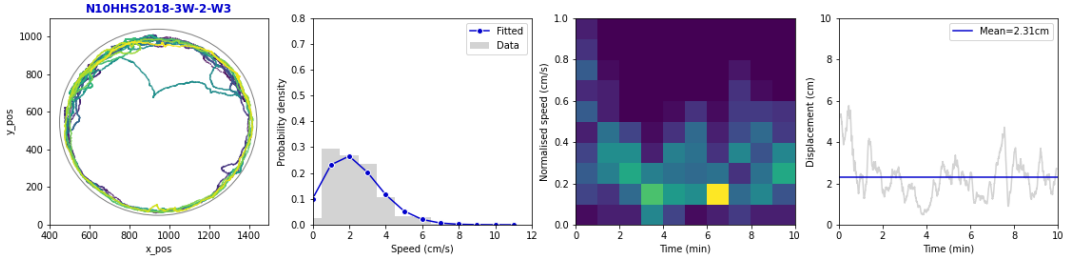

N10HHS2018-3W-1-W1
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.0cm

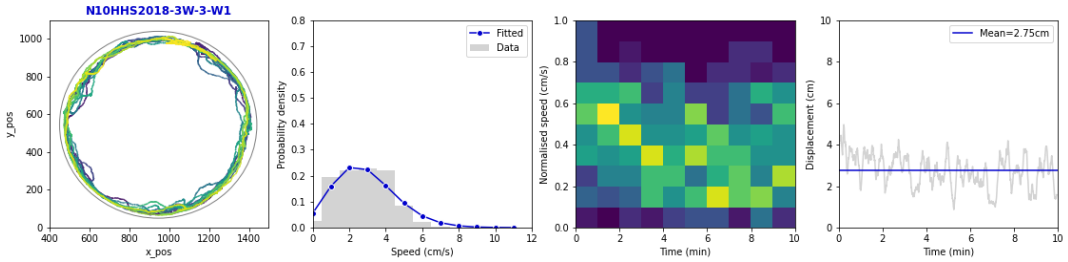

N10HHS2018-3W-1-W2
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=3.12cm

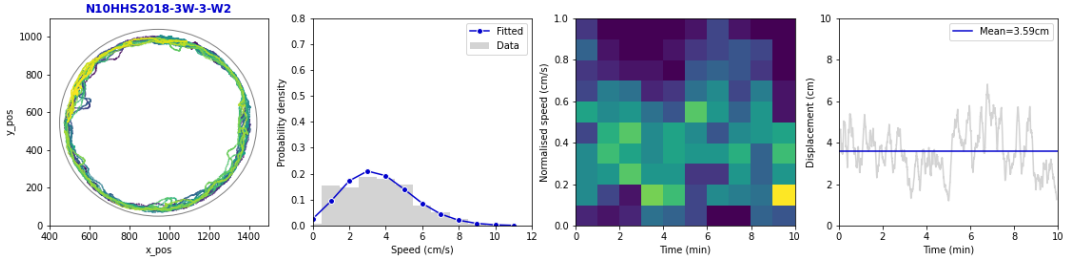

N10HHS2018-3W-1-W3
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=4.24cm

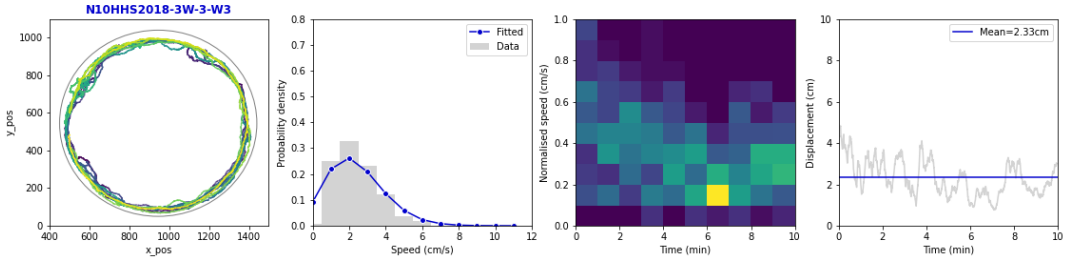

N10HHS2018-3W-2-W1
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.48cm

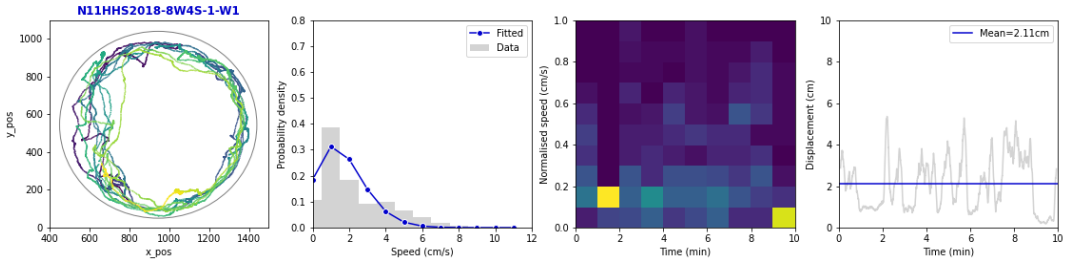

N10HHS2018-3W-2-W2
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=4.0cm

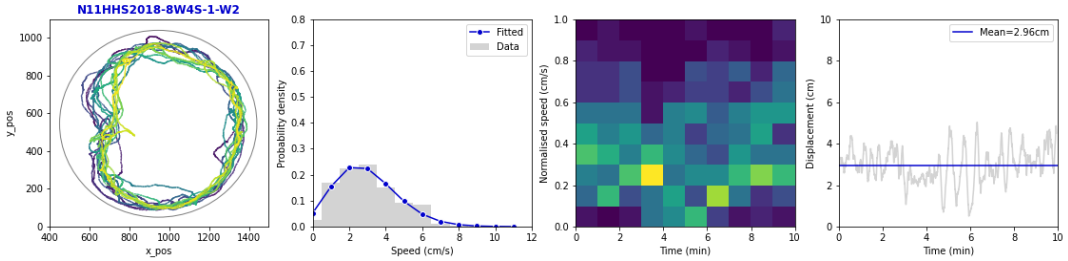

N10HHS2018-3W-2-W3
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.31cm

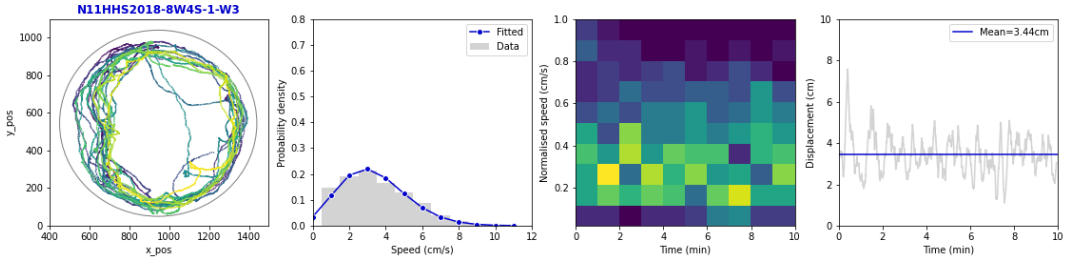

N10HHS2018-3W-3-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.75cm
Displacement (cm)
Time (min)

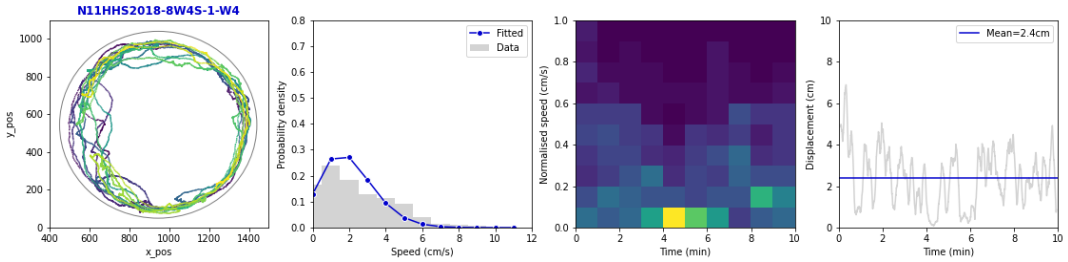

N10HHS2018-3W-3-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.59cm
Displacement (cm)
Time (min)

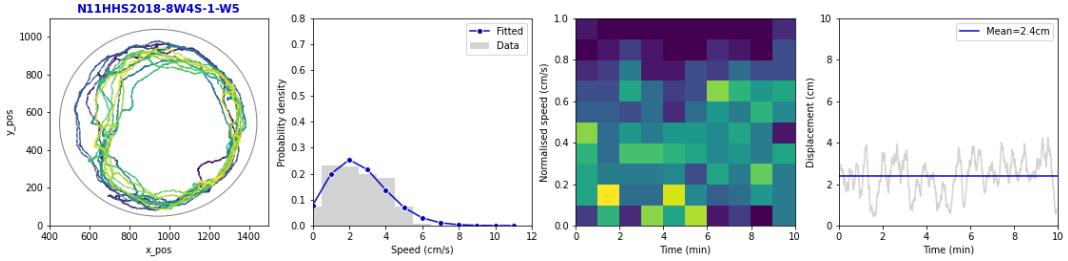

N10HHS2018-3W-3-W3
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.33cm
Displacement (cm)
Time (min)

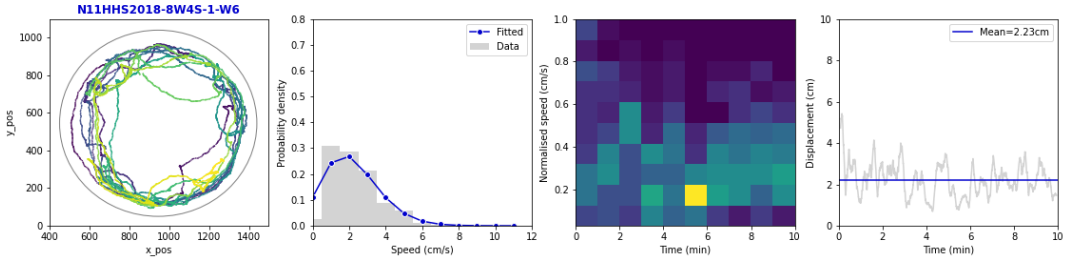

N11HHS2018-8W4S-1-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.11cm
Displacement (cm)
Time (min)

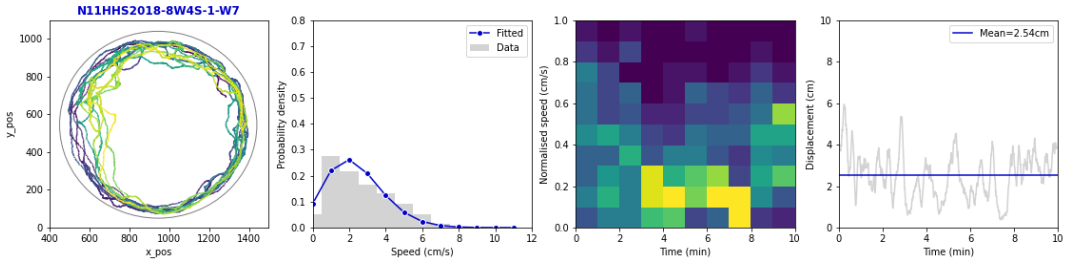

N11HHS2018-8W4S-1-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.96cm
Displacement (cm)
Time (min)

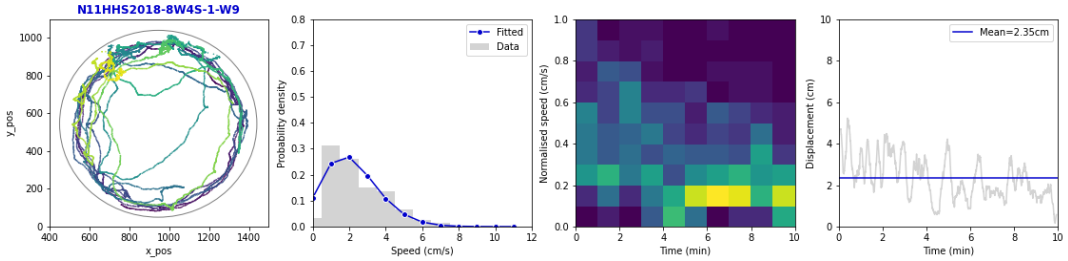

N11HHS2018-8W4S-1-W3
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.44cm
Displacement (cm)
Time (min)

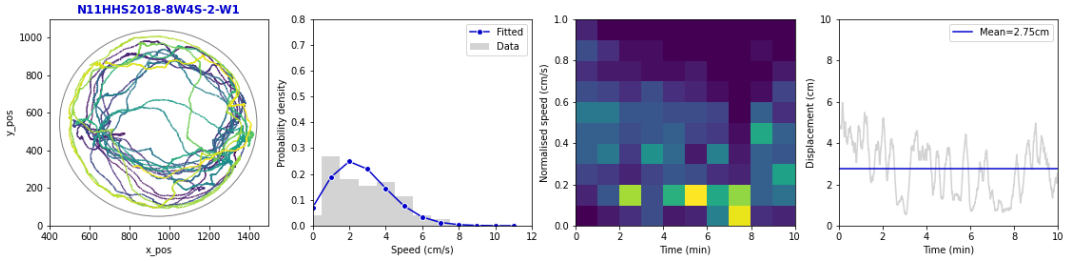

N11HHS2018-8W4S-1-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.4cm

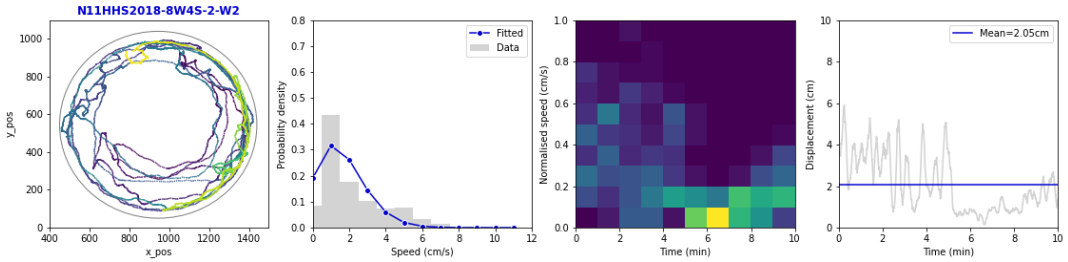

N11HHS2018-8W4S-1-W5
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.4cm

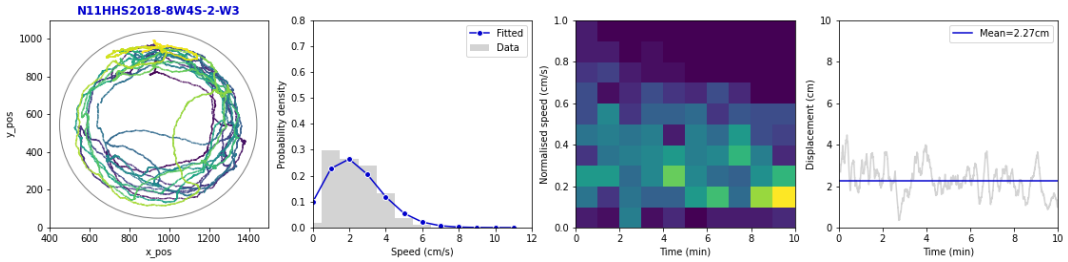

N11HHS2018-8W4S-1-W6
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.23cm

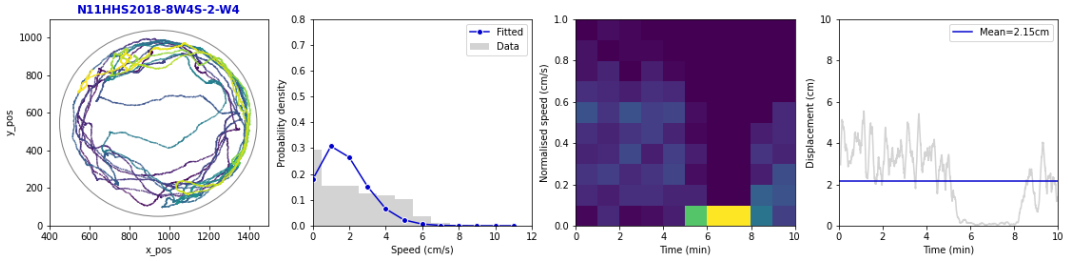

N11HHS2018-8W4S-1-W7
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.54cm

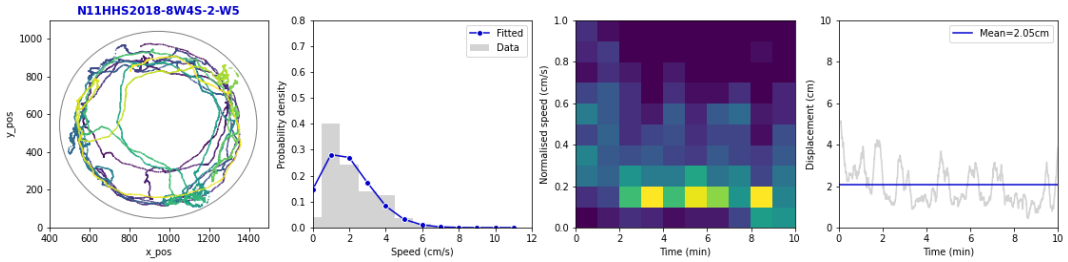

N11HHS2018-8W4S-1-W9
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.35cm

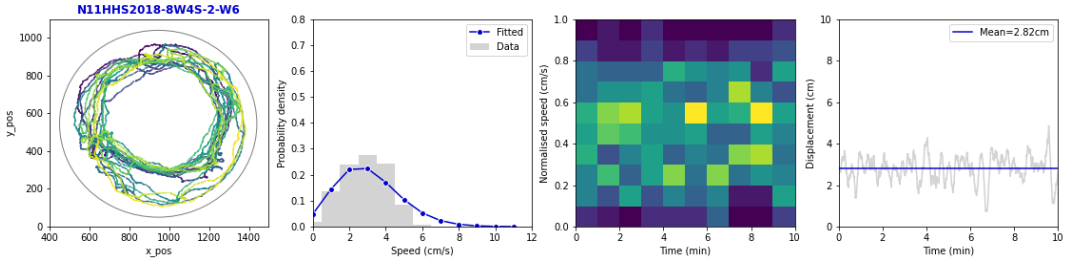

N11HHS2018-8W4S-2-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.75cm

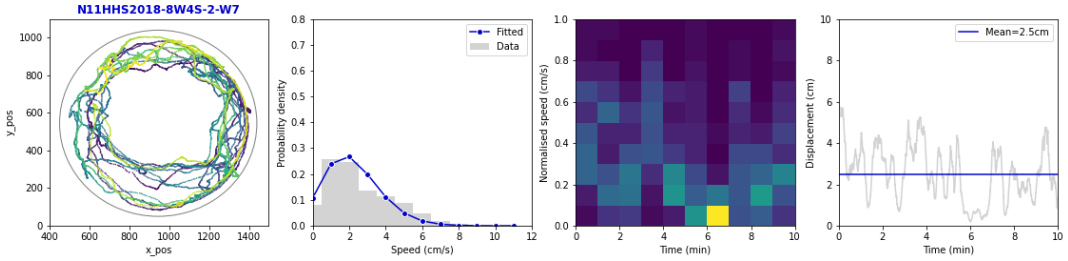

N11HHS2018-8W4S-2-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.05cm
Displacement (cm)
Time (min)

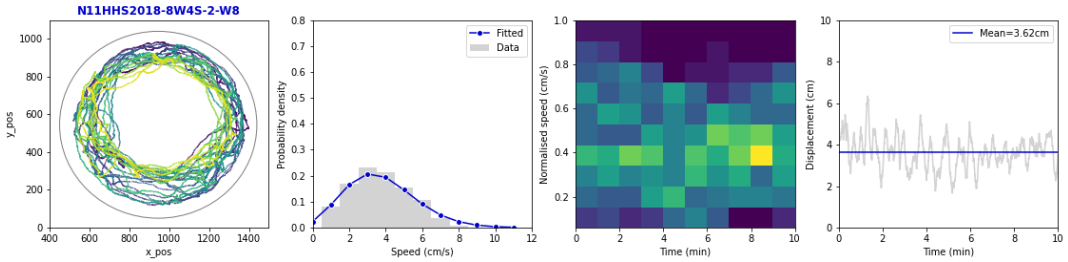

N11HHS2018-8W4S-2-W3
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.27cm
Displacement (cm)
Time (min)

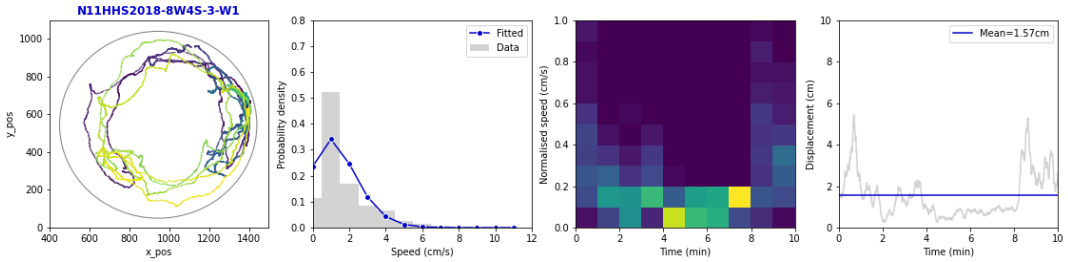

N11HHS2018-8W4S-2-W4
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.15cm
Displacement (cm)
Time (min)

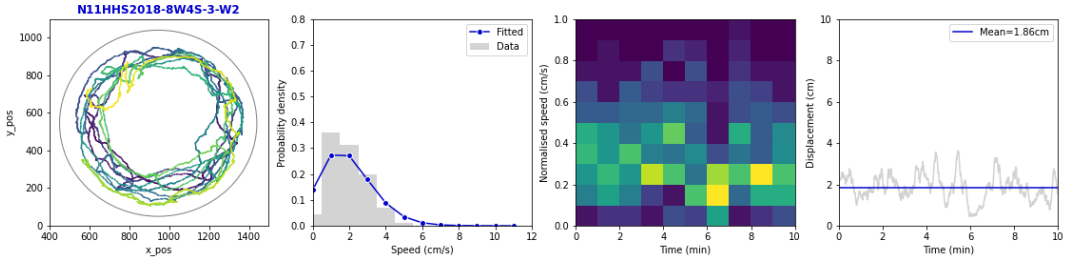

N11HHS2018-8W4S-2-W5
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.05cm
Displacement (cm)
Time (min)

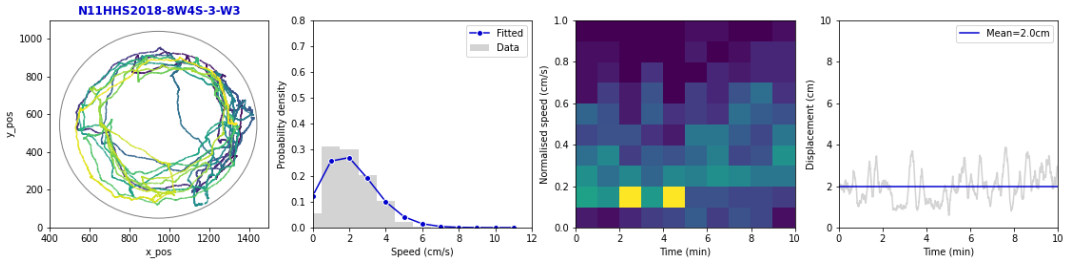

N11HHS2018-8W4S-2-W6
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.82cm
Displacement (cm)
Time (min)

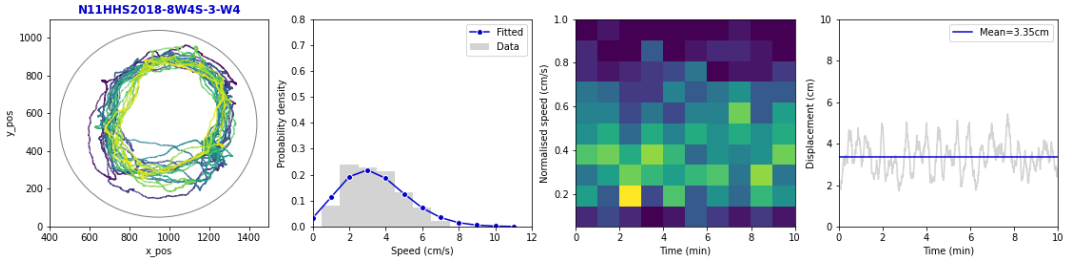

N11HHS2018-8W4S-2-W7
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.5cm
Displacement (cm)
Time (min)

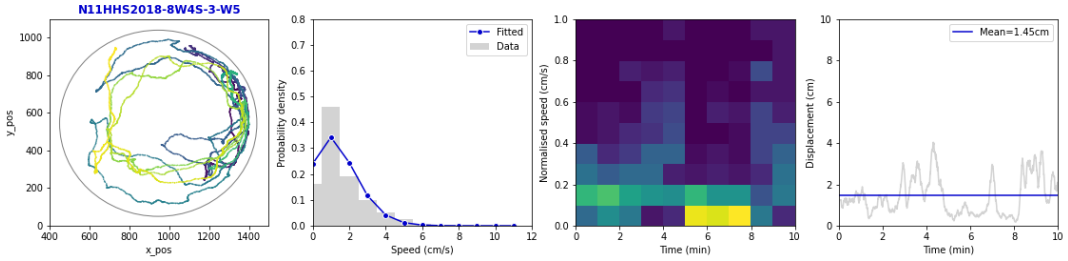

N11HHS2018-8W4S-2-W8
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.62cm
Displacement (cm)
Time (min)

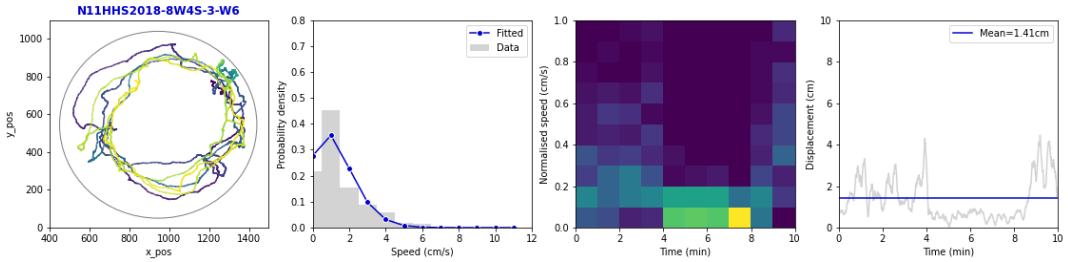

N11HHS2018-8W4S-3-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.57cm
Displacement (cm)
Time (min)

