EIGHT LEGS AND A BRAIN:
LOCOMOTOR MIMICRY OF ANTS AND NEUROETHOLOGY OF JUMPING SPIDERS

A Dissertation
Presented to the Faculty of the Graduate School
of Cornell University
In Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

by
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August 2015

EIGHT LEGS AND A BRAIN: LOCOMOTOR MIMICRY OF ANTS AND NEUROETHOLOGY OF JUMPING SPIDERS

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Cornell University 2015

This thesis explores the ecology, behavior, and sensory neurobiology of jumping spiders (Araneae: Salticidae). The first section (Chapters 1 and 2) investigates ant-mimicry by the jumping spider *Myrmarachne formicaria*. The second section (Chapter 3) takes a neuroethological approach to sound perception in the jumping spider *Phidippus audax*. Chapter 1 uses observations and collections from sites across France to study the relationship between mimics and co-occurring ant species. Behavioral trials also explore how individual jumping spiders and ants interact. Results from these studies suggest that *M. formicaria* is a general mimic of multiple ant species and that it does not positively associate with ants. Chapter 2 takes a quantitative approach to the study of locomotor mimicry by *M. formicaria*. It measures and compares aspects of gait and overall movement across non-mimetic jumping spiders, mimics, and multiple ant species. Against widely held beliefs, this work suggests that these ant mimics do not move on six legs. In their overall motion, however, *M. formicaria* do imitate ants. Chapter 3 uses behavioral trials and extracellular recordings of electrical activity from neurons in the jumping spider brain to explore responses to airborne acoustic stimuli. This work demonstrates that jumping spiders perceive airborne sound at distances far greater than previously believed possible (> 2 m).

BIOGRAPHICAL SKETCH

Paul Scott Shamble was born and raised in northern California. He is a 2008 graduate of the University of California at Berkeley, where he studied biology and English. He has since worked on spiders and other invertebrates at the University of Nebraska at Lincoln, Yale University, and the Royal Veterinary College, London. Shamble began his doctoral studies in 2009 at Cornell University under Profs. Ron Hoy and Rob Raguso. He has taught extensively at Cornell, including courses in chemical ecology, introductory biology, and a cross-disciplinary seminar on the senses. His thesis included research in England, France, and the United States.

ACKNOWLEDGMENTS

I am grateful to my advisors at Cornell—Ron Hoy and Rob Raguso— for their advice, encouragement, and support over the years. I am also grateful to the other members of my committee—Cole Gilbert, Eileen Hebets, and Andy Ruina (the latter as a member of my pre-dissertation committee). Eileen Hebets has been essential to my development as a scientist; I thank her for her support since my undergraduate days in her lab at Berkeley, where I fed her spiders and asked her questions