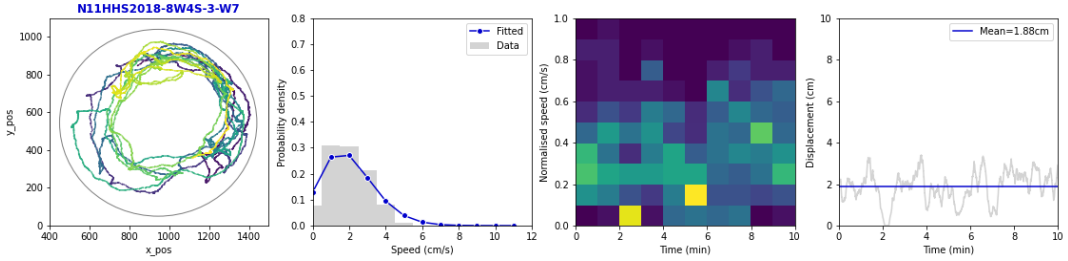

N11HHS2018-8W4S-3-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.86cm
Displacement (cm)
Time (min)

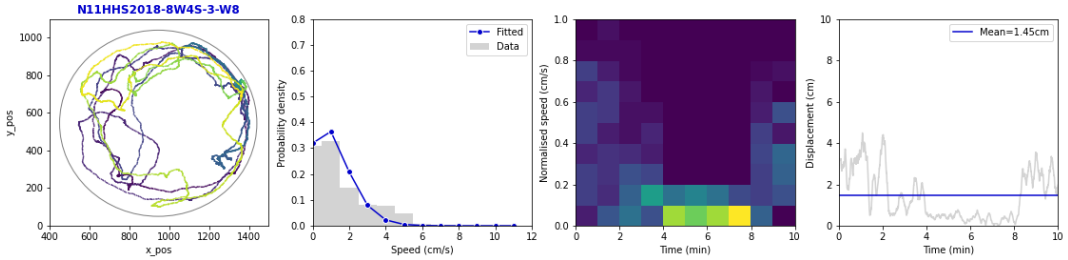

N11HHS2018-8W4S-3-W3
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.0cm
Displacement (cm)
Time (min)

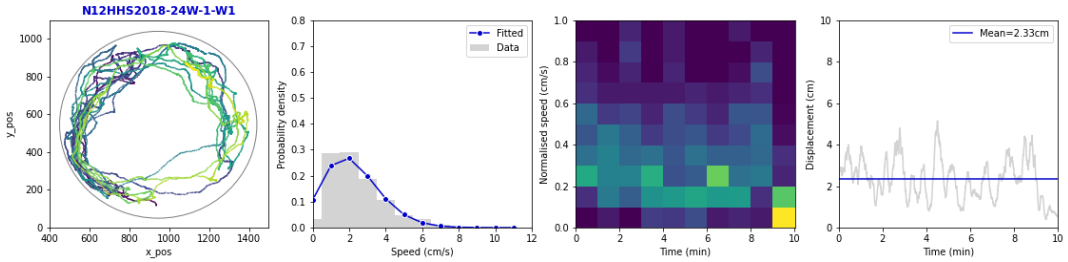

N11HHS2018-8W4S-3-W4
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.35cm
Displacement (cm)
Time (min)

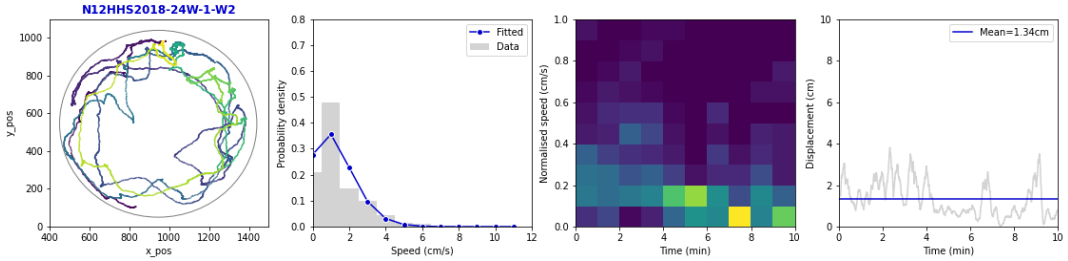

N11HHS2018-8W4S-3-W5
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.45cm
Displacement (cm)
Time (min)

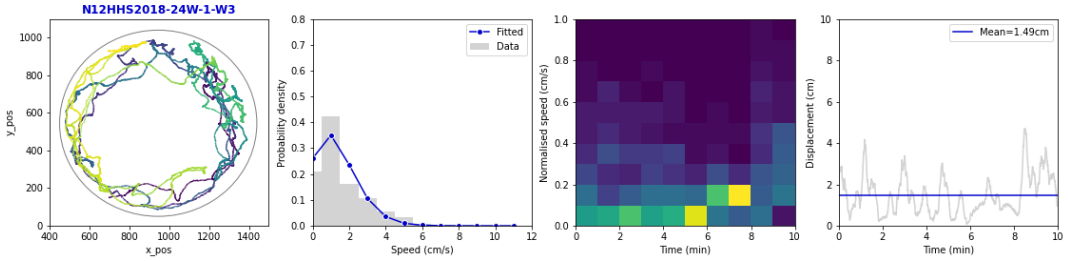

N11HHS2018-8W4S-3-W6
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.41cm
Displacement (cm)
Time (min)

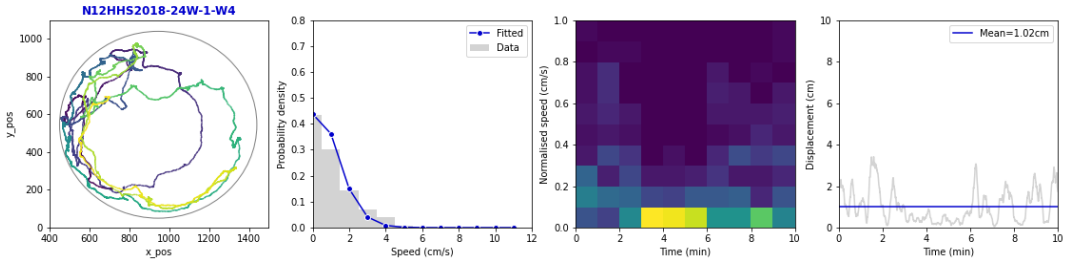

N11HHS2018-8W4S-3-W7
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.88cm
Displacement (cm)
Time (min)

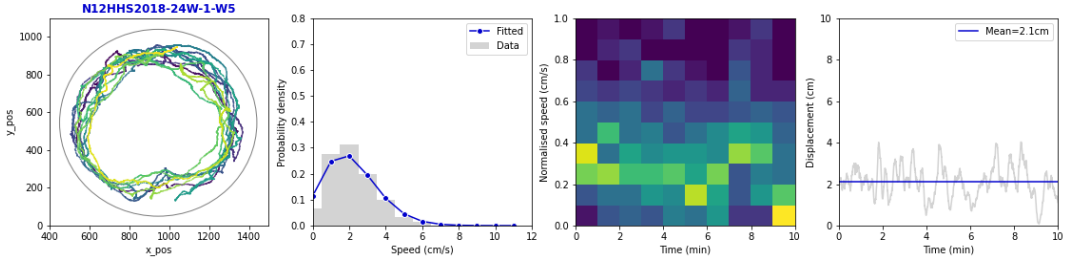

N11HHS2018-8W4S-3-W8
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.45cm
Displacement (cm)
Time (min)

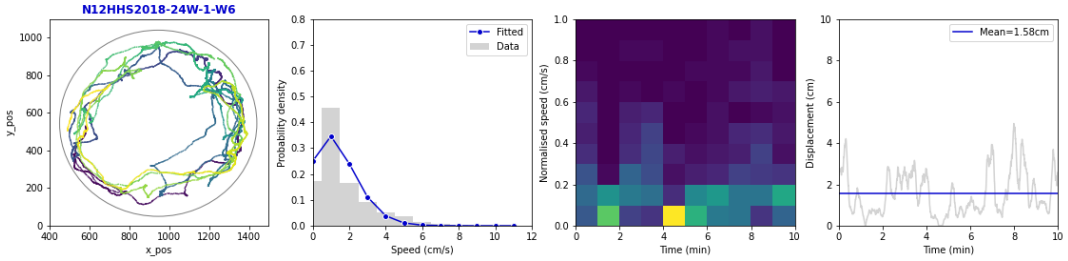

N12HHS2018-24W-1-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.33cm
Displacement (cm)
Time (min)

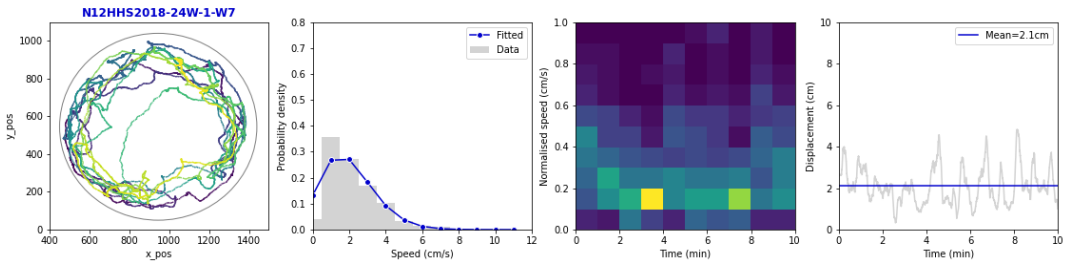

N12HHS2018-24W-1-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.34cm
Displacement (cm)
Time (min)

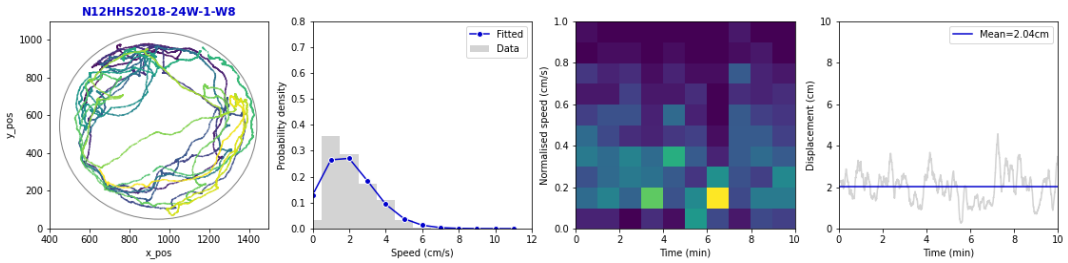

N12HHS2018-24W-1-W3
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.49cm
Displacement (cm)
Time (min)

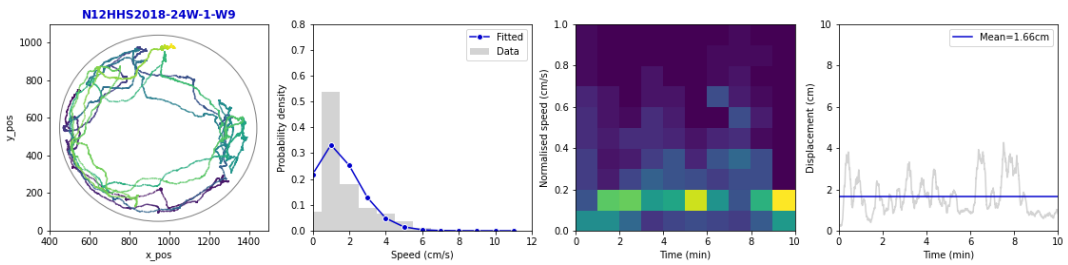

N12HHS2018-24W-1-W4
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.02cm

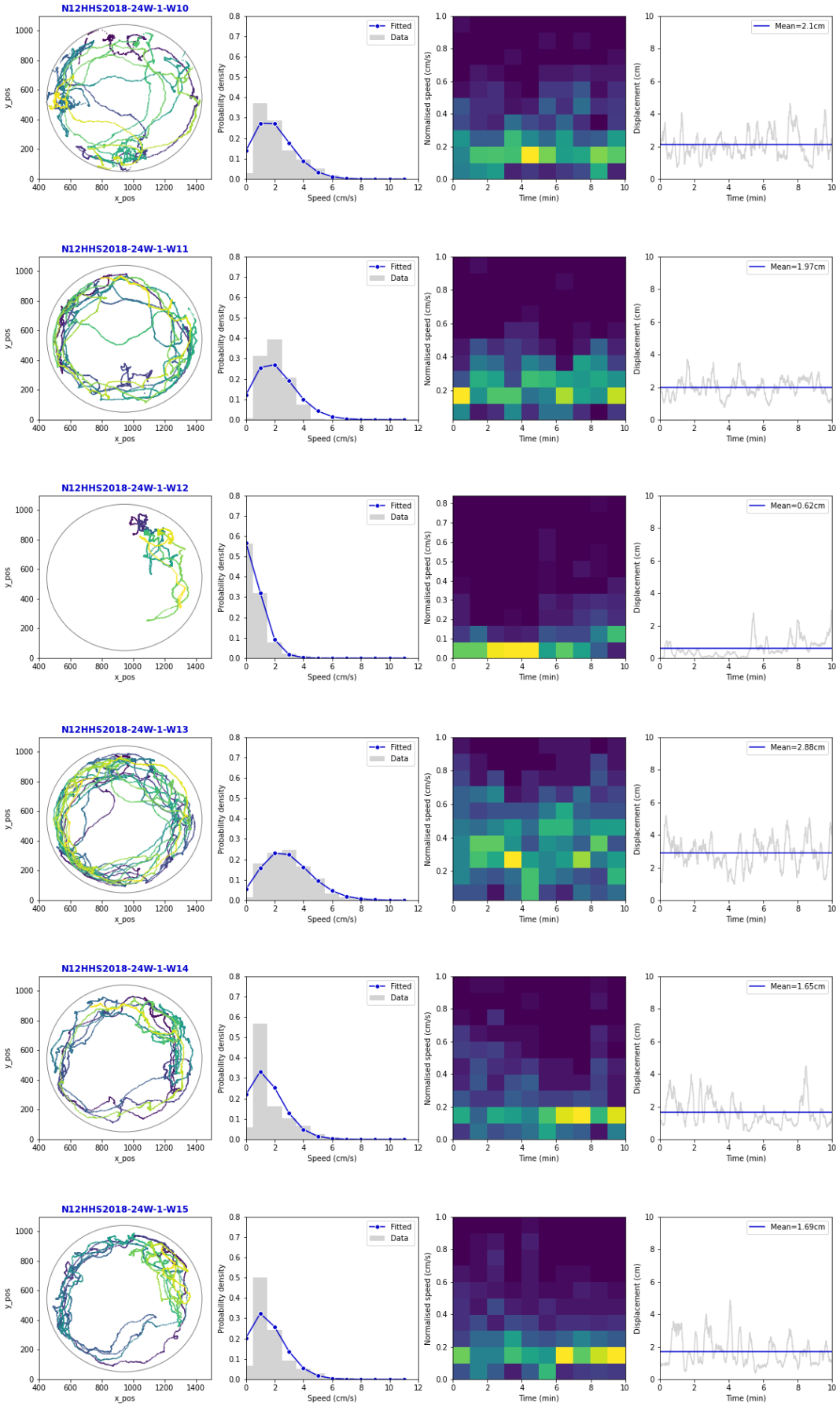

N12HHS2018-24W-1-W5
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.1cm

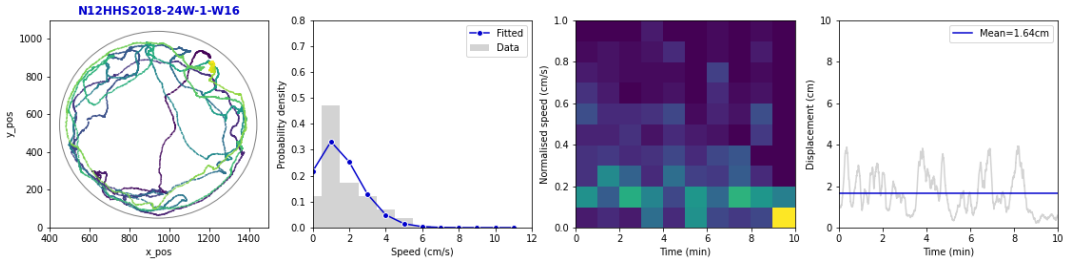

N12HHS2018-24W-1-W6
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.58cm

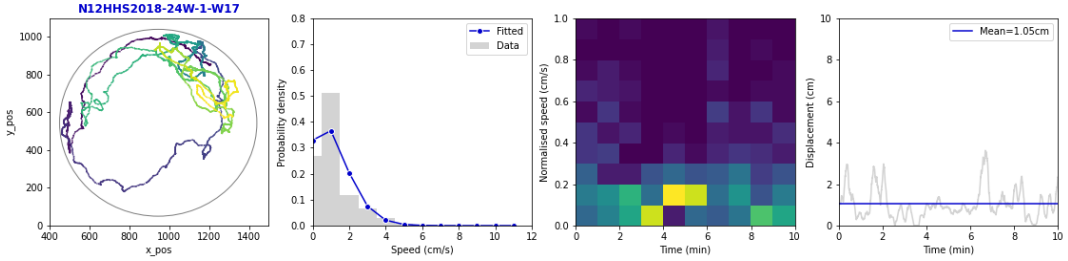

N12HHS2018-24W-1-W7
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.1cm

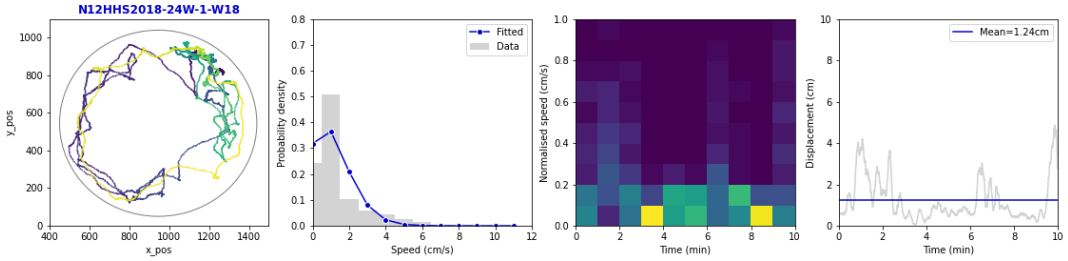

N12HHS2018-24W-1-W8
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.04cm

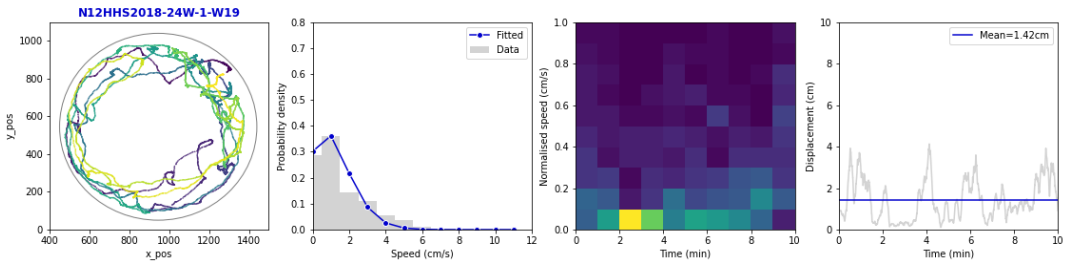

N12HHS2018-24W-1-W9
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.66cm

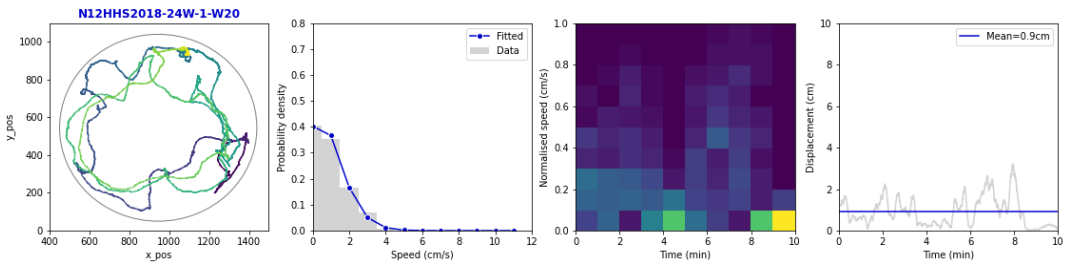

N12HHS2018-24W-1-W10
N12HHS2018-24W-1-W11
N12HHS2018-24W-1-W12
N12HHS2018-24W-1-W13
N12HHS2018-24W-1-W14
N12HHS2018-24W-1-W15
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.1cm
Mean=1.97cm
Mean=0.62cm
Mean=2.88cm
Mean=1.65cm
Mean=1.69cm

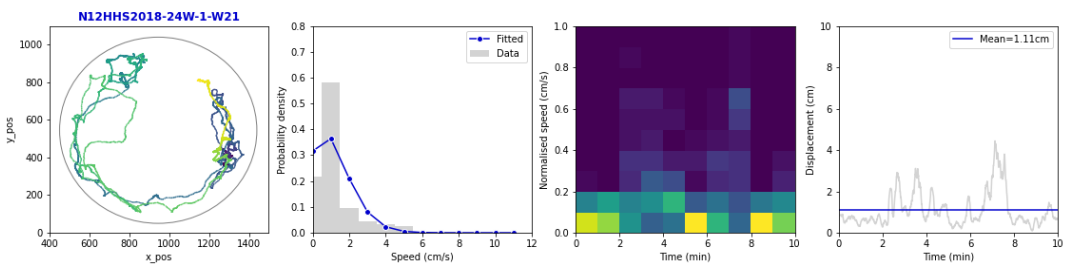

N12HHS2018-24W-1-W16
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.64cm

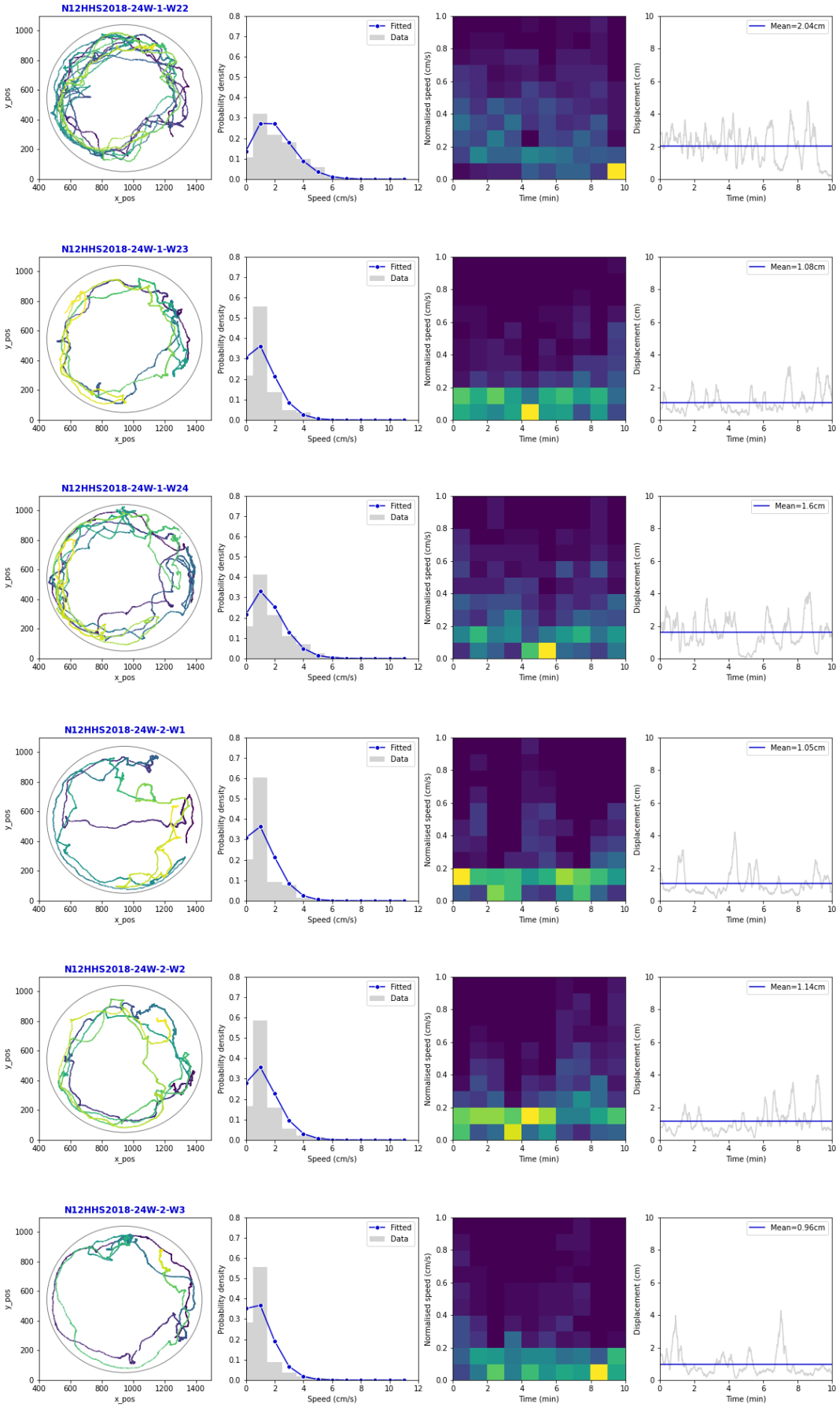

N12HHS2018-24W-1-W17
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.05cm

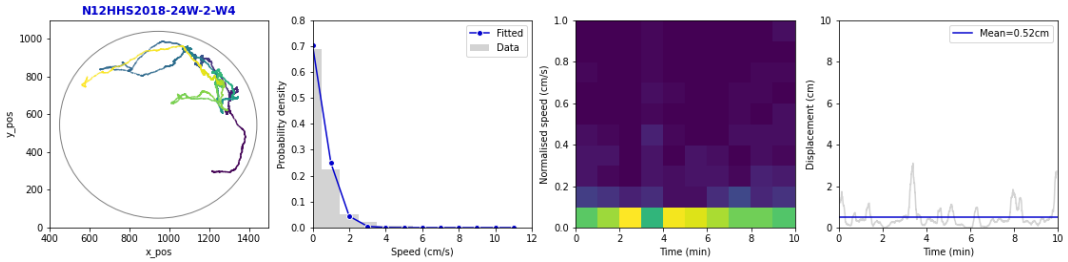

N12HHS2018-24W-1-W18
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.24cm

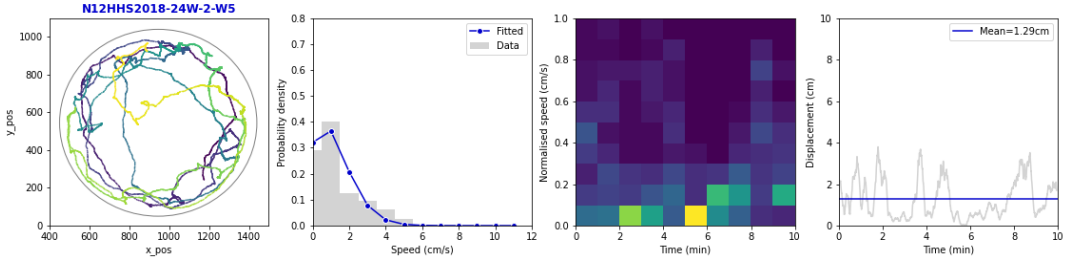

N12HHS2018-24W-1-W19
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.42cm

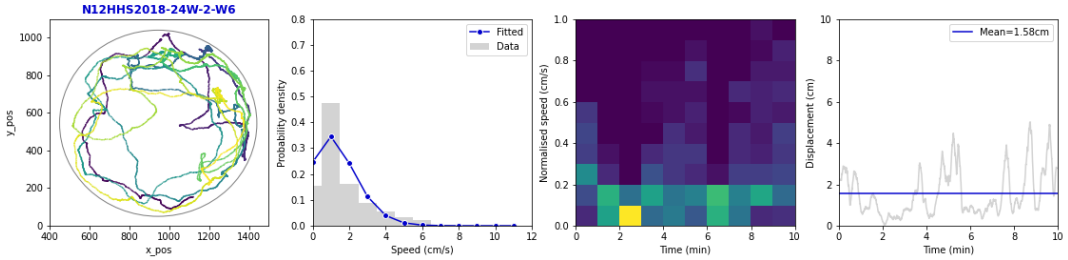

N12HHS2018-24W-1-W20
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.9cm

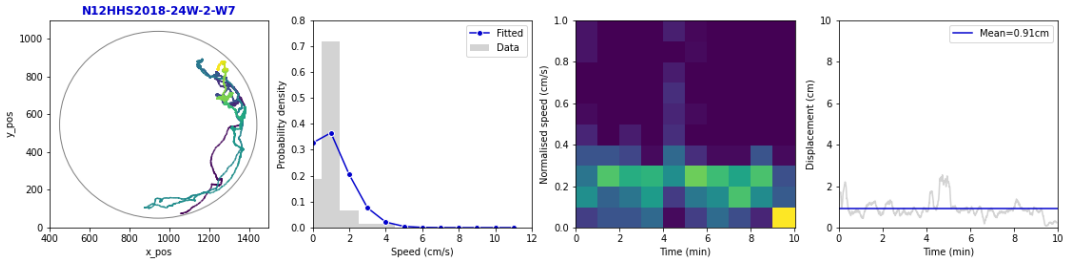

N12HHS2018-24W-1-W21
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.11cm

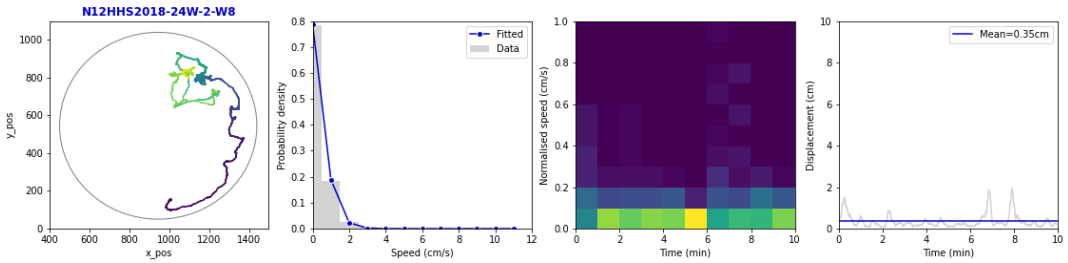

N12HHS2018-24W-1-W22
N12HHS2018-24W-1-W23
N12HHS2018-24W-1-W24
N12HHS2018-24W-2-W1
N12HHS2018-24W-2-W2
N12HHS2018-24W-2-W3
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.04cm
Mean=1.08cm
Mean=1.6cm
Mean=1.05cm
Mean=1.14cm
Mean=0.96cm

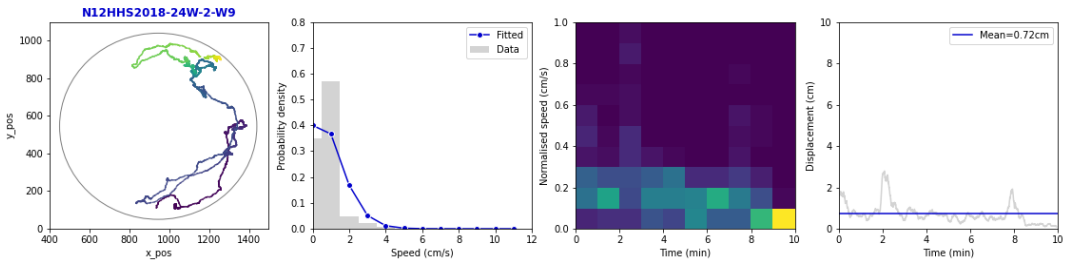

N12HHS2018-24W-2-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.52cm

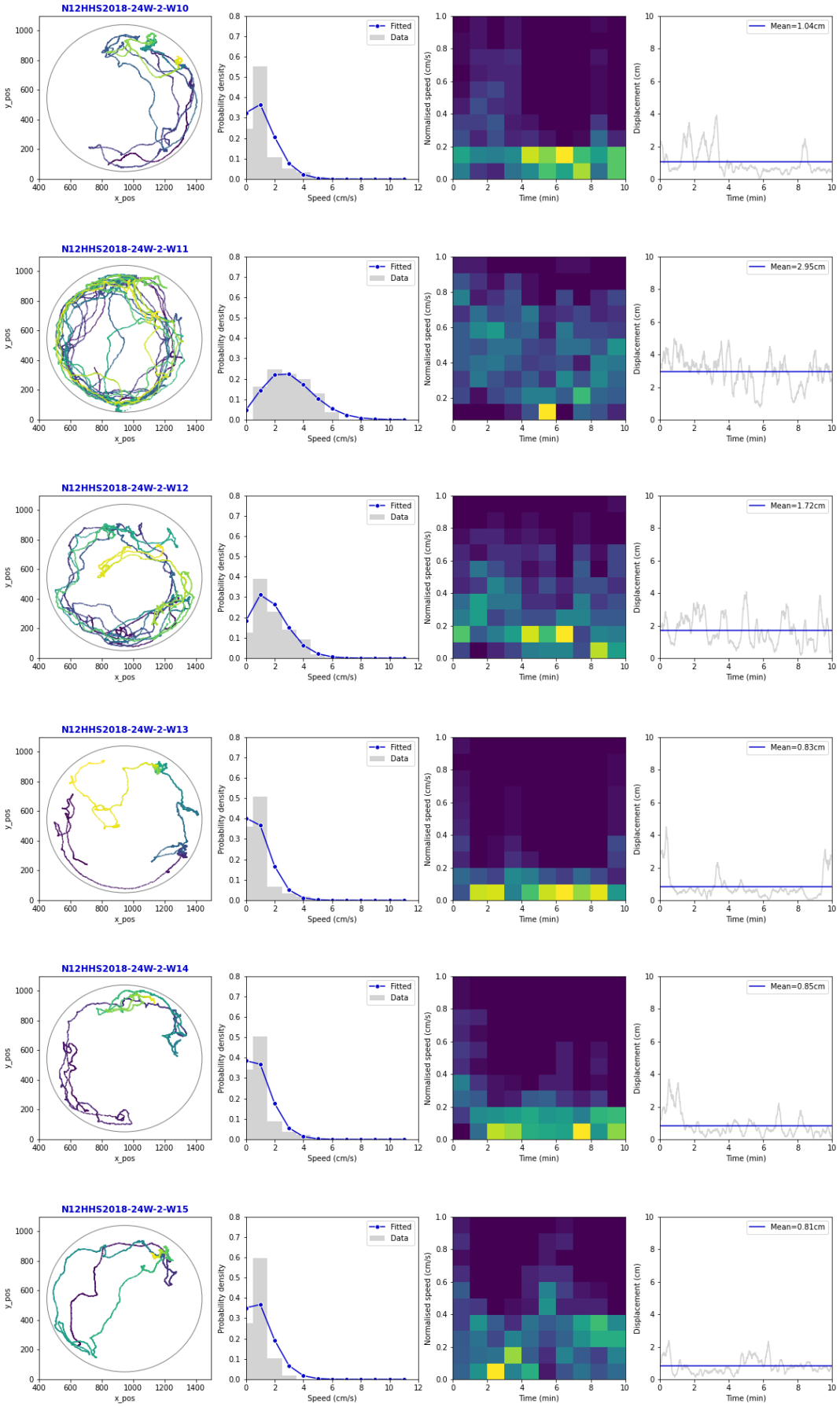

N12HHS2018-24W-2-W5
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.29cm

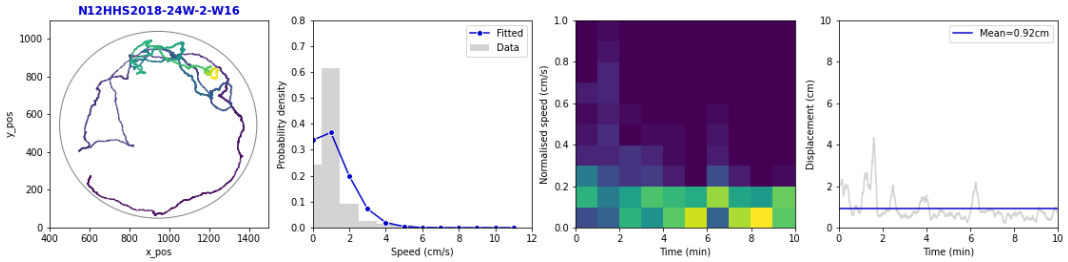

N12HHS2018-24W-2-W6
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.58cm

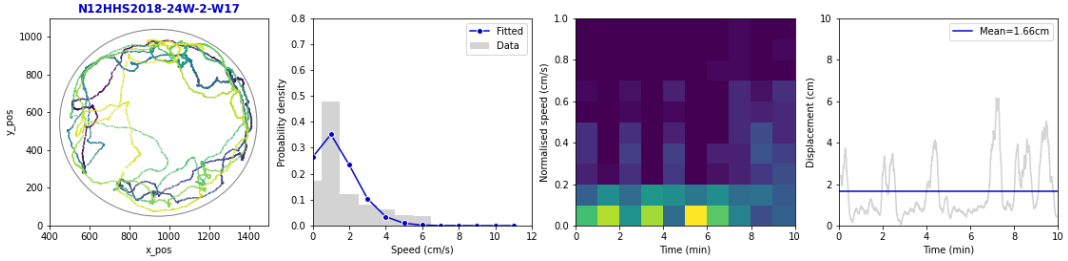

N12HHS2018-24W-2-W7
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.91cm

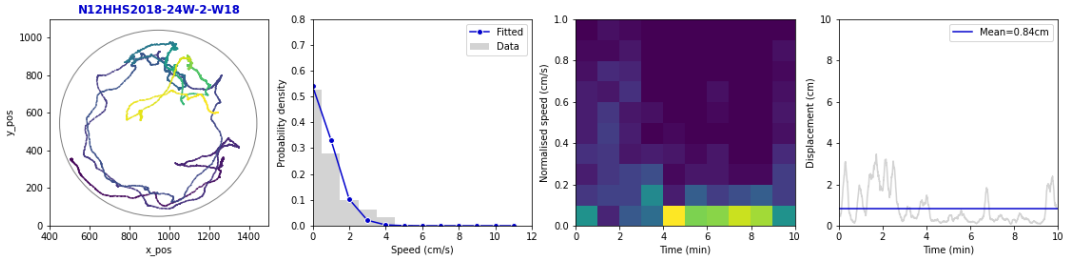

N12HHS2018-24W-2-W8
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.35cm

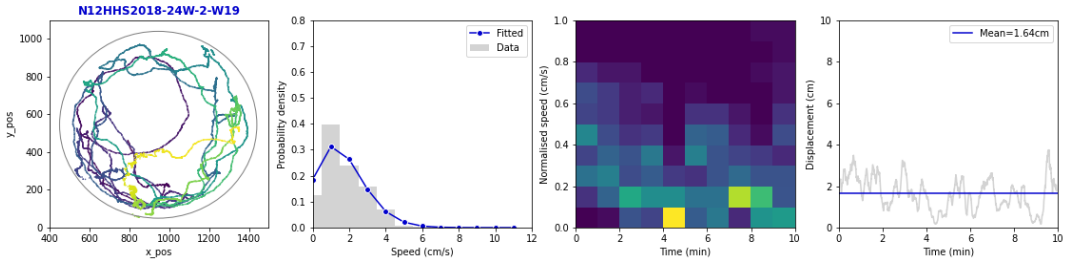

N12HHS2018-24W-2-W9
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.72cm

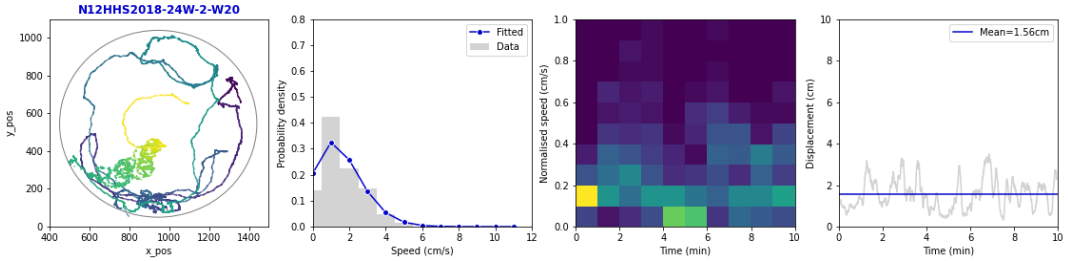

N12HHS2018-24W-2-W10
N12HHS2018-24W-2-W11
N12HHS2018-24W-2-W12
N12HHS2018-24W-2-W13
N12HHS2018-24W-2-W14
N12HHS2018-24W-2-W15
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.04cm
Mean=2.95cm
Mean=1.72cm
Mean=0.83cm
Mean=0.85cm
Mean=0.81cm

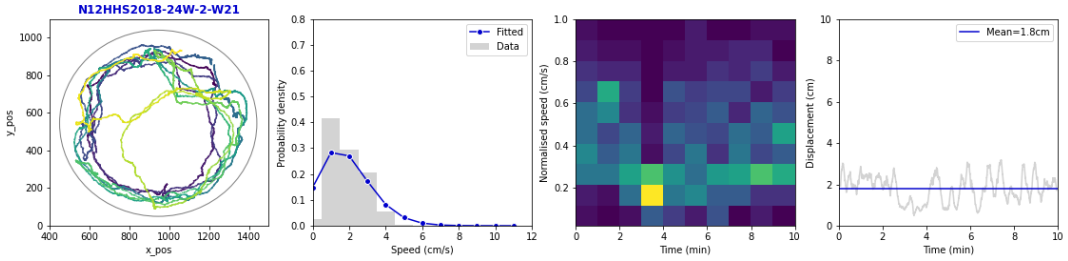

N12HHS2018-24W-2-W16
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.92cm

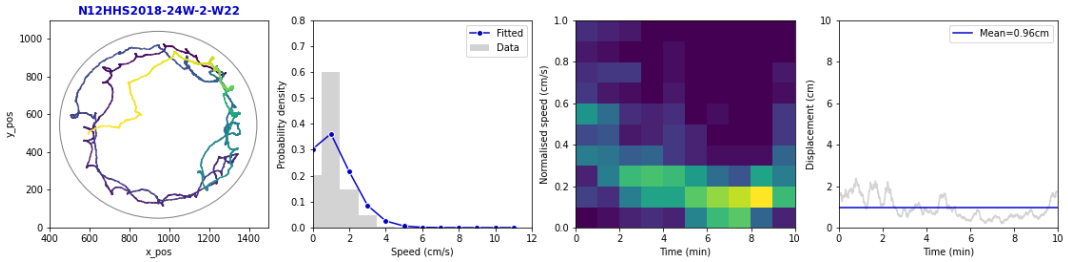

N12HHS2018-24W-2-W17
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.66cm

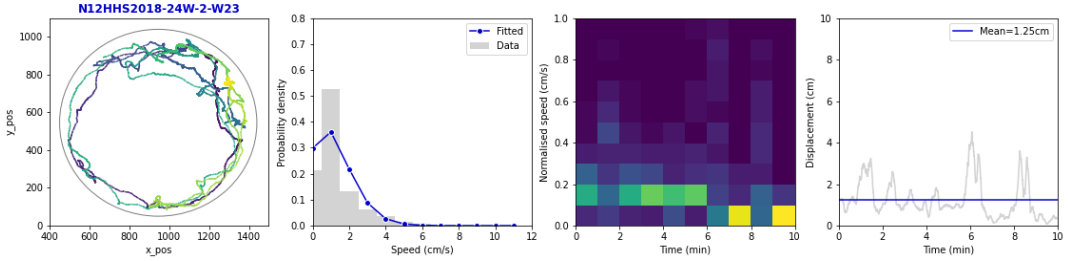

N12HHS2018-24W-2-W18
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.84cm

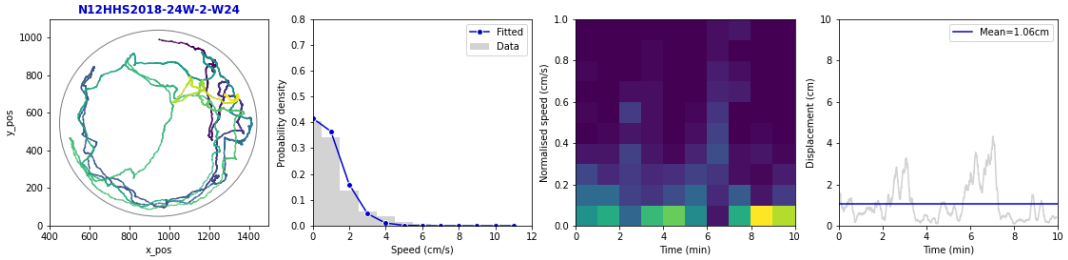

N12HHS2018-24W-2-W19
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.64cm

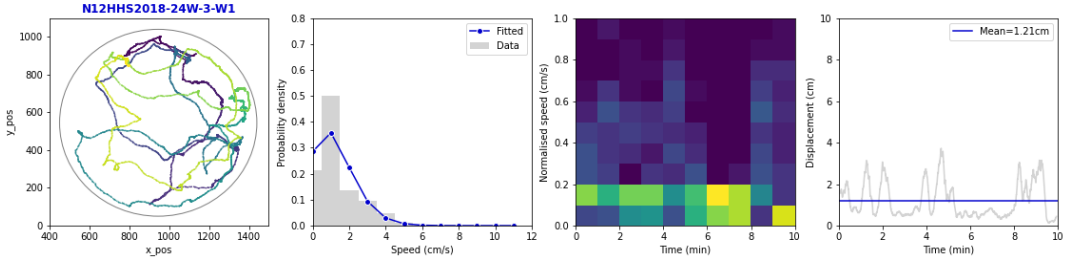

N12HHS2018-24W-2-W20
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.56cm

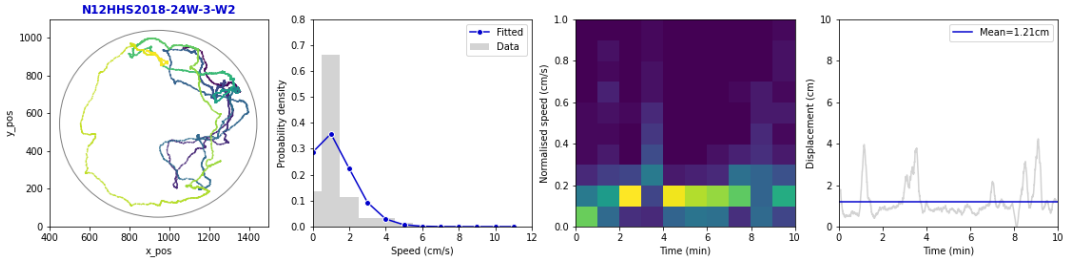

N12HHS2018-24W-2-W21
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.8cm

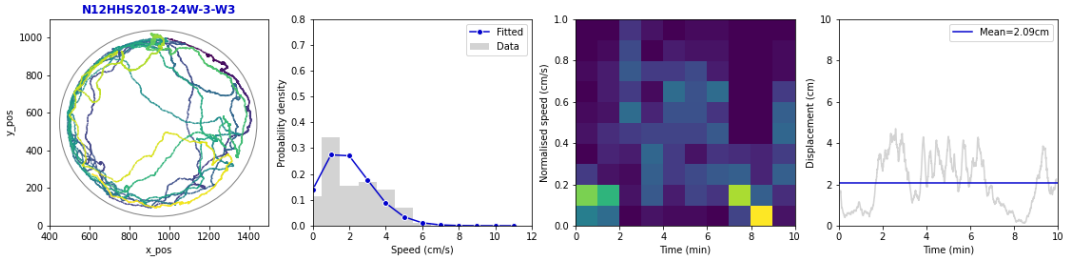

N12HHS2018-24W-2-W22
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.96cm

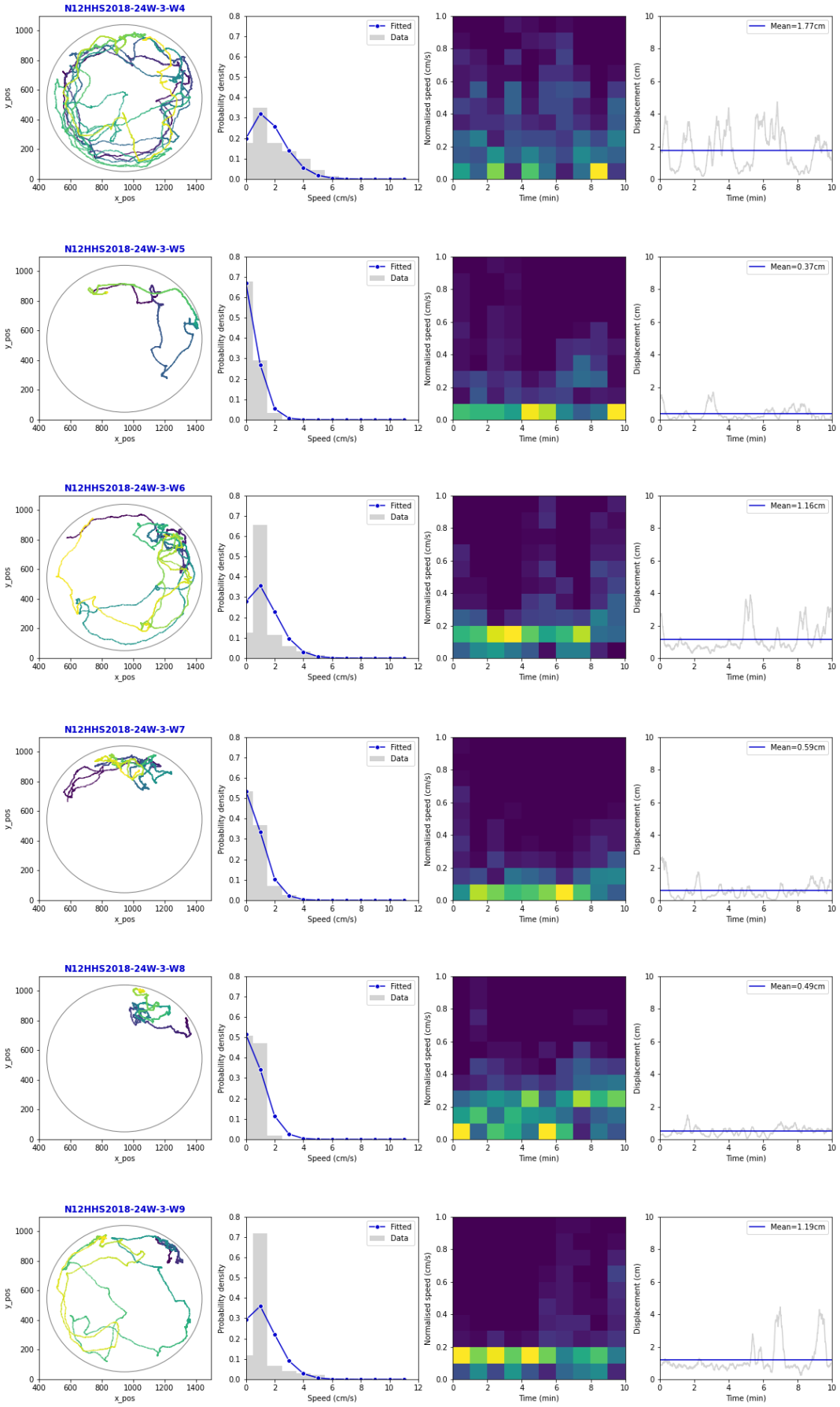

N12HHS2018-24W-2-W23
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.25cm

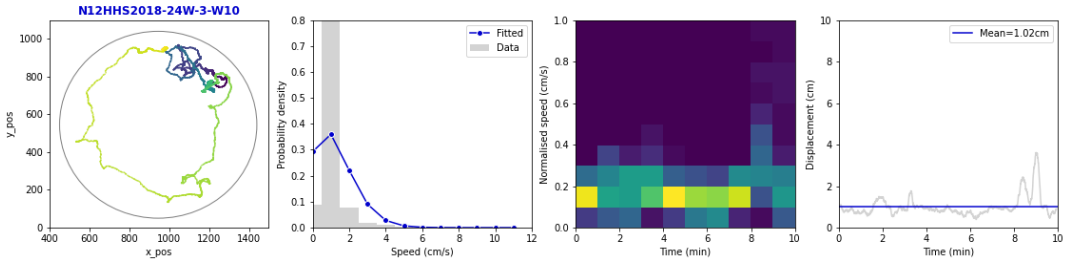

N12HHS2018-24W-2-W24
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.06cm

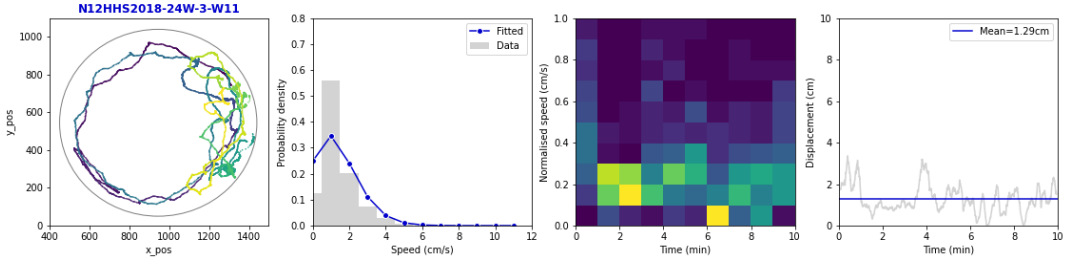

N12HHS2018-24W-3-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.21cm

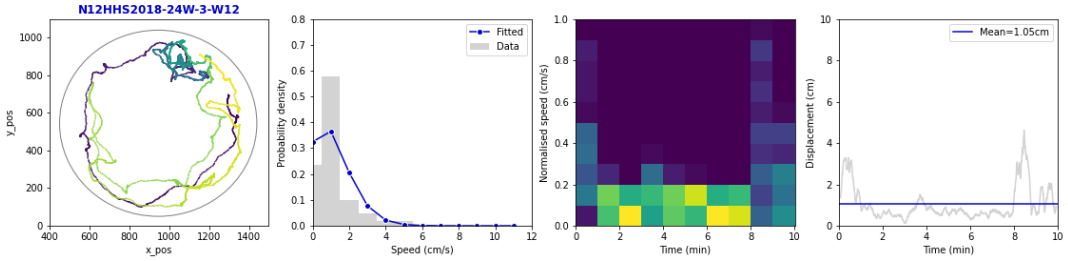

N12HHS2018-24W-3-W2
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.21cm

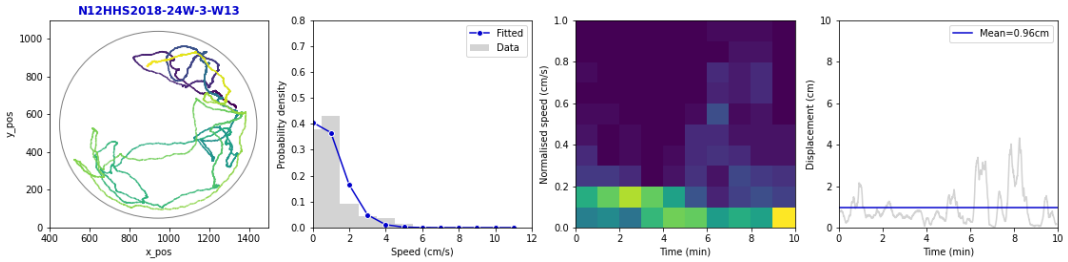

N12HHS2018-24W-3-W3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.09cm

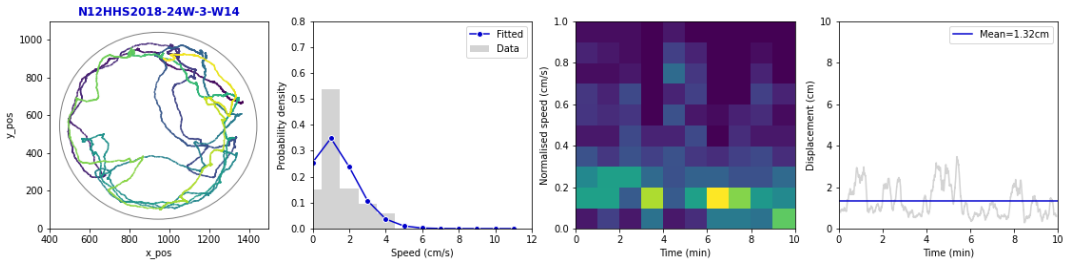

N12HHS2018-24W-3-W4
N12HHS2018-24W-3-W5
N12HHS2018-24W-3-W6
N12HHS2018-24W-3-W7
N12HHS2018-24W-3-W8
N12HHS2018-24W-3-W9
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.77cm
Mean=0.37cm
Mean=1.16cm
Mean=0.59cm
Mean=0.49cm
Mean=1.19cm

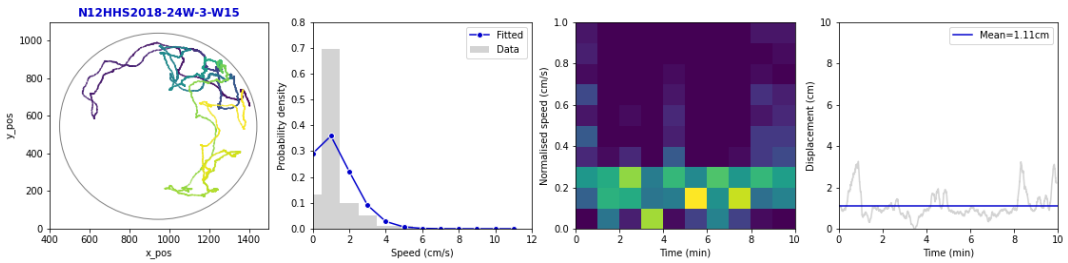

N12HHS2018-24W-3-W10
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.02cm

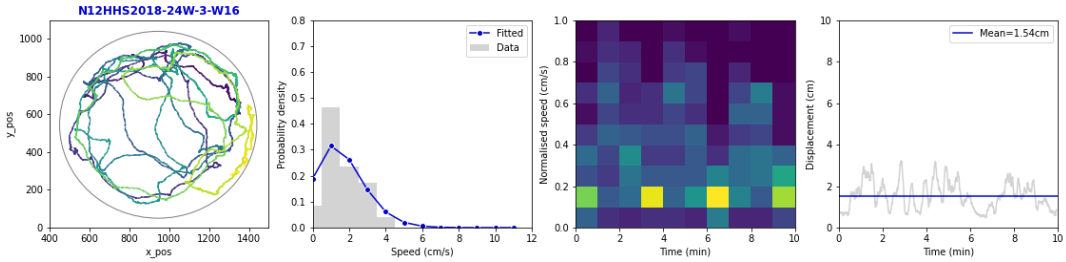

N12HHS2018-24W-3-W11
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.29cm

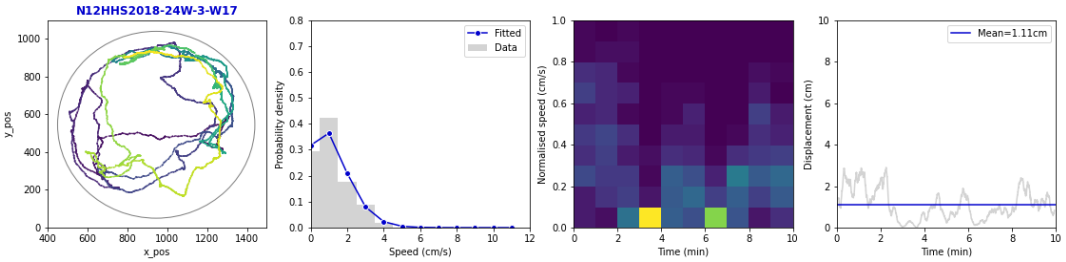

N12HHS2018-24W-3-W12
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.05cm

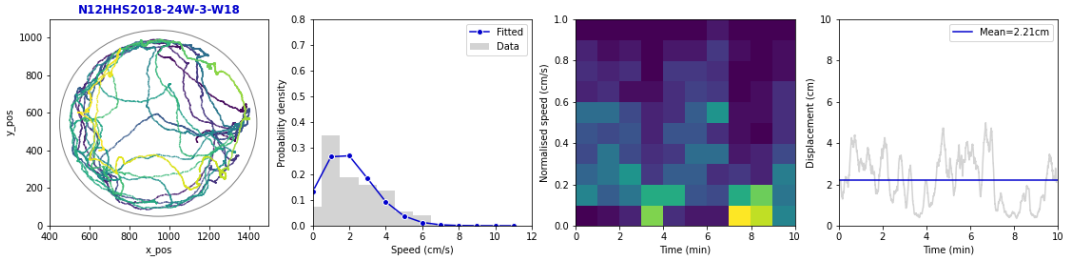

N12HHS2018-24W-3-W13
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.96cm

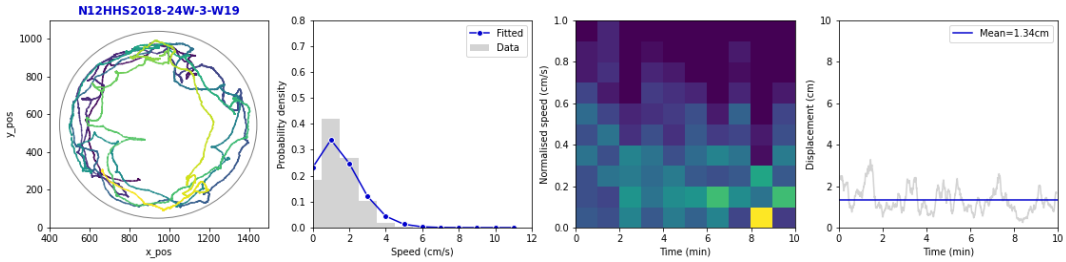

N12HHS2018-24W-3-W14
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.32cm

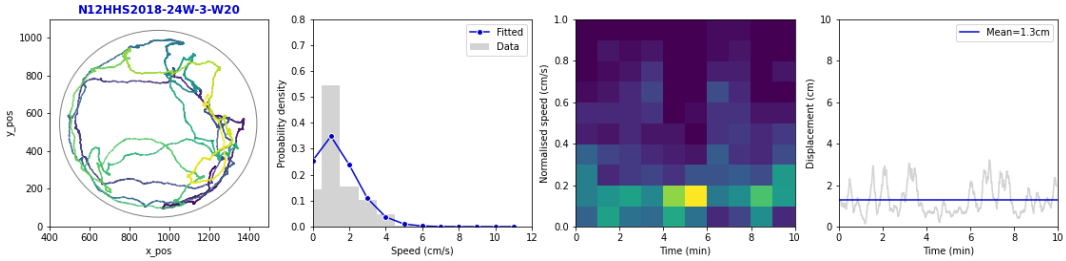

N12HHS2018-24W-3-W15
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.11cm

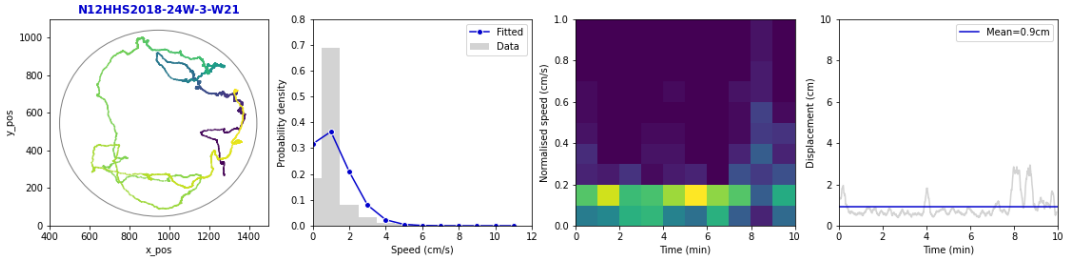

N12HHS2018-24W-3-W16
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.54cm
Displacement (cm)
Time (min)

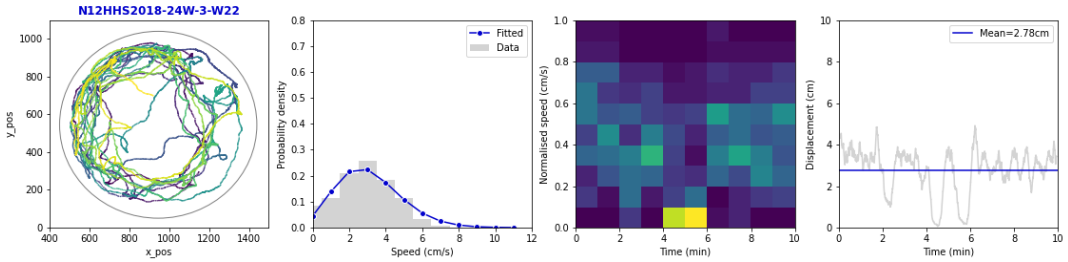

N12HHS2018-24W-3-W17
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.11cm
Displacement (cm)
Time (min)

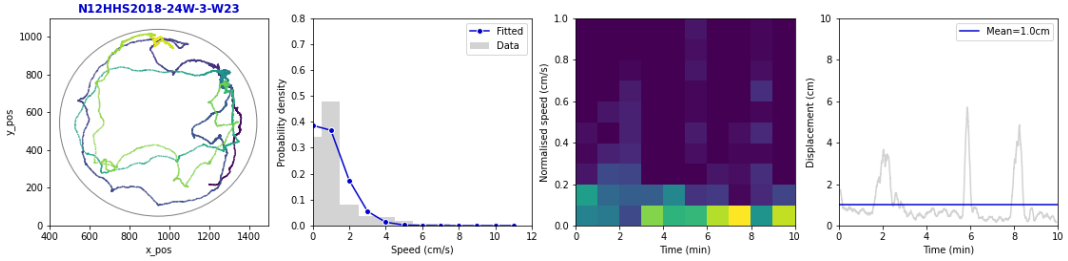

N12HHS2018-24W-3-W18
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.21cm
Displacement (cm)
Time (min)

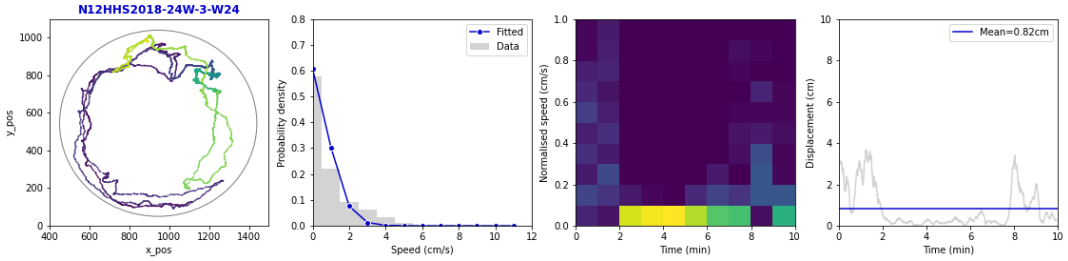

N12HHS2018-24W-3-W19
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.34cm
Displacement (cm)
Time (min)

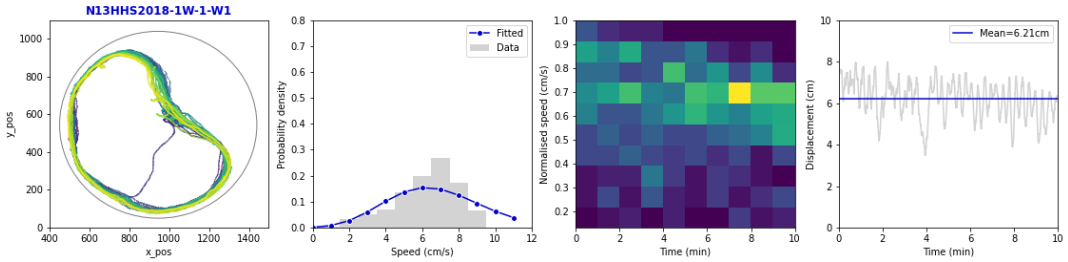

N12HHS2018-24W-3-W20
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.3cm
Displacement (cm)
Time (min)

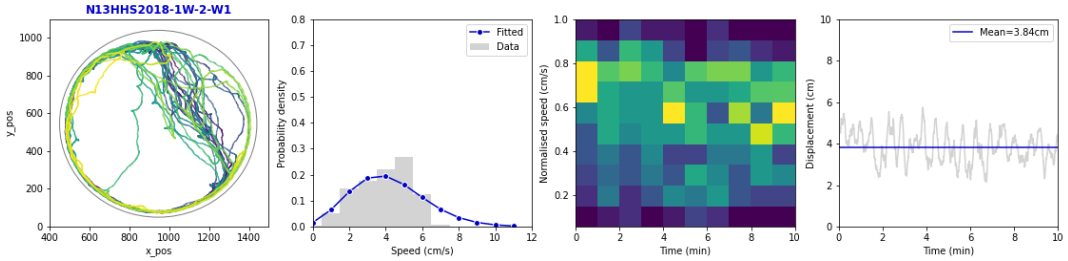

N12HHS2018-24W-3-W21
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.9cm
Displacement (cm)
Time (min)

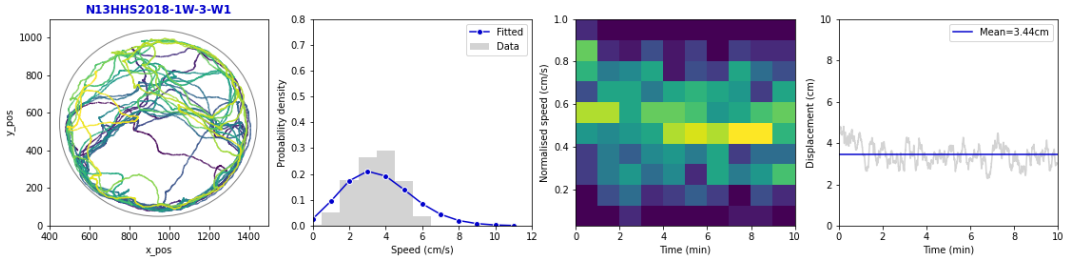

N12HHS2018-24W-3-W22
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.78cm

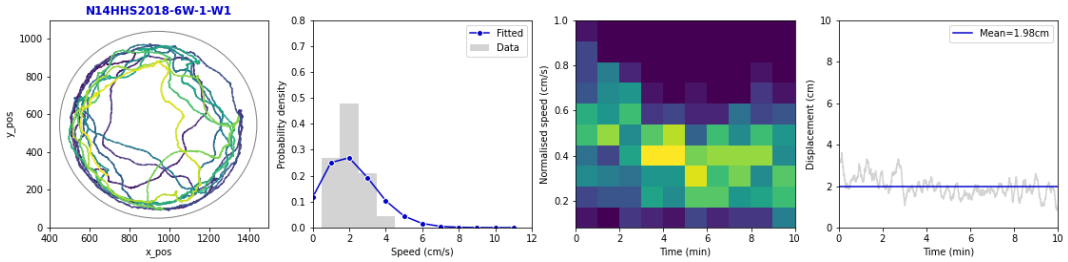

N12HHS2018-24W-3-W23
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.0cm

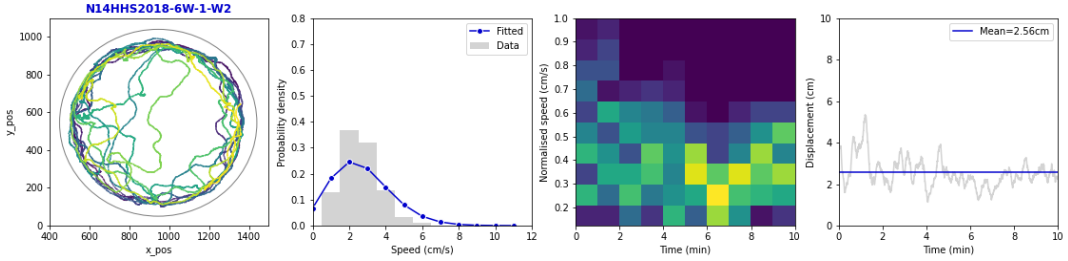

N12HHS2018-24W-3-W24
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.82cm

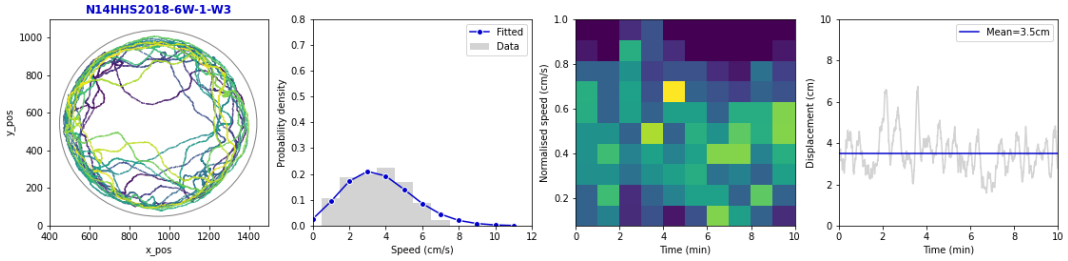

N13HHS2018-1W-1-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=6.21cm

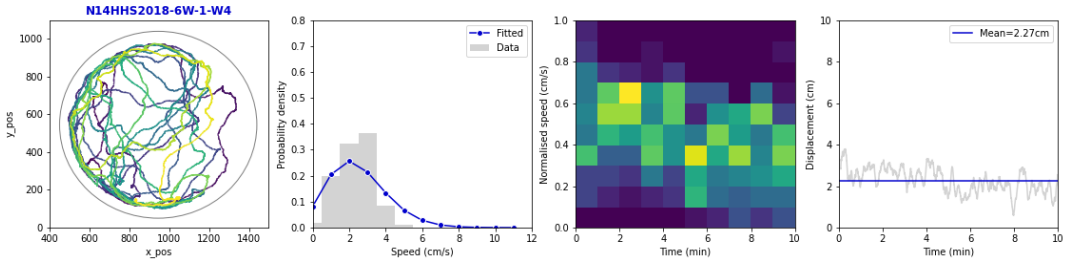

N13HHS2018-1W-2-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.84cm

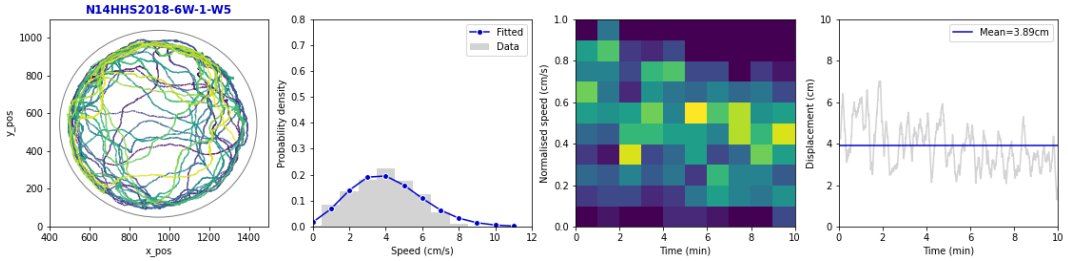

N13HHS2018-1W-3-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.44cm

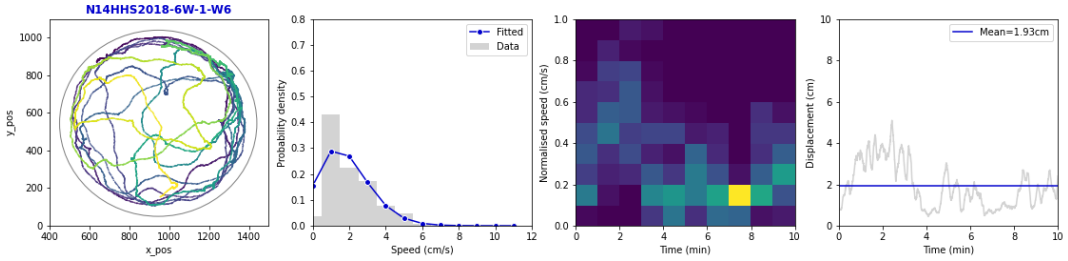

N14HHS2018-6W-1-W1
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.98cm

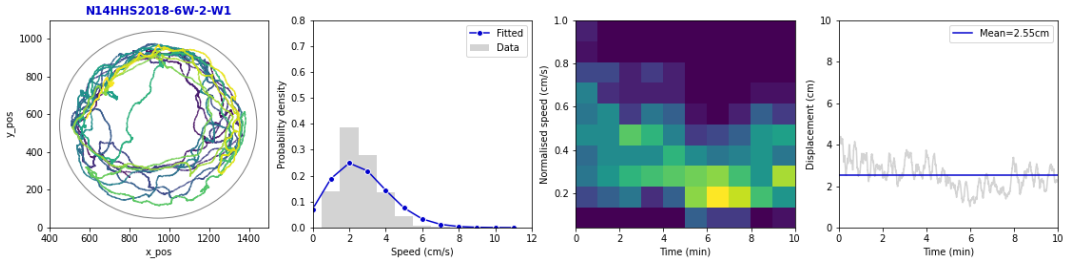

N14HHS2018-6W-1-W2
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.56cm

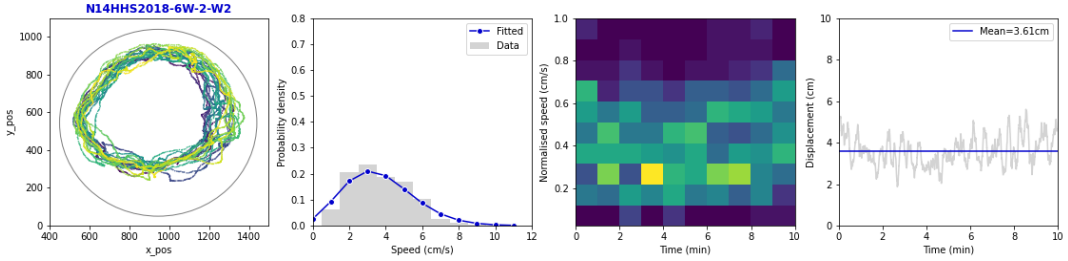

N14HHS2018-6W-1-W3
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=3.5cm

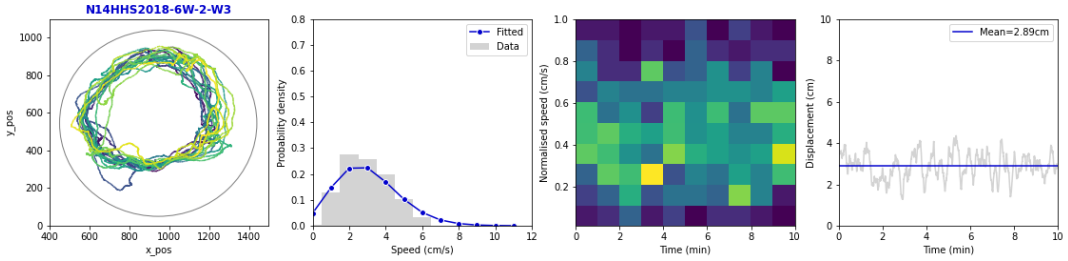

N14HHS2018-6W-1-W4
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.27cm

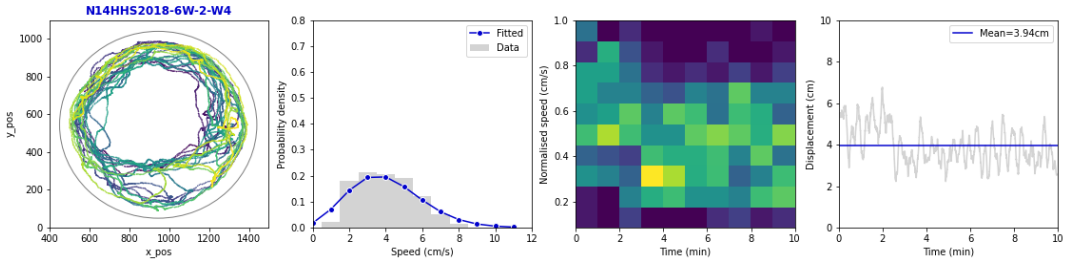

N14HHS2018-6W-1-W5
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=3.89cm

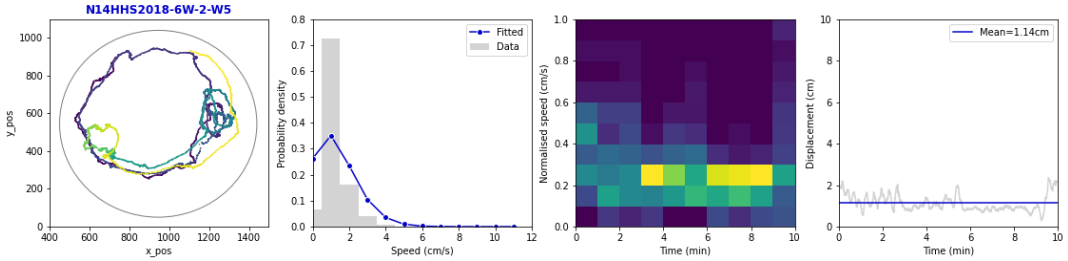

N14HHS2018-6W-1-W6
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.93cm

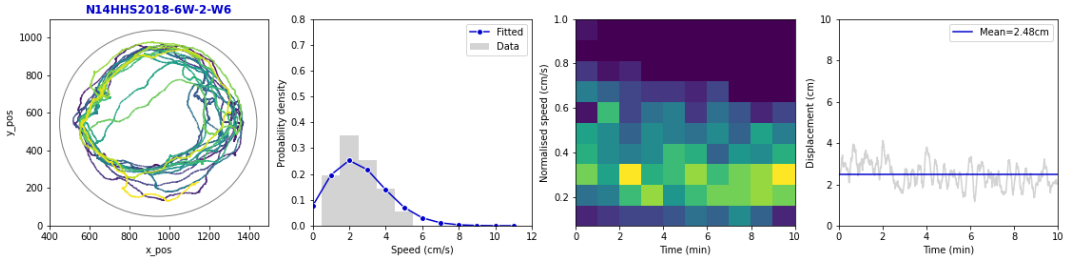

N14HHS2018-6W-2-W1
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=2.55cm
Displacement (cm)

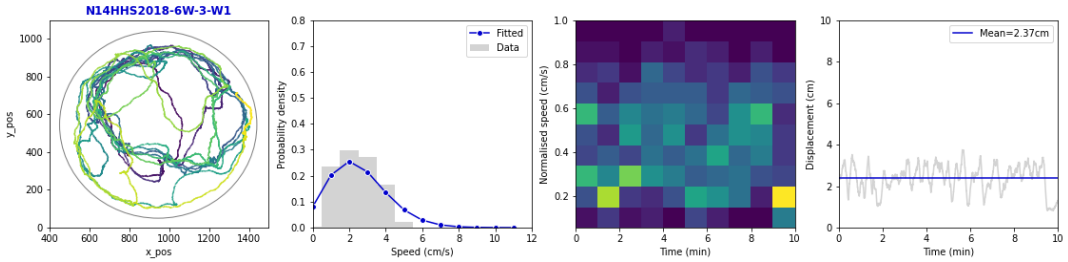

N14HHS2018-6W-2-W2
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=3.61cm
Displacement (cm)

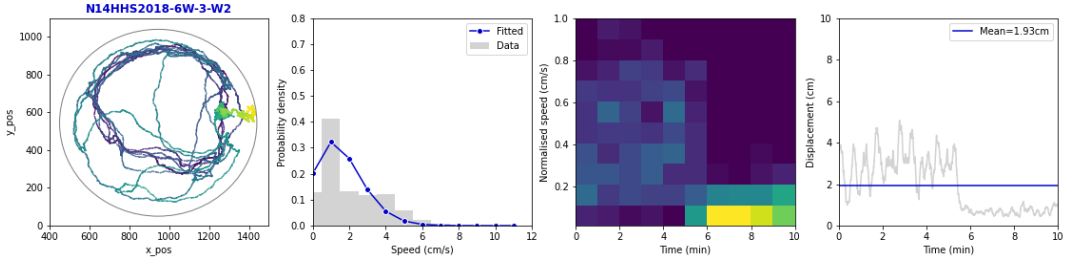

N14HHS2018-6W-2-W3
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=2.89cm
Displacement (cm)

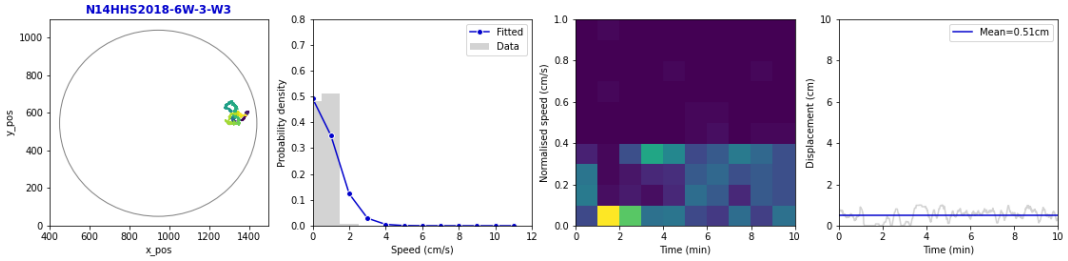

N14HHS2018-6W-2-W4
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=3.94cm
Displacement (cm)

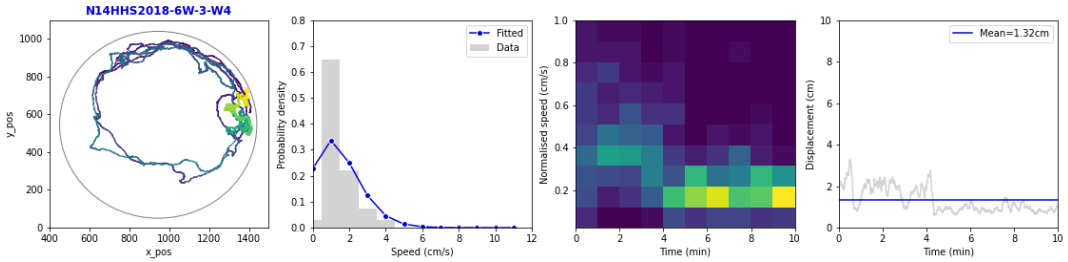

N14HHS2018-6W-2-W5
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=1.14cm
Displacement (cm)

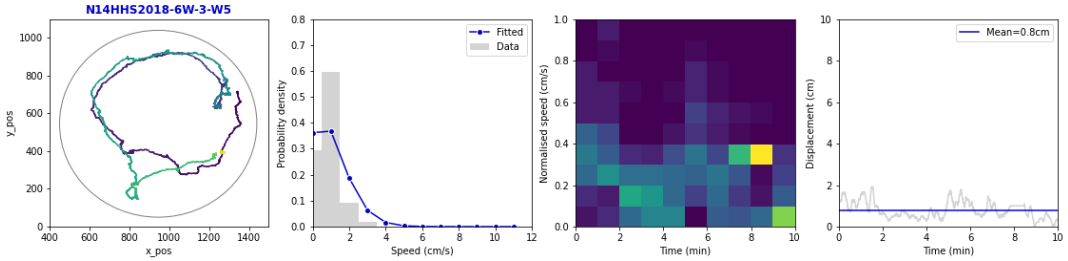

N14HHS2018-6W-2-W6
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=2.48cm
Displacement (cm)

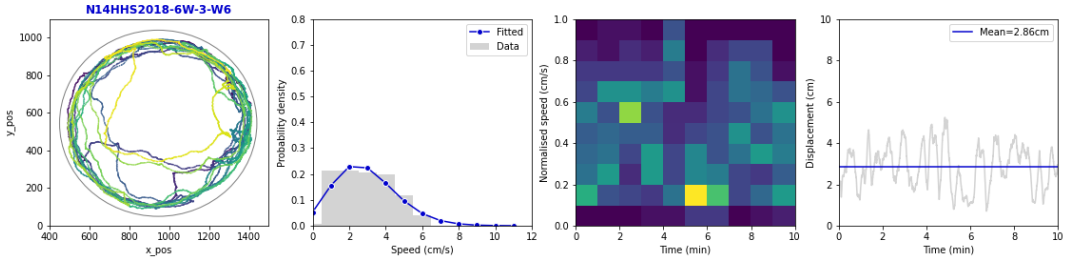

N14HHS2018-6W-3-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.37cm

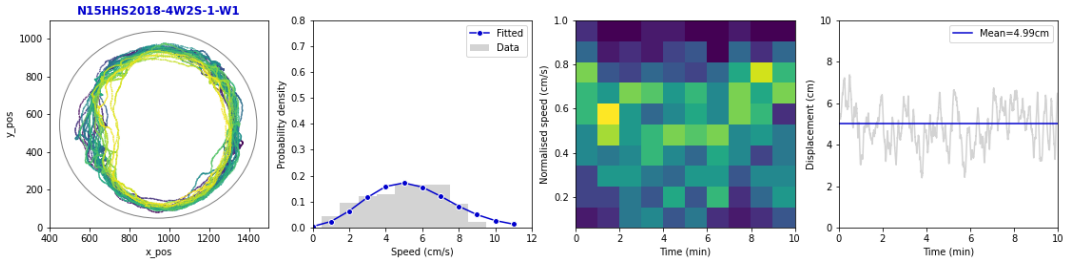

N14HHS2018-6W-3-W2
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.93cm

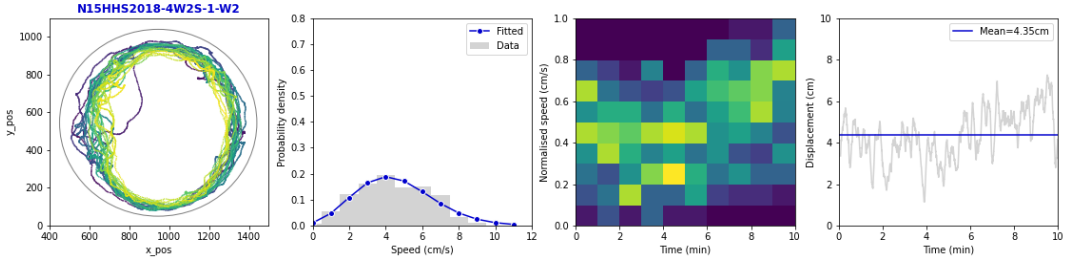

N14HHS2018-6W-3-W3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.51cm

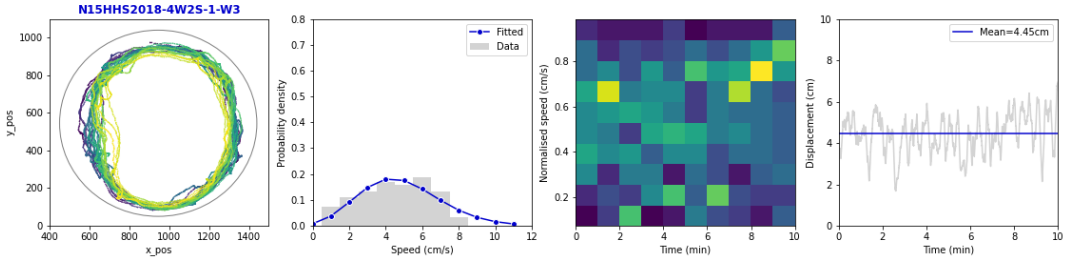

N14HHS2018-6W-3-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.32cm

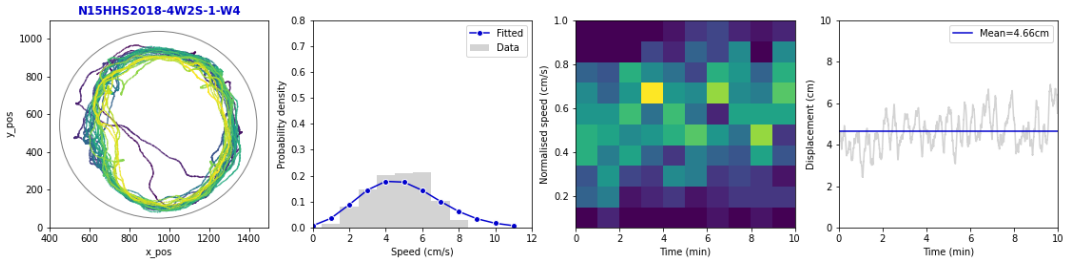

N14HHS2018-6W-3-W5
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.8cm

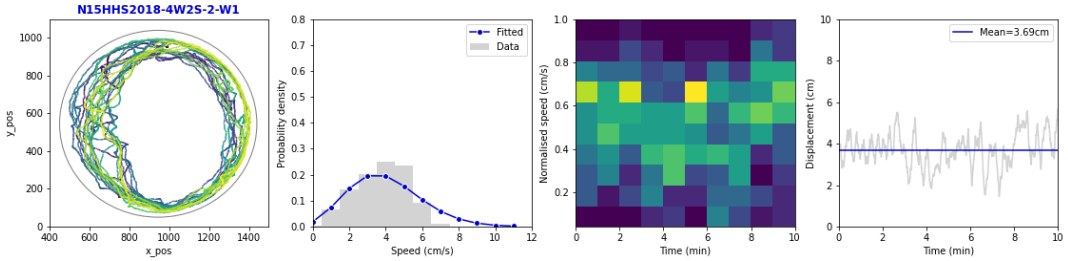

N14HHS2018-6W-3-W6
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.86cm

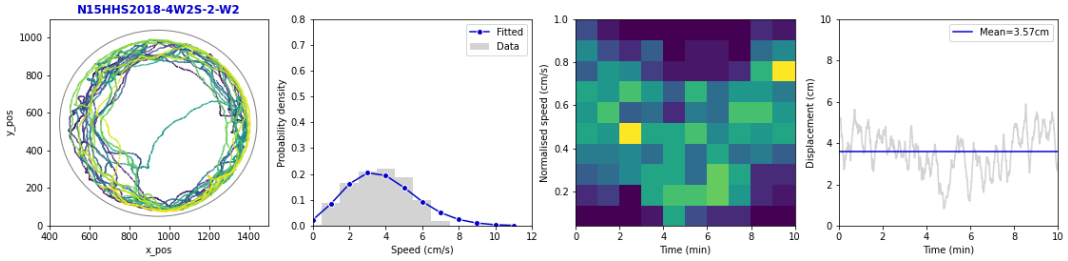

N15HHS2018-4W2S-1-W1
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=4.99cm
Displacement (cm)

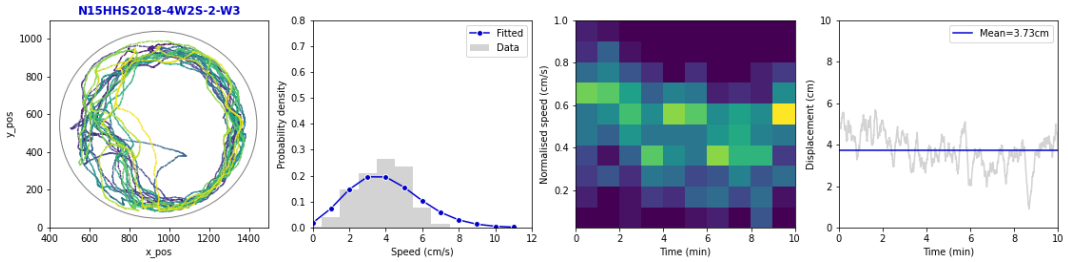

N15HHS2018-4W2S-1-W2
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=4.35cm
Displacement (cm)

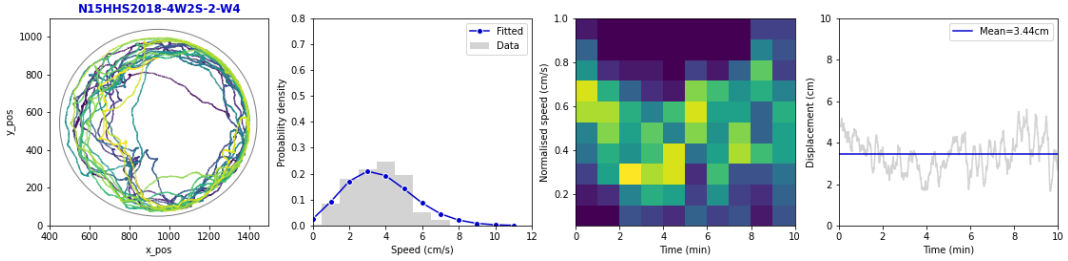

N15HHS2018-4W2S-1-W3
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=4.45cm
Displacement (cm)

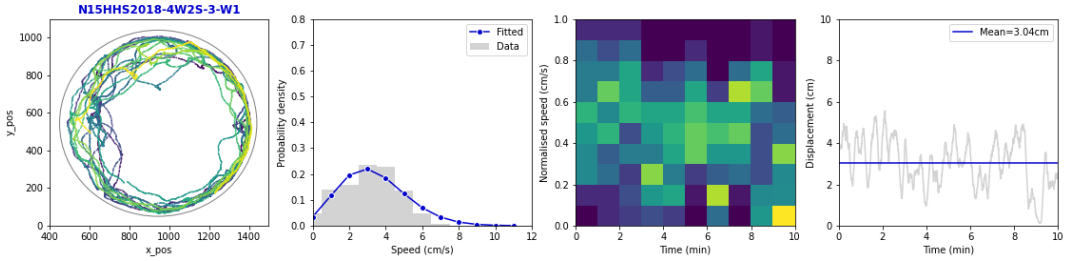

N15HHS2018-4W2S-1-W4
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=4.66cm
Displacement (cm)

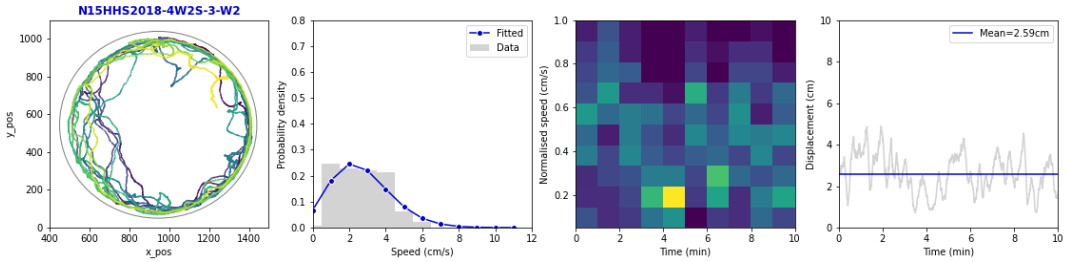

N15HHS2018-4W2S-2-W1
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=3.69cm
Displacement (cm)

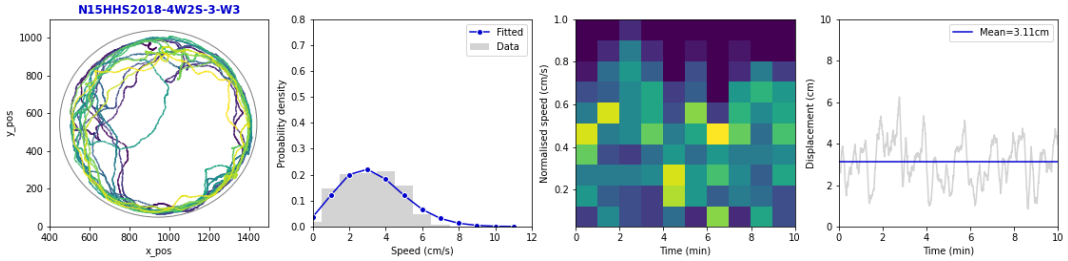

N15HHS2018-4W2S-2-W2
x_pos
y_pos
Fitted
Data
Speed (cm/s)
Probability density
Time (min)
Normalised speed (cm/s)
Mean=3.57cm
Displacement (cm)

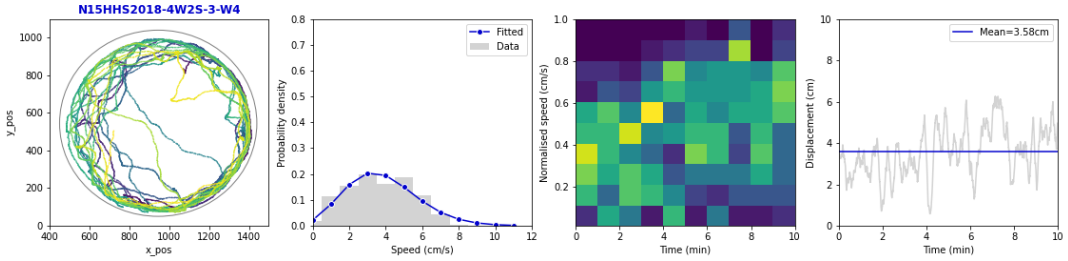

N15HHS2018-4W2S-2-W3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.73cm

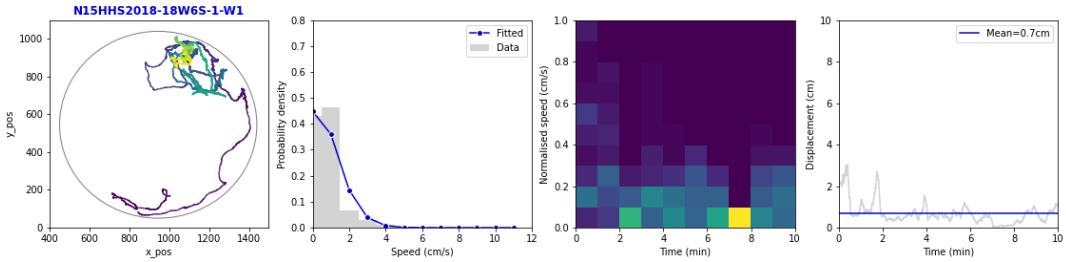

N15HHS2018-4W2S-2-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.44cm

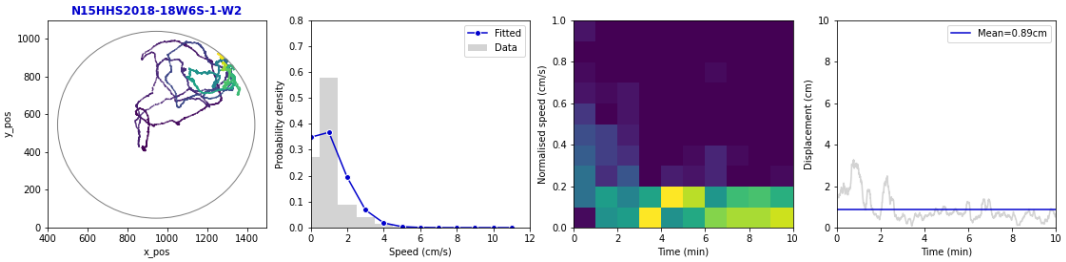

N15HHS2018-4W2S-3-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.04cm

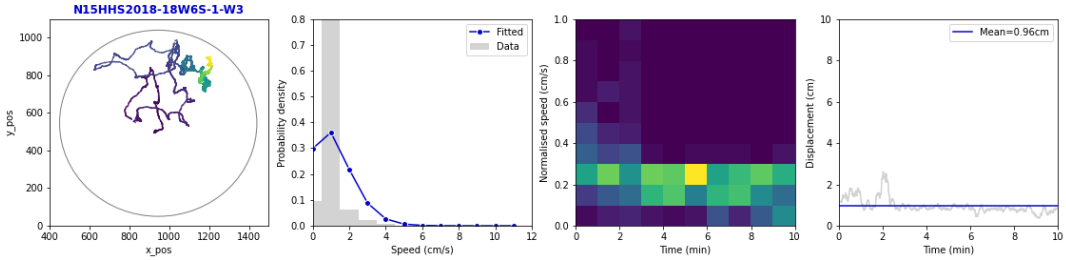

N15HHS2018-4W2S-3-W2
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.59cm

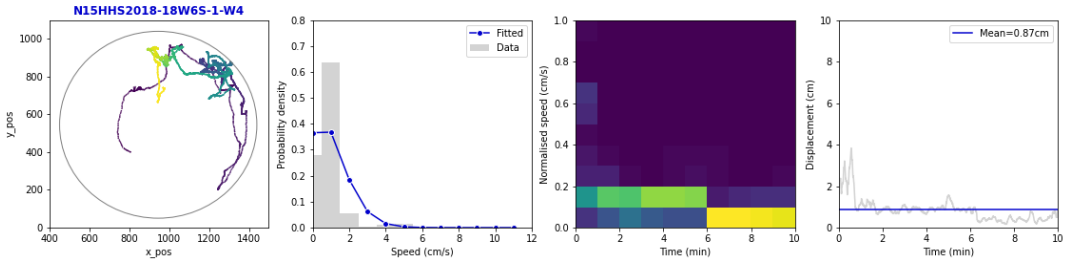

N15HHS2018-4W2S-3-W3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.11cm

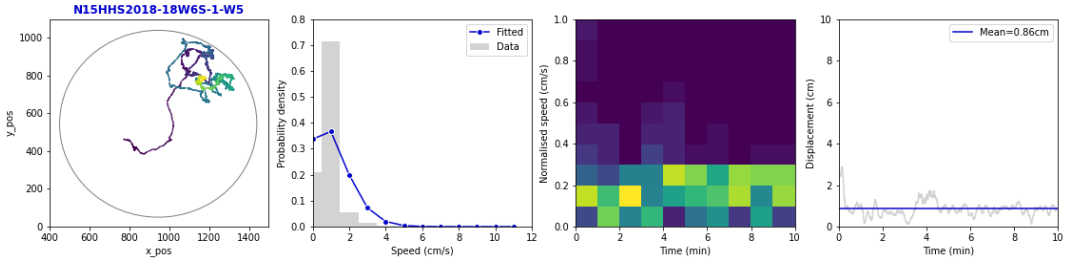

N15HHS2018-4W2S-3-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=3.58cm

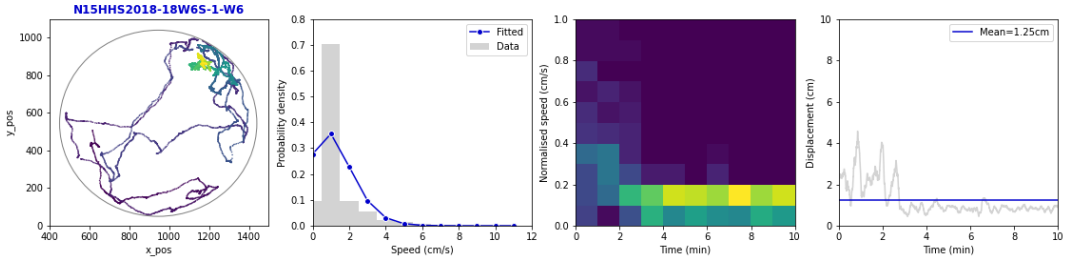

N15HHS2018-18W6S-1-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.7cm
Displacement (cm)
Time (min)

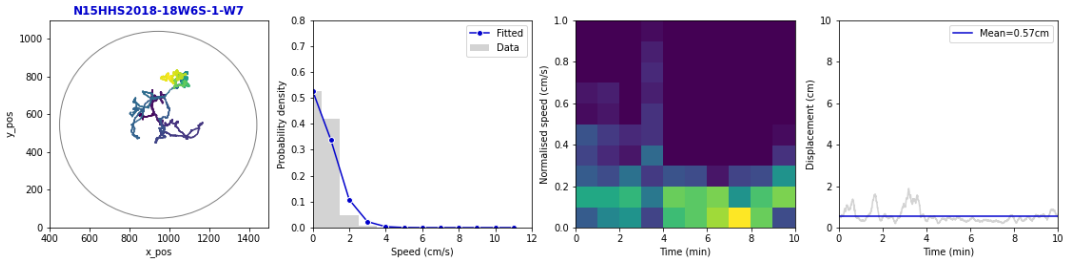

N15HHS2018-18W6S-1-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.89cm
Displacement (cm)
Time (min)

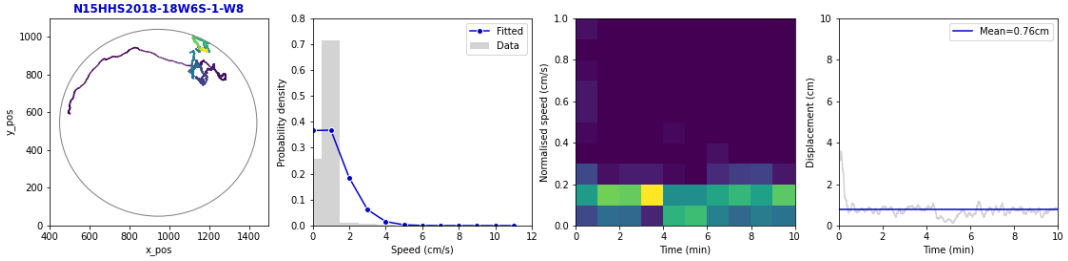

N15HHS2018-18W6S-1-W3
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.96cm
Displacement (cm)
Time (min)

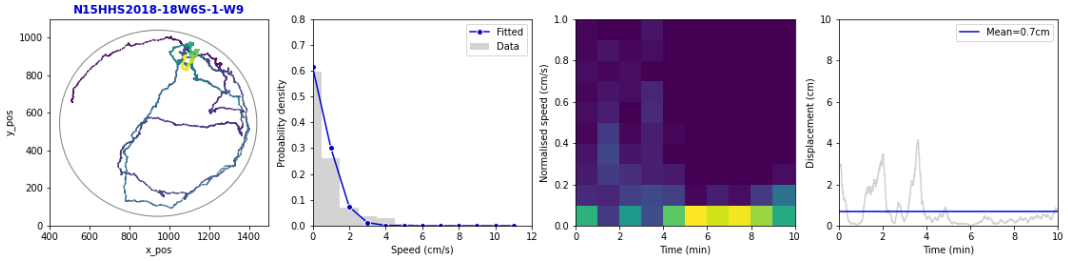

N15HHS2018-18W6S-1-W4
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.87cm
Displacement (cm)
Time (min)

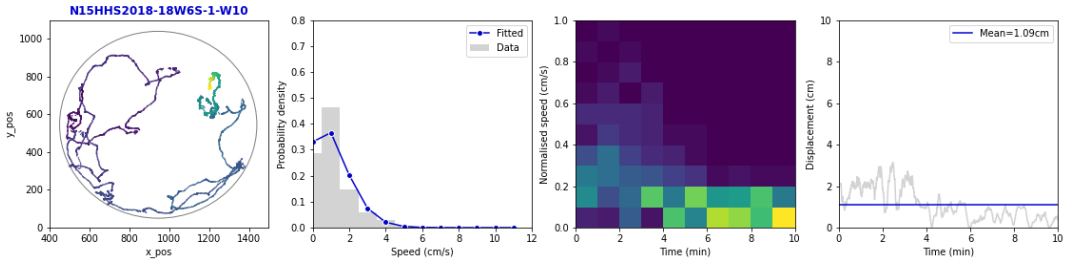

N15HHS2018-18W6S-1-W5
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.86cm
Displacement (cm)
Time (min)

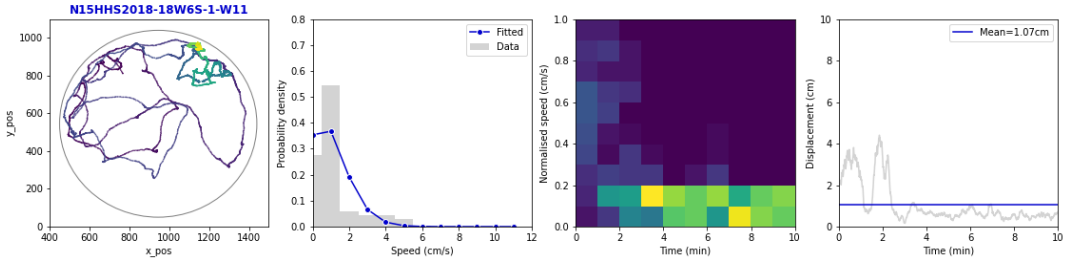

N15HHS2018-18W6S-1-W6
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.25cm
Displacement (cm)
Time (min)

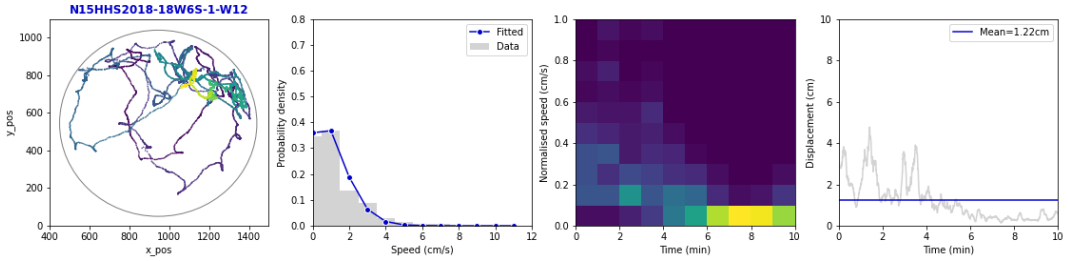

N15HHS2018-18W6S-1-W7
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.57cm

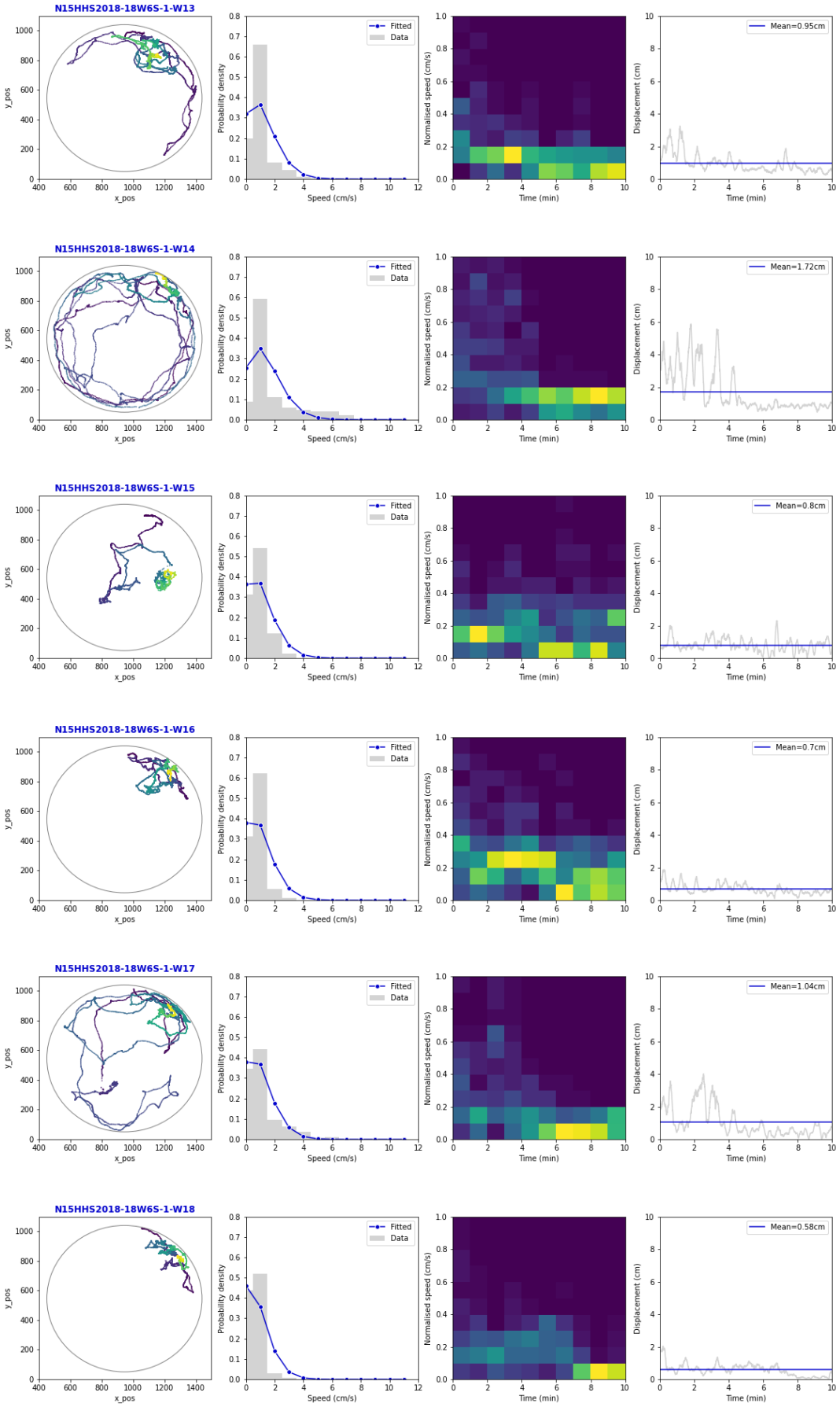

N15HHS2018-18W6S-1-W8
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.76cm

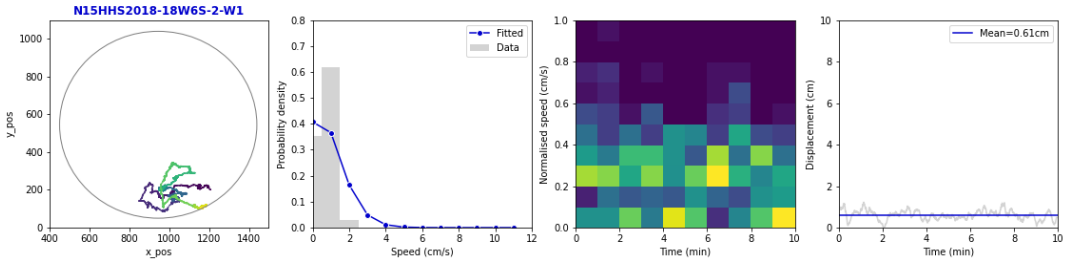

N15HHS2018-18W6S-1-W9
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.7cm

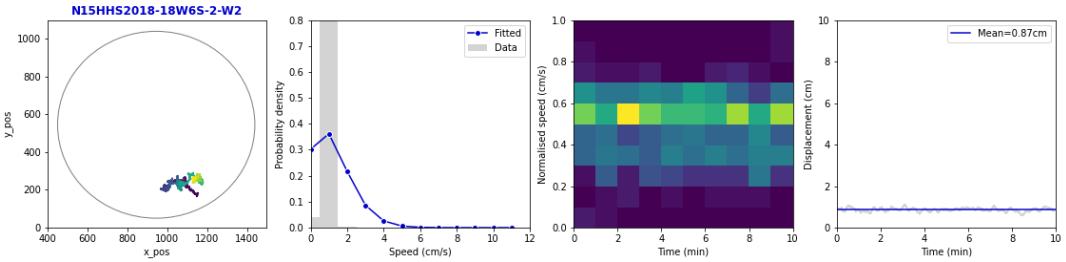

N15HHS2018-18W6S-1-W10
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.09cm

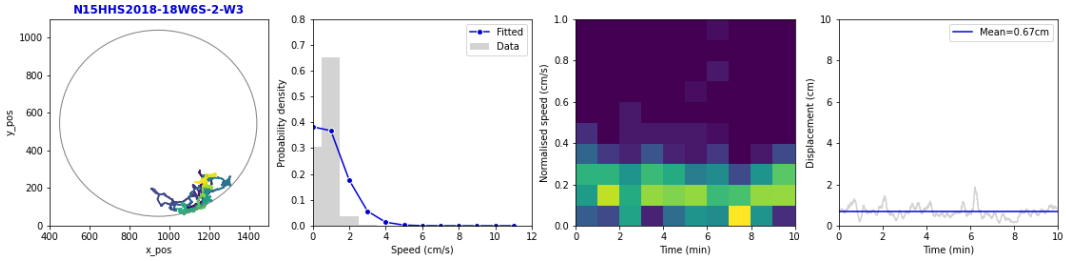

N15HHS2018-18W6S-1-W11
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.07cm

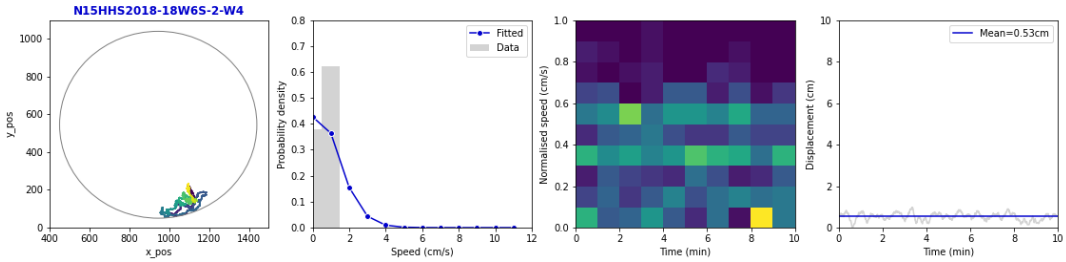

N15HHS2018-18W6S-1-W12
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.22cm

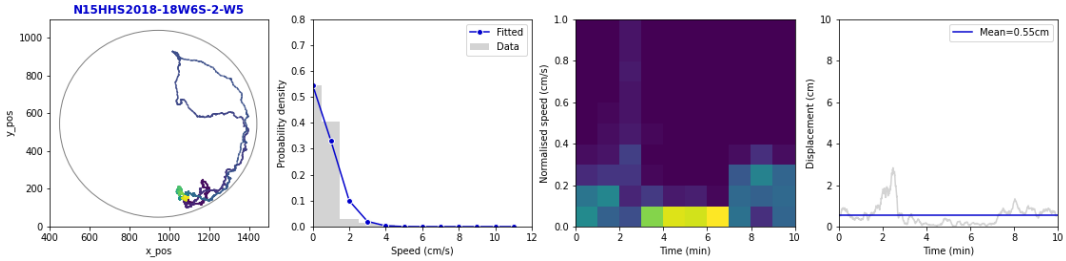

N15HHS2018-18W6S-1-W13
N15HHS2018-18W6S-1-W14
N15HHS2018-18W6S-1-W15
N15HHS2018-18W6S-1-W16
N15HHS2018-18W6S-1-W17
N15HHS2018-18W6S-1-W18
x_pos
y_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.95cm
Mean=1.72cm
Mean=0.8cm
Mean=0.7cm
Mean=1.04cm
Mean=0.58cm

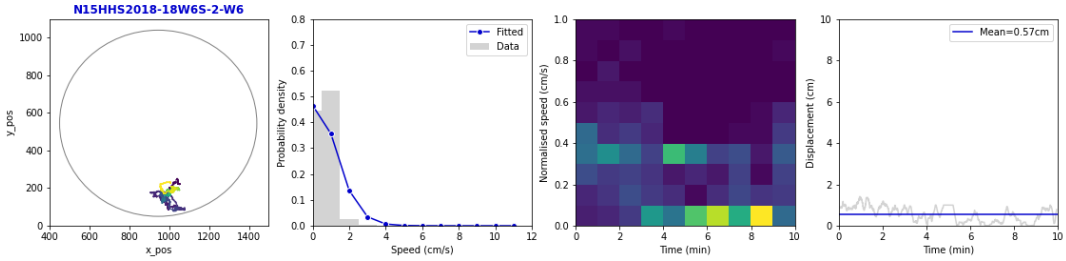

N15HHS2018-18W6S-2-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.61cm

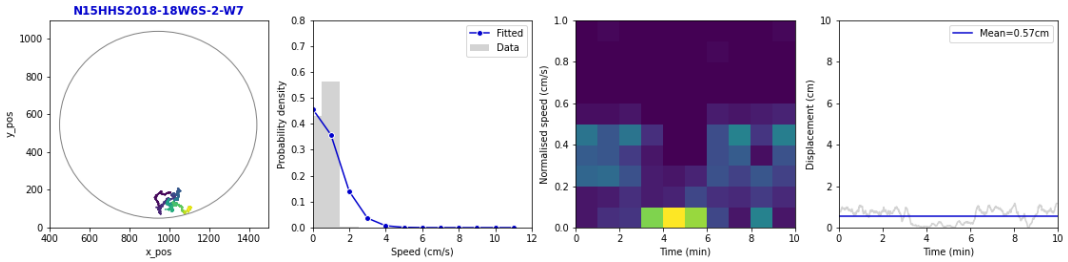

N15HHS2018-18W6S-2-W2
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.87cm

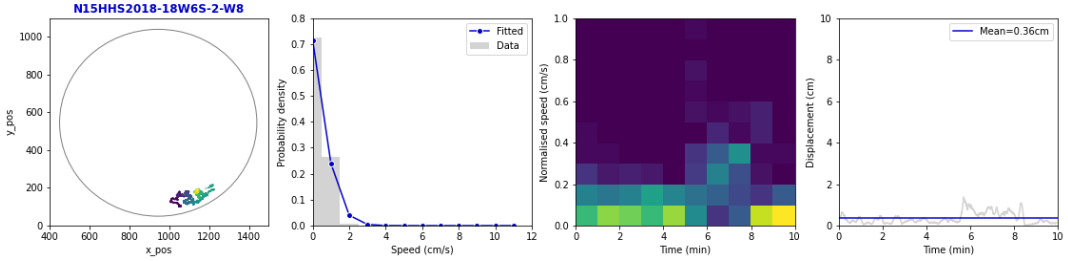

N15HHS2018-18W6S-2-W3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.67cm

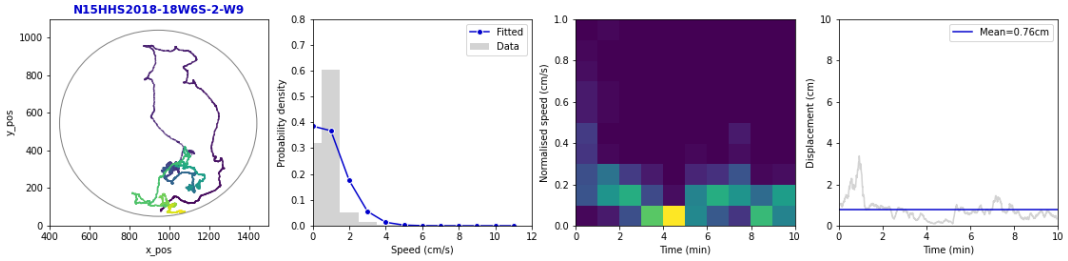

N15HHS2018-18W6S-2-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.53cm

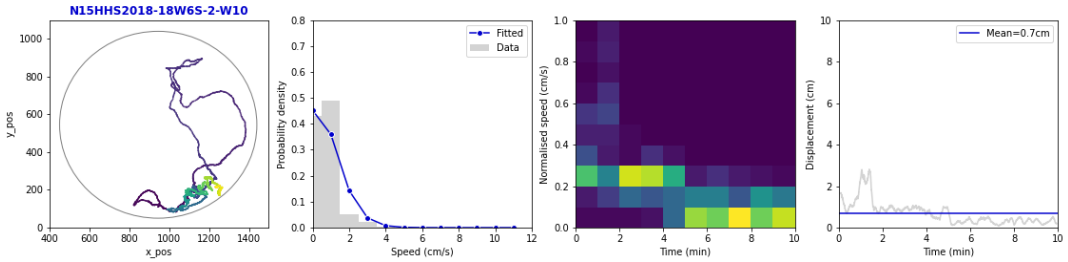

N15HHS2018-18W6S-2-W5
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.55cm

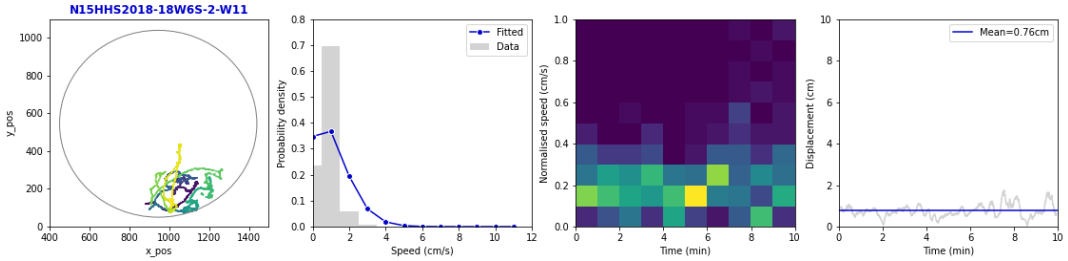

N15HHS2018-18W6S-2-W6
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.57cm

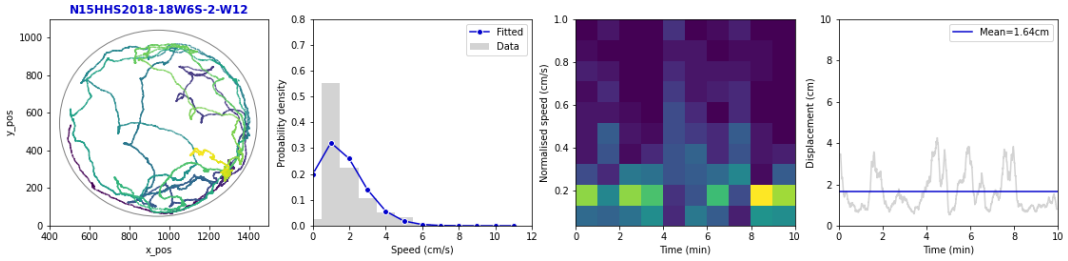

N15HHS2018-18W6S-2-W7
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.57cm

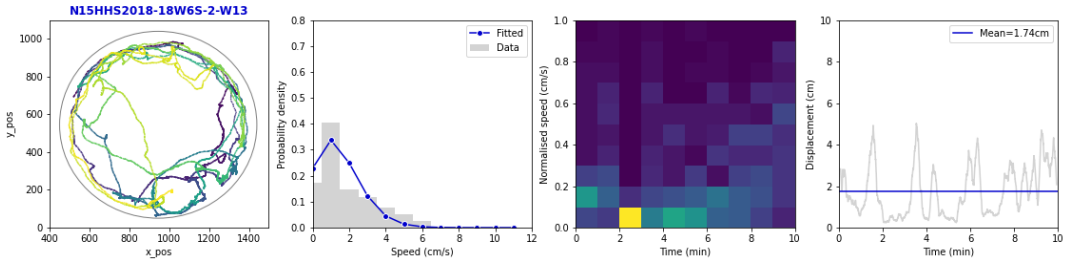

N15HHS2018-18W6S-2-W8
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.36cm

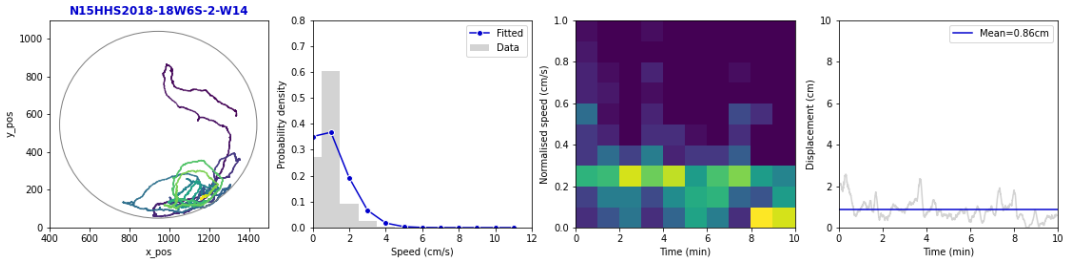

N15HHS2018-18W6S-2-W9
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.76cm

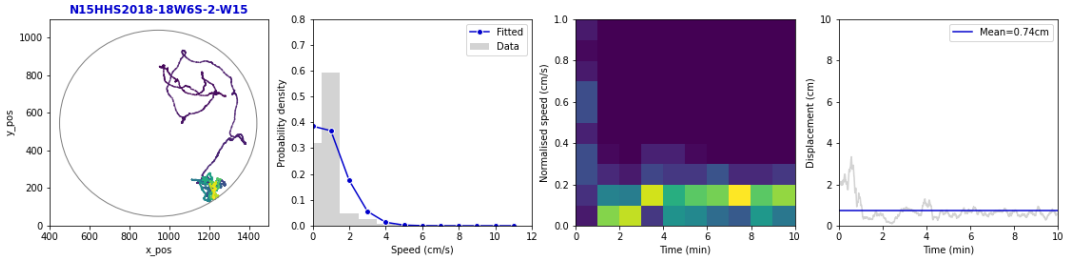

N15HHS2018-18W6S-2-W10
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.7cm

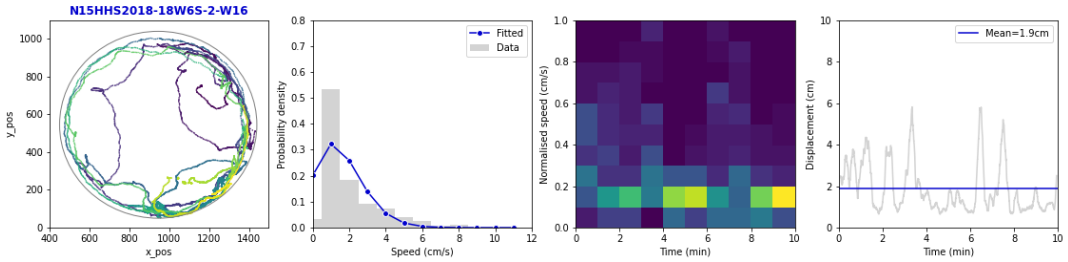

N15HHS2018-18W6S-2-W11
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.76cm

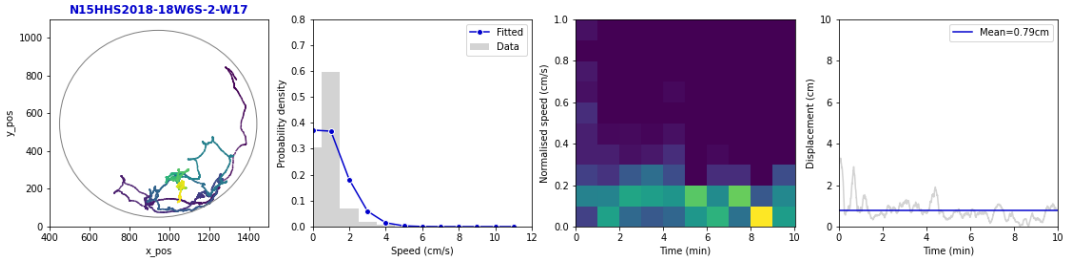

N15HHS2018-18W6S-2-W12
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.64cm

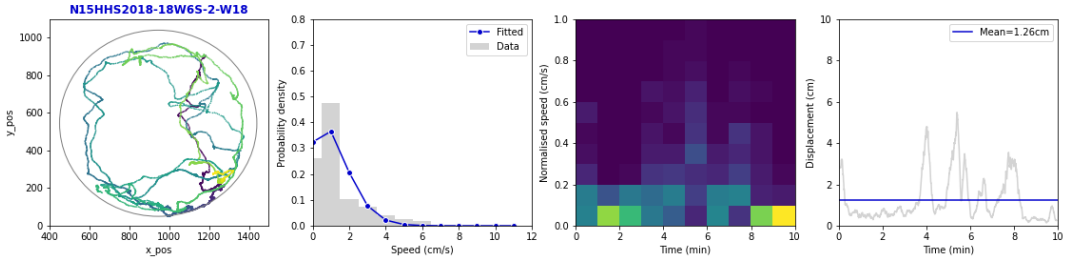

N15HHS2018-18W6S-2-W13
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.74cm

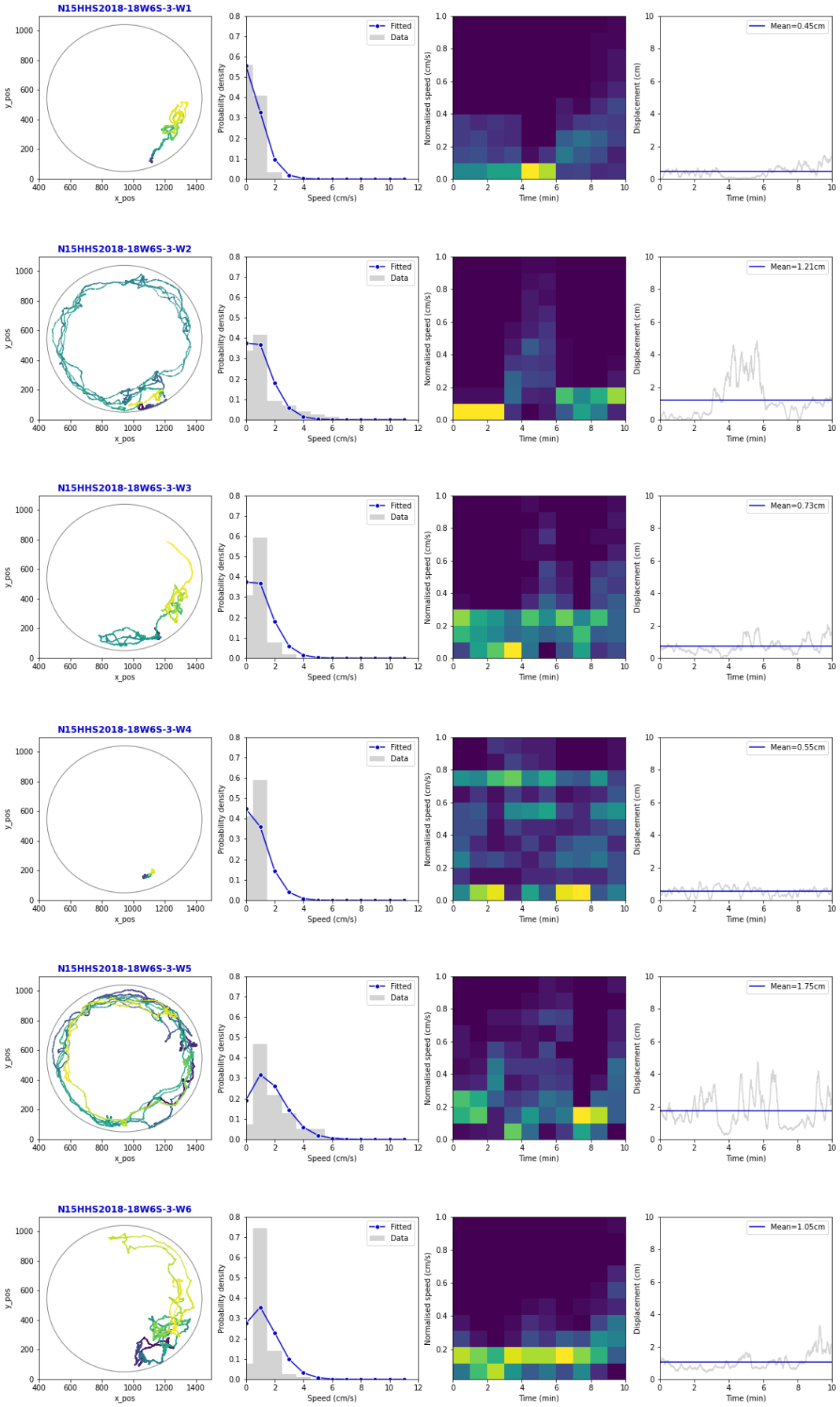

N15HHS2018-18W6S-2-W14
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.86cm

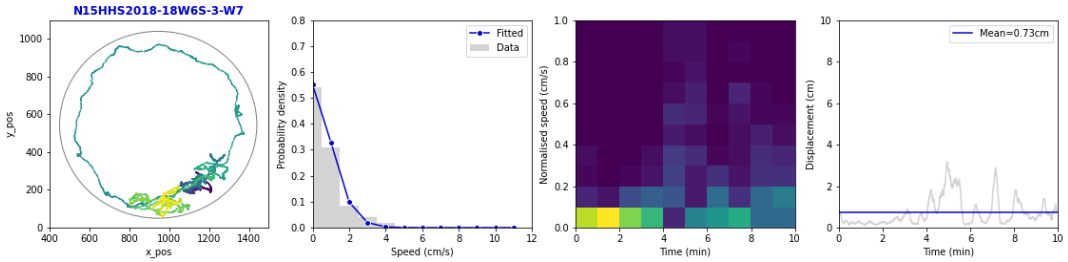

N15HHS2018-18W6S-2-W15
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.74cm

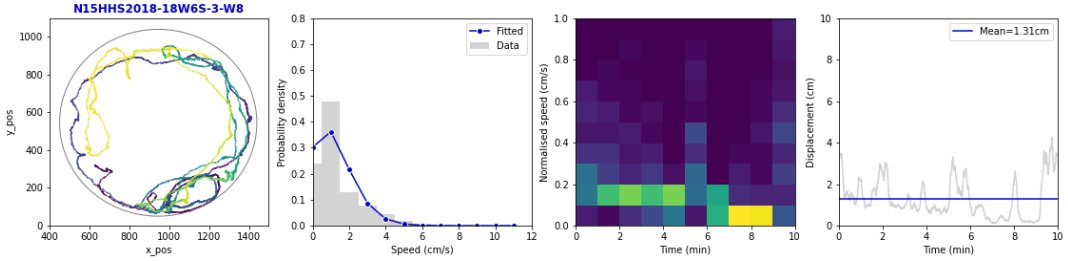

N15HHS2018-18W6S-2-W16
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.9cm

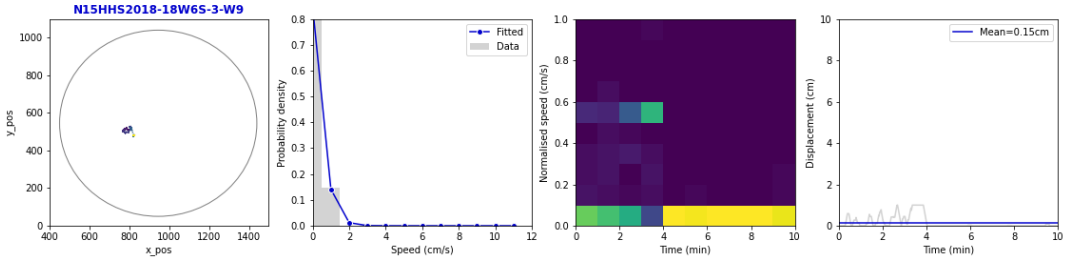

N15HHS2018-18W6S-2-W17
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=0.79cm

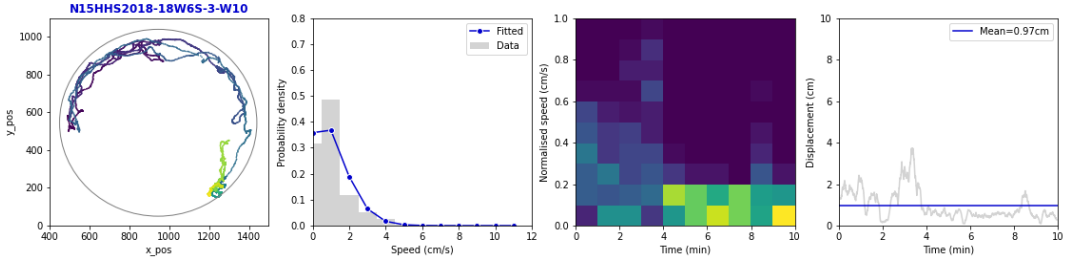

N15HHS2018-18W6S-2-W18
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.26cm

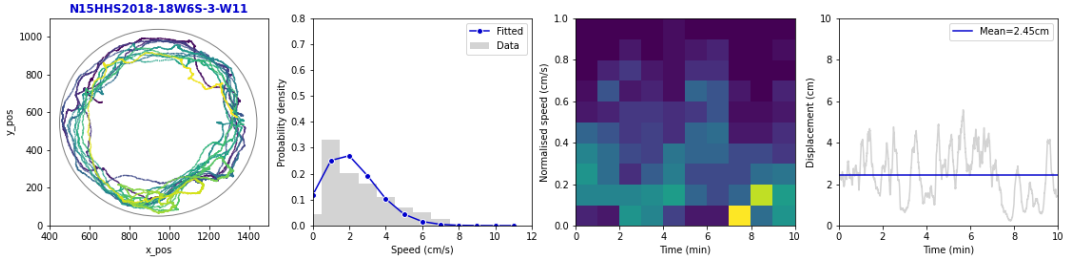

N15HHS2018-18W6S-3-W1
N15HHS2018-18W6S-3-W2
N15HHS2018-18W6S-3-W3
N15HHS2018-18W6S-3-W4
N15HHS2018-18W6S-3-W5
N15HHS2018-18W6S-3-W6
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.45cm
Mean=1.21cm
Mean=0.73cm
Mean=0.55cm
Mean=1.75cm
Mean=1.05cm

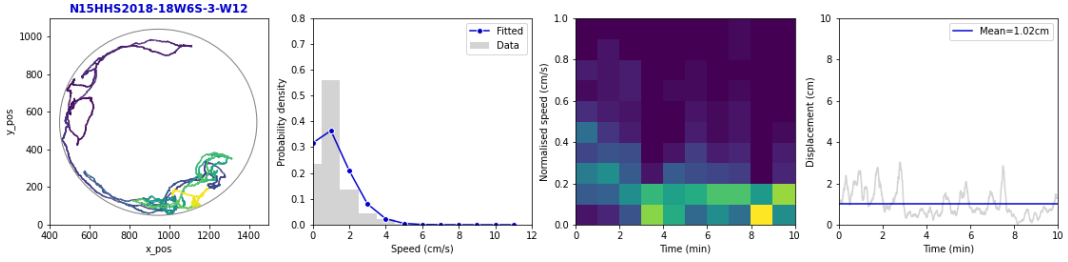

N15HHS2018-18W6S-3-W7
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.73cm
Displacement (cm)
Time (min)

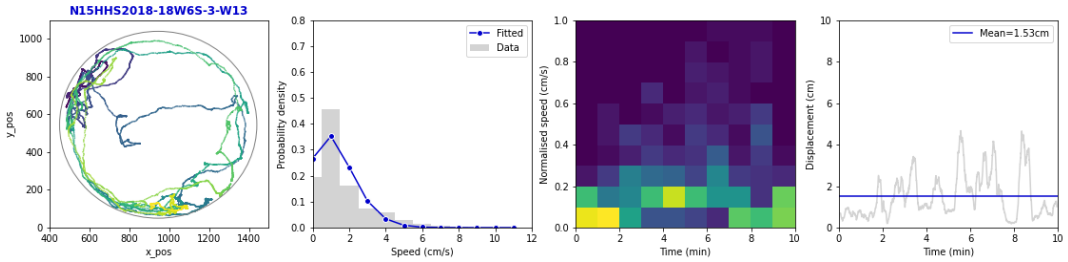

N15HHS2018-18W6S-3-W8
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.31cm
Displacement (cm)
Time (min)

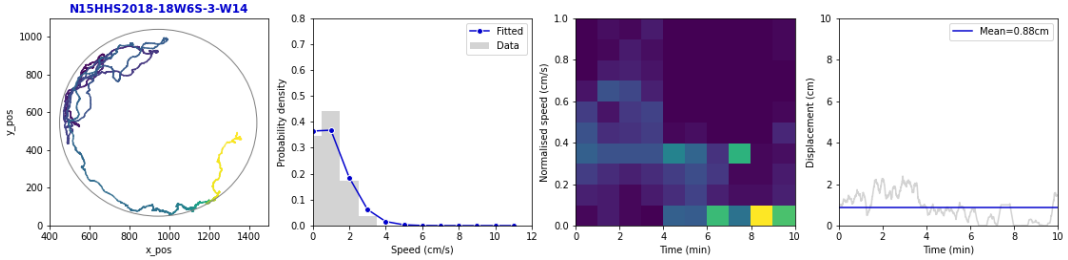

N15HHS2018-18W6S-3-W9
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.15cm
Displacement (cm)
Time (min)

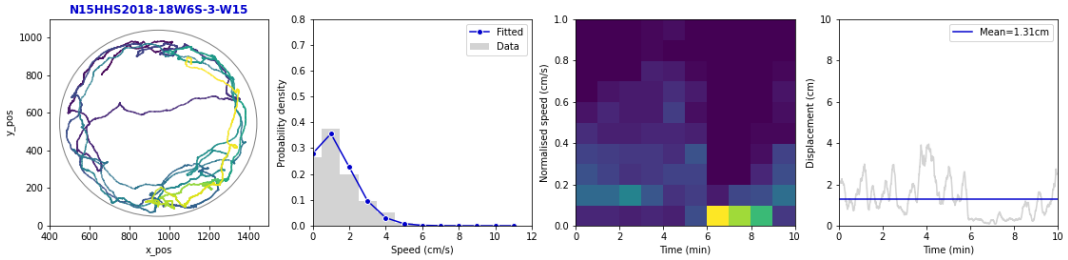

N15HHS2018-18W6S-3-W10
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.97cm
Displacement (cm)
Time (min)

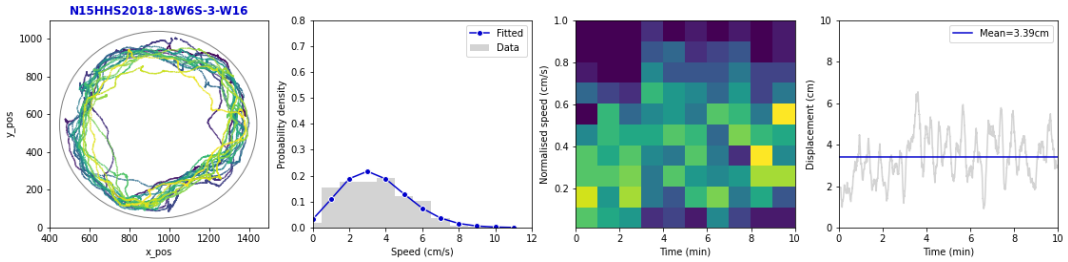

N15HHS2018-18W6S-3-W11
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=2.45cm
Displacement (cm)
Time (min)

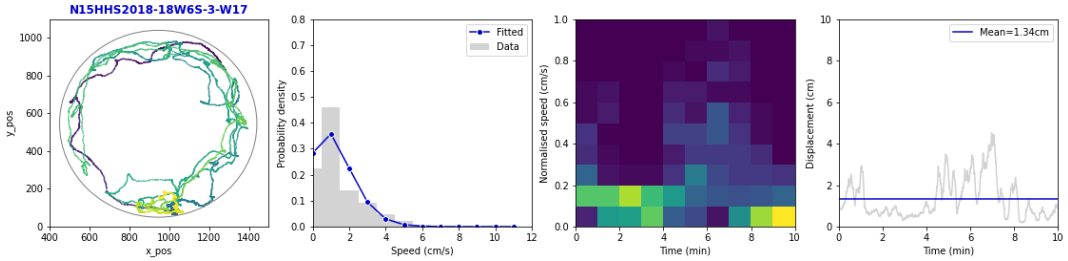

N15HHS2018-18W6S-3-W12
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.02cm
Displacement (cm)
Time (min)

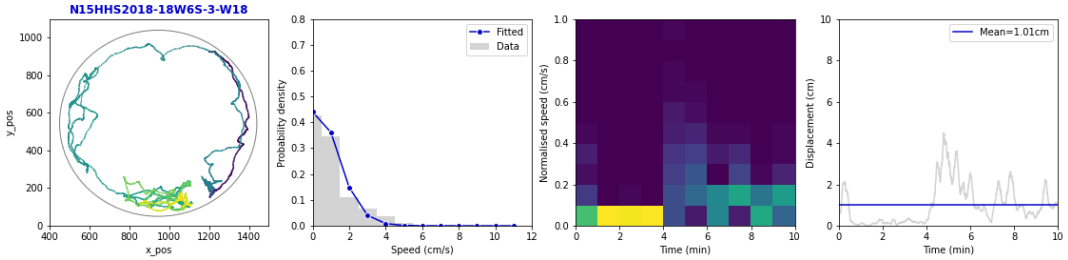

N15HHS2018-18W6S-3-W13
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.53cm
Displacement (cm)
Time (min)

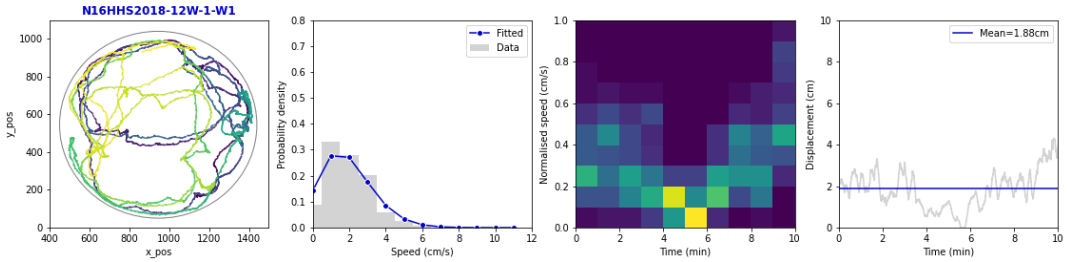

N15HHS2018-18W6S-3-W14
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=0.88cm
Displacement (cm)
Time (min)

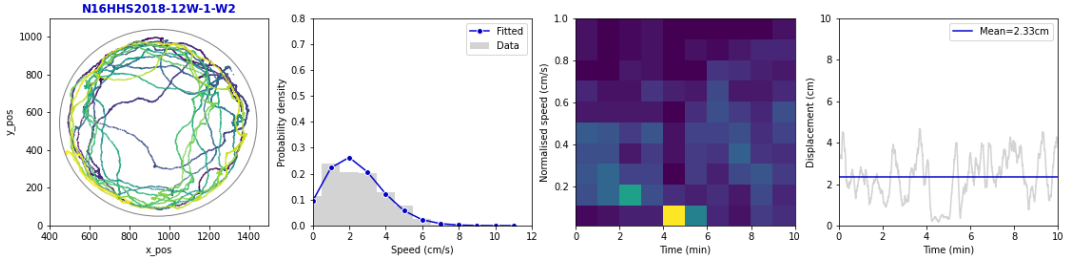

N15HHS2018-18W6S-3-W15
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.31cm
Displacement (cm)
Time (min)

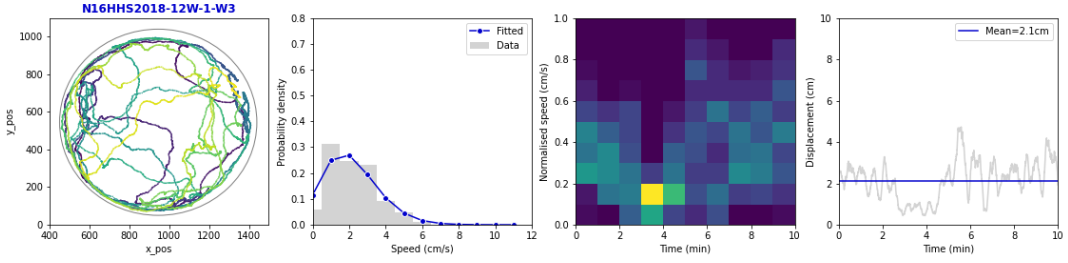

N15HHS2018-18W6S-3-W16
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=3.39cm
Displacement (cm)
Time (min)

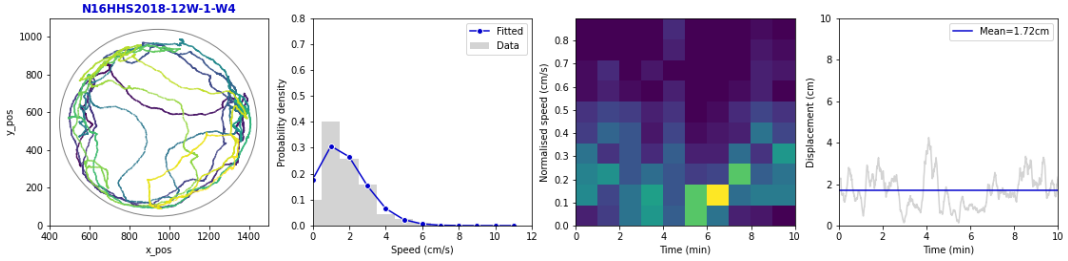

N15HHS2018-18W6S-3-W17
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.34cm
Displacement (cm)
Time (min)

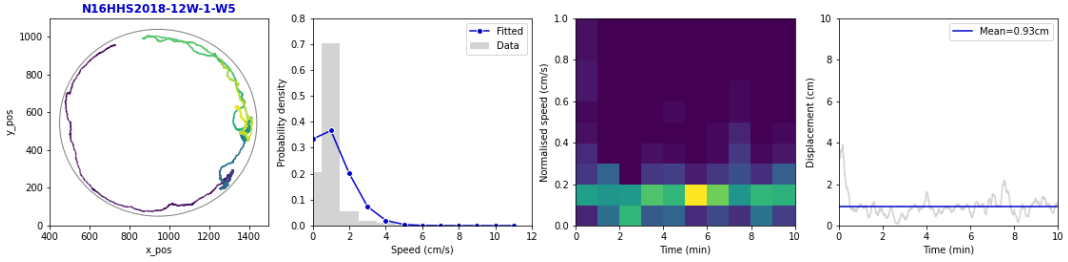

N15HHS2018-18W6S-3-W18
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.01cm
Displacement (cm)
Time (min)

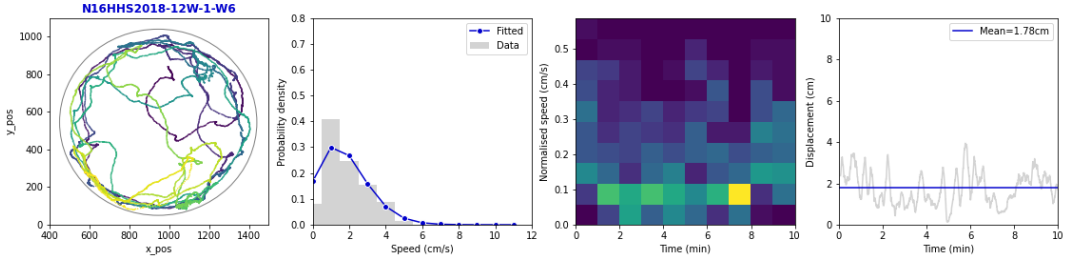

N16HHS2018-12W-1-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.88cm

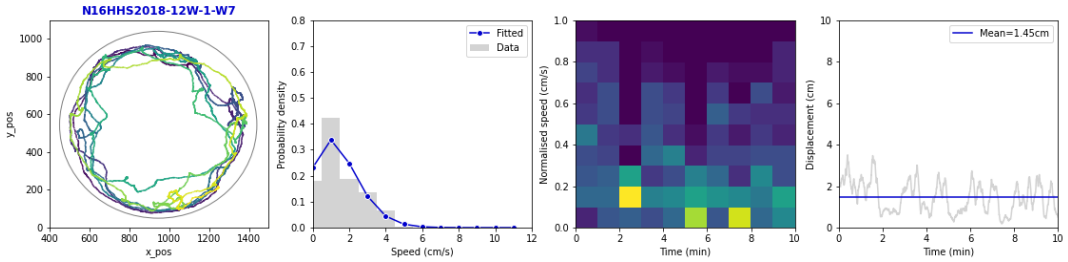

N16HHS2018-12W-1-W2
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.33cm

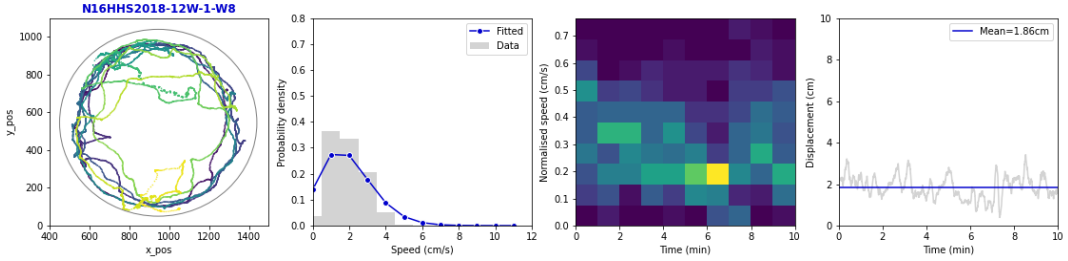

N16HHS2018-12W-1-W3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=2.1cm

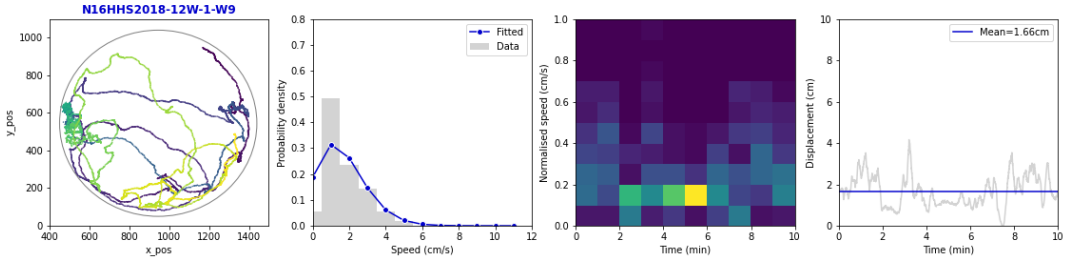

N16HHS2018-12W-1-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.72cm

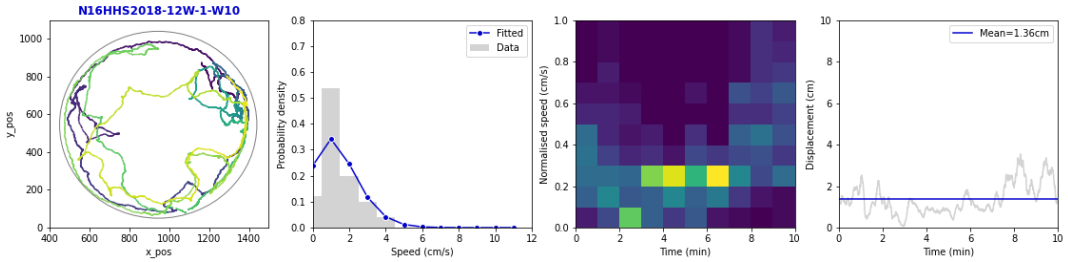

N16HHS2018-12W-1-W5
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=0.93cm

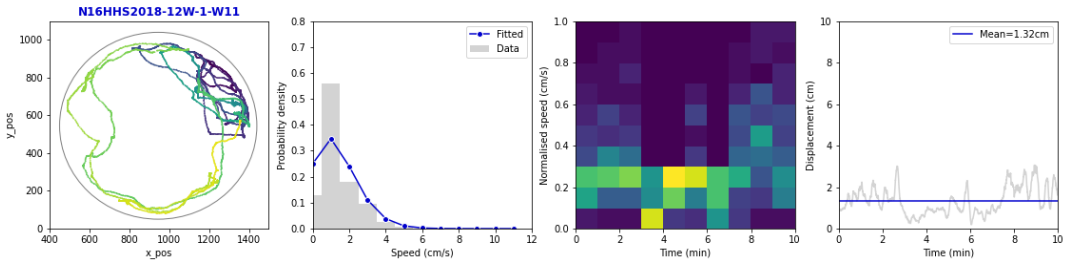

N16HHS2018-12W-1-W6
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.78cm

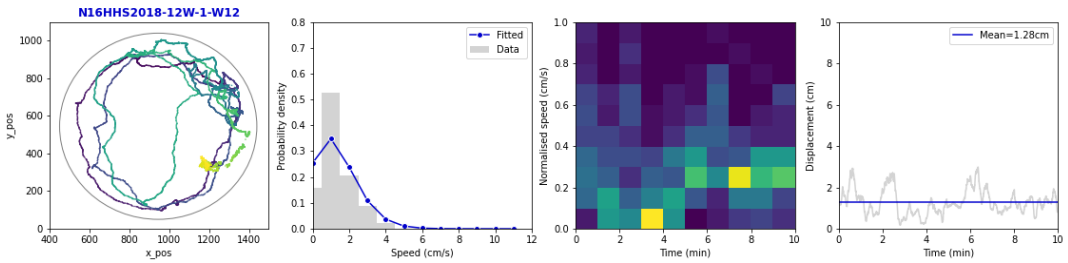

N16HHS2018-12W-1-W7
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.45cm

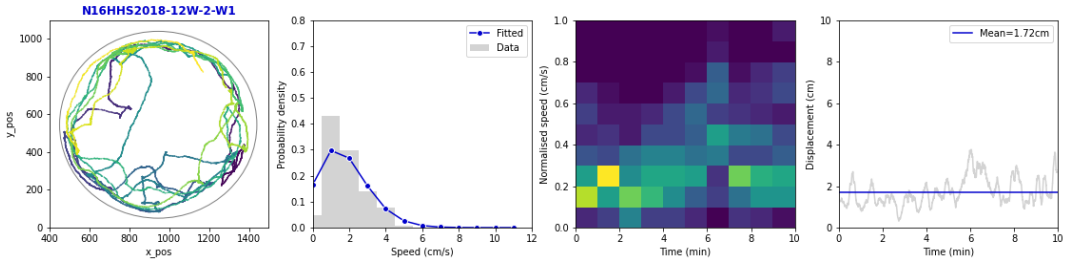

N16HHS2018-12W-1-W8
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.86cm

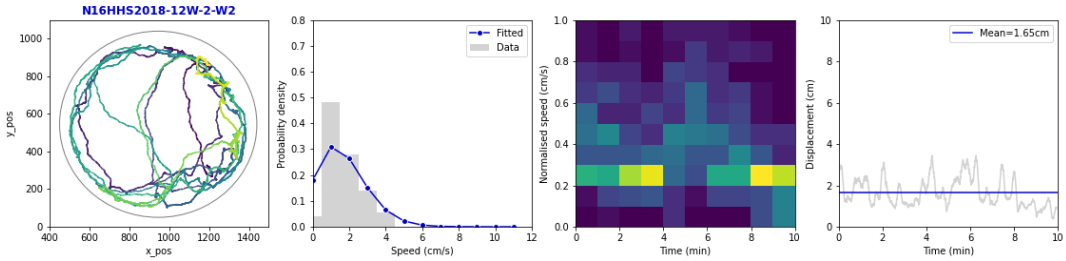

N16HHS2018-12W-1-W9
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.66cm

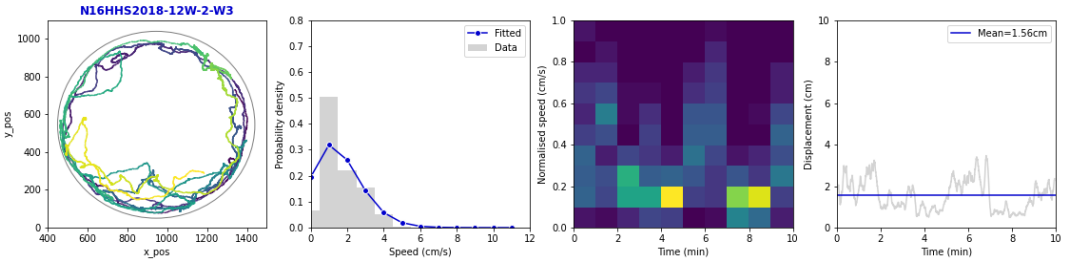

N16HHS2018-12W-1-W10
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.36cm

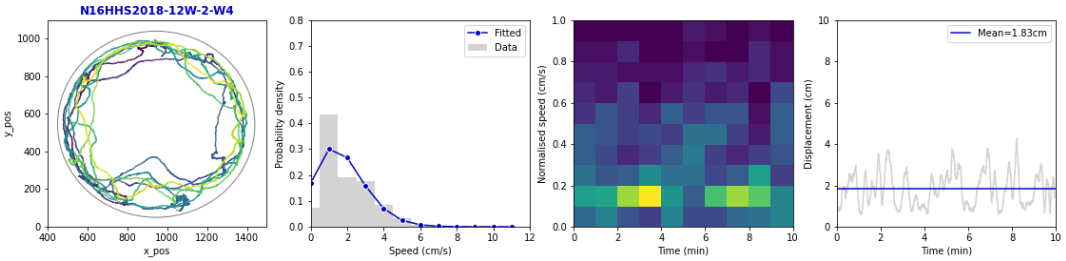

N16HHS2018-12W-1-W11
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.32cm

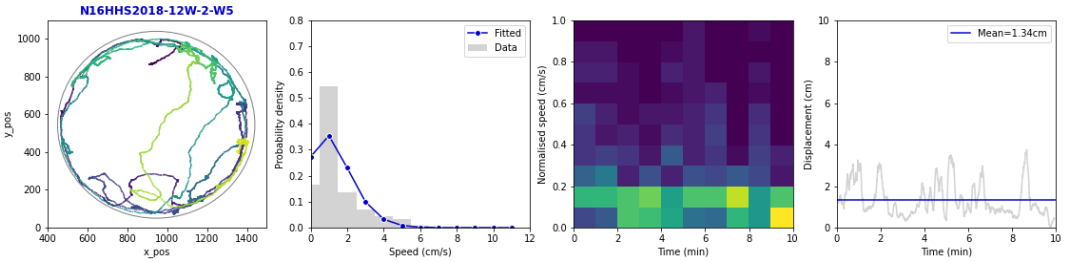

N16HHS2018-12W-1-W12
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.28cm

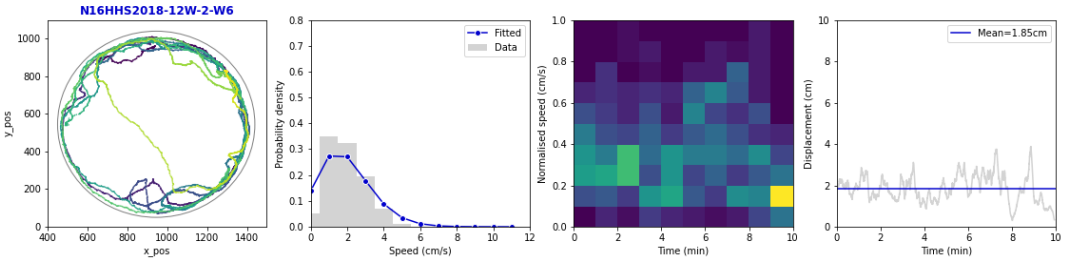

N16HHS2018-12W-2-W1
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.72cm
Displacement (cm)

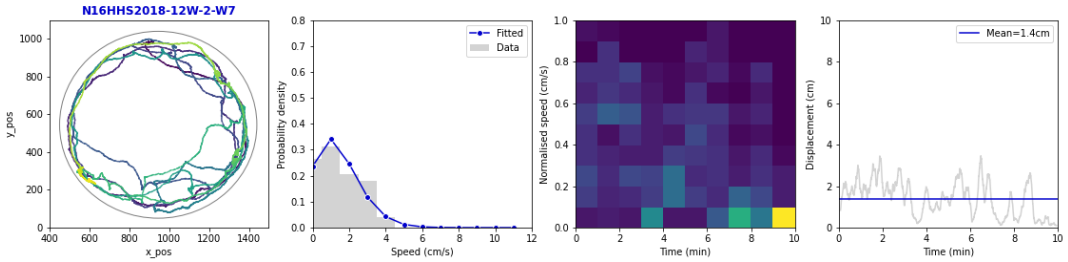

N16HHS2018-12W-2-W2
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.65cm
Displacement (cm)

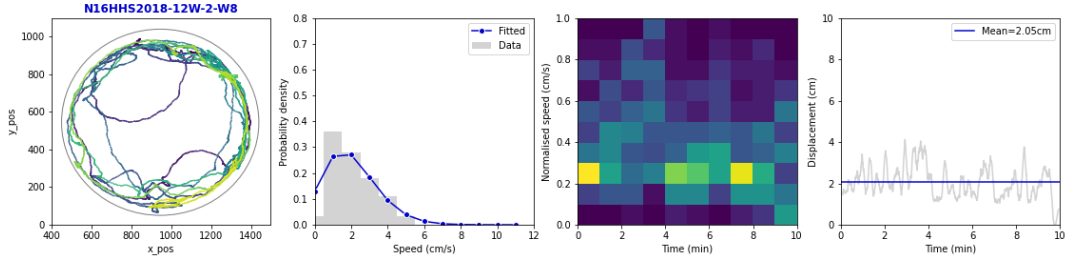

N16HHS2018-12W-2-W3
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.56cm
Displacement (cm)

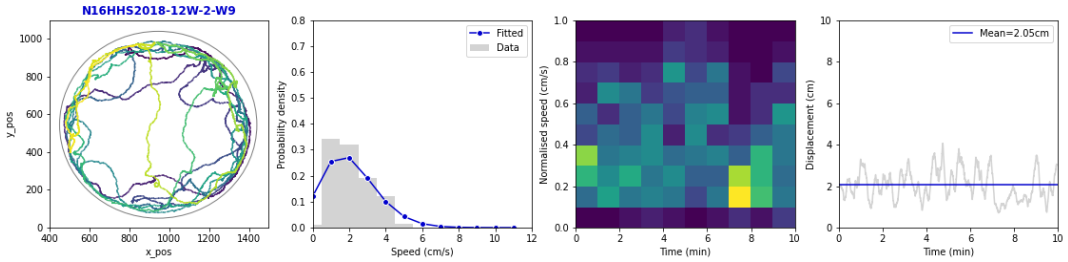

N16HHS2018-12W-2-W4
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.83cm
Displacement (cm)

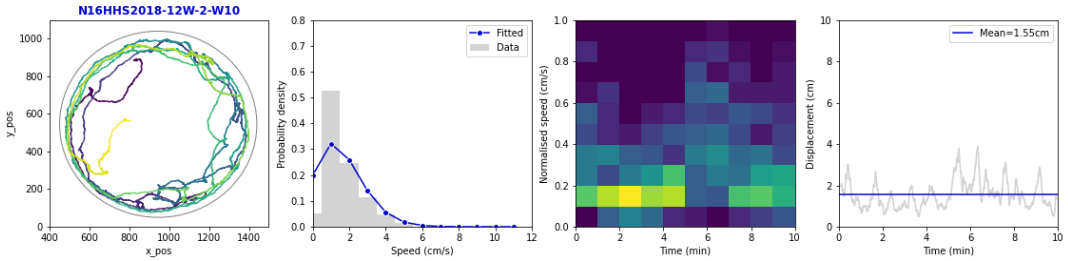

N16HHS2018-12W-2-W5
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.34cm
Displacement (cm)

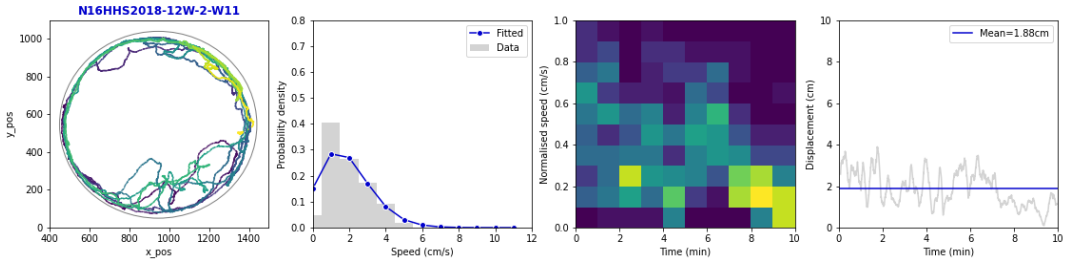

N16HHS2018-12W-2-W6
x_pos
y_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.85cm
Displacement (cm)

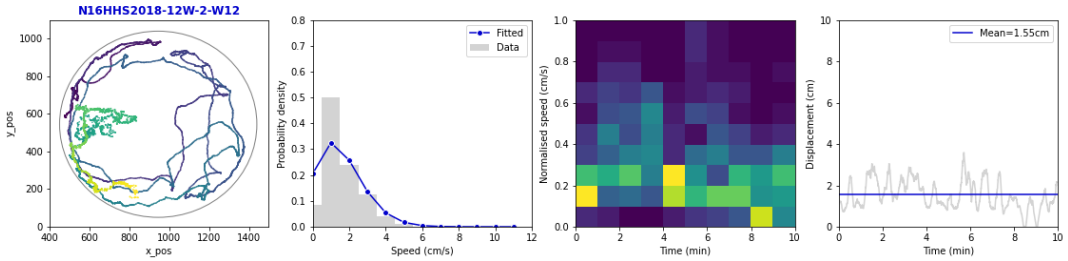

N16HHS2018-12W-2-W7
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.4cm

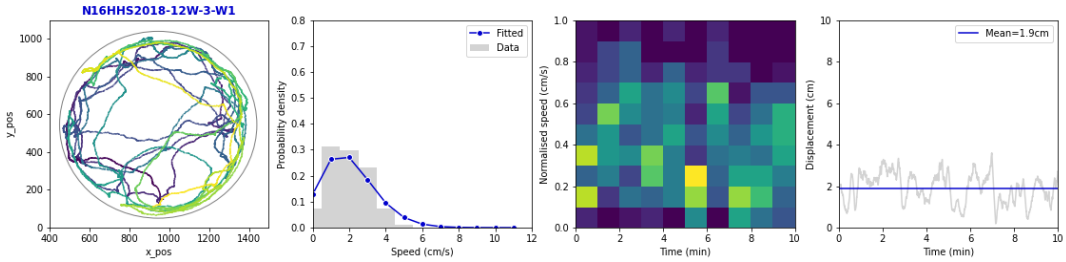

N16HHS2018-12W-2-W8
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.05cm

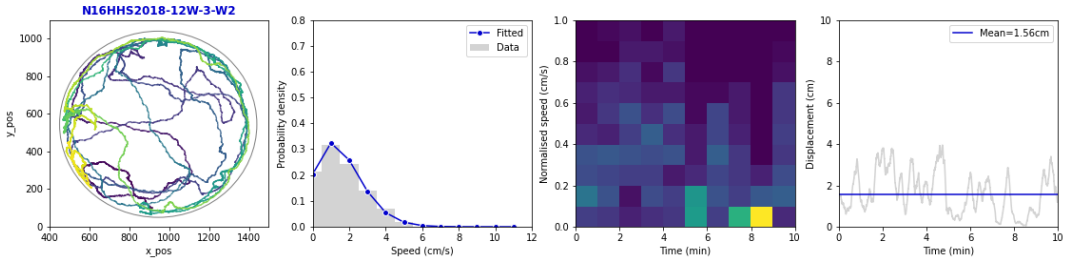

N16HHS2018-12W-2-W9
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=2.05cm

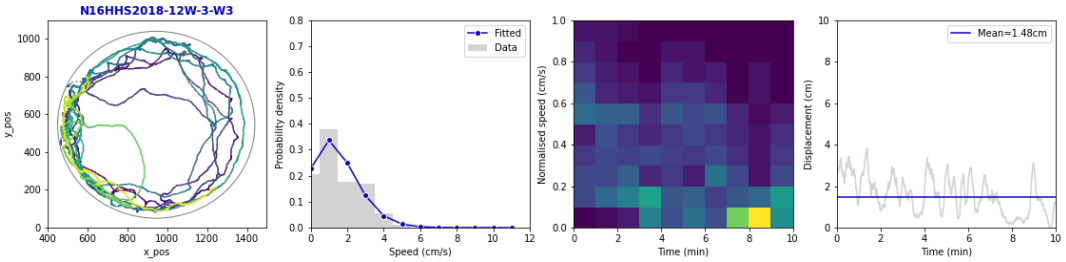

N16HHS2018-12W-2-W10
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.55cm

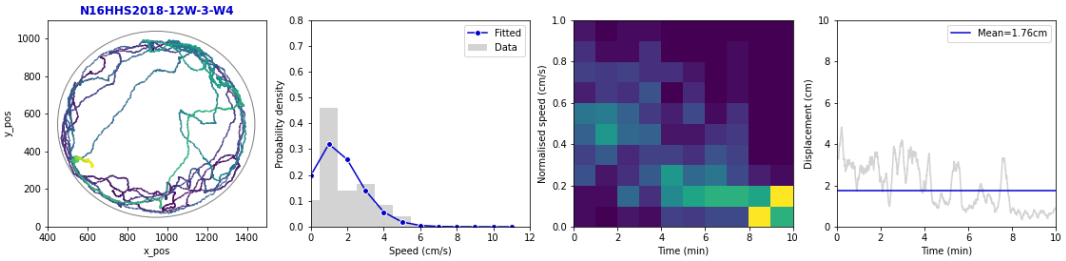

N16HHS2018-12W-2-W11
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.88cm

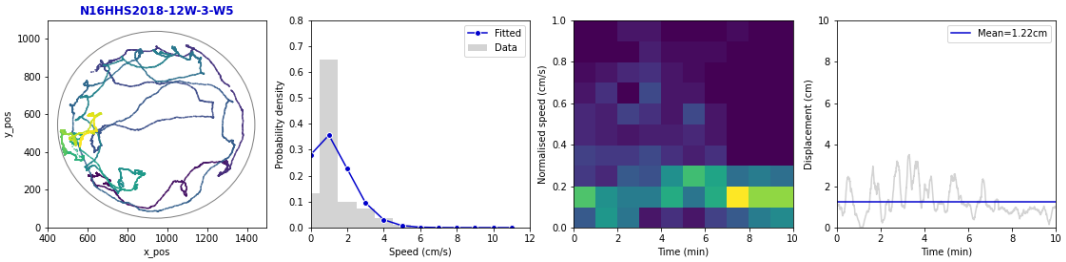

N16HHS2018-12W-2-W12
y_pos
x_pos
Probability density
Speed (cm/s)
Fitted
Data
Normalised speed (cm/s)
Time (min)
Displacement (cm)
Mean=1.55cm

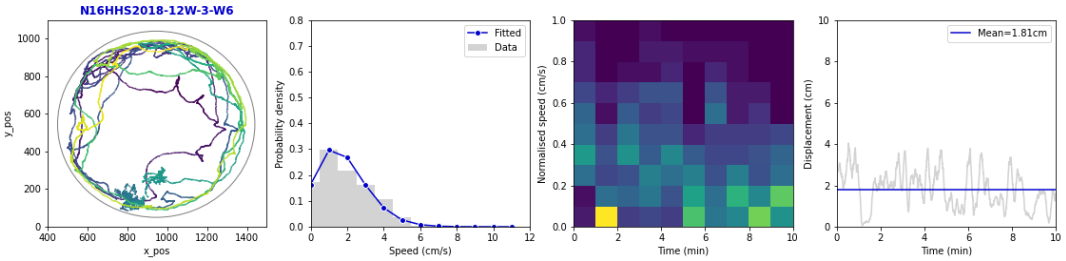

N16HHS2018-12W-3-W1
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.9cm

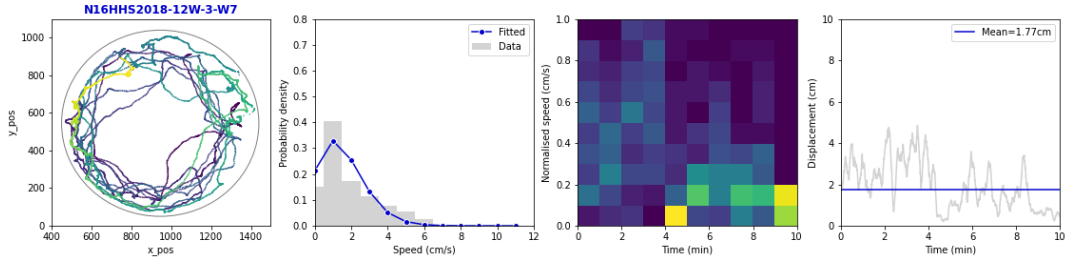

N16HHS2018-12W-3-W2
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.56cm

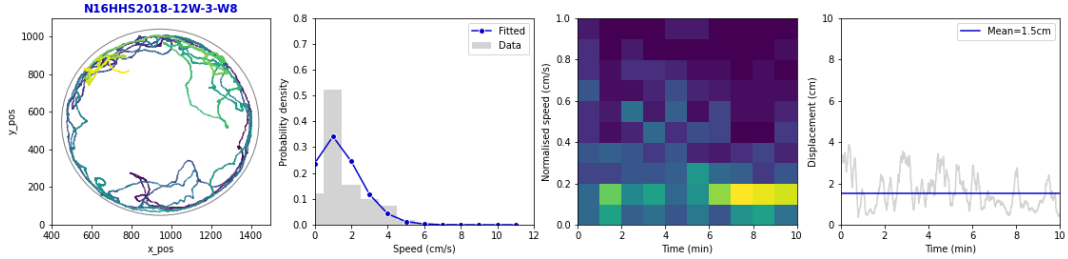

N16HHS2018-12W-3-W3
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.48cm

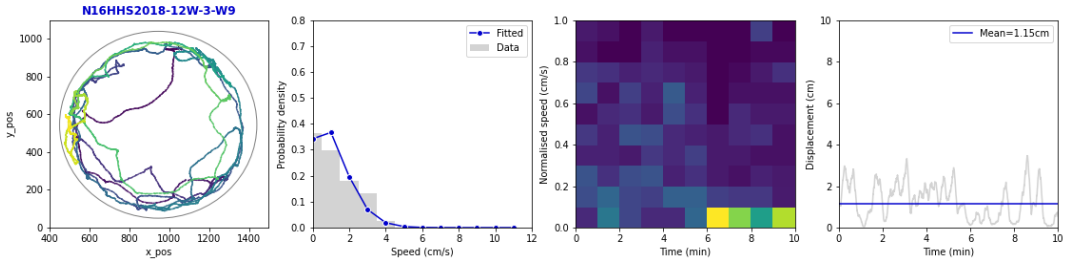

N16HHS2018-12W-3-W4
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.76cm

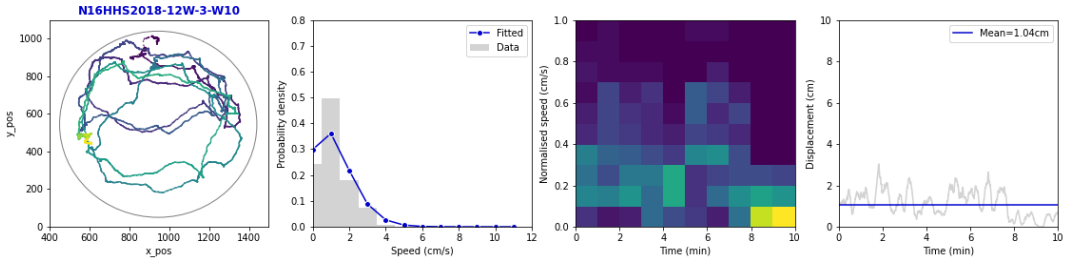

N16HHS2018-12W-3-W5
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.22cm

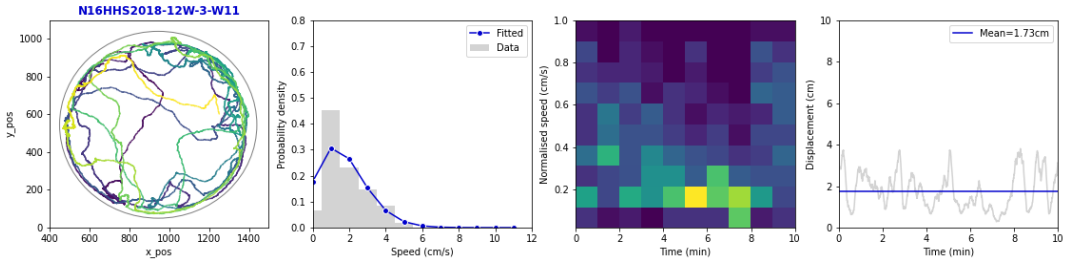

N16HHS2018-12W-3-W6
x_pos
y_pos
Speed (cm/s)
Probability density
Fitted
Data
Time (min)
Normalised speed (cm/s)
Displacement (cm)
Mean=1.81cm

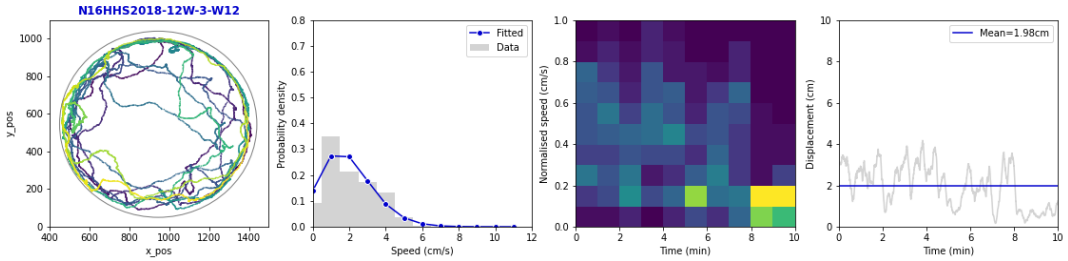

N16HHS2018-12W-3-W7
y_pos
x_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.77cm
Displacement (cm)
Time (min)


N16HHS2018-12W-3-W8
y_pos
x_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.5cm
Displacement (cm)
Time (min)


N16HHS2018-12W-3-W9
y_pos
x_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.15cm
Displacement (cm)
Time (min)


N16HHS2018-12W-3-W10
y_pos
x_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.04cm
Displacement (cm)
Time (min)


N16HHS2018-12W-3-W11
y_pos
x_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.73cm
Displacement (cm)
Time (min)


N16HHS2018-12W-3-W12
y_pos
x_pos
Fitted
Data
Probability density
Speed (cm/s)
Normalised speed (cm/s)
Time (min)
Mean=1.98cm
Displacement (cm)
Time (min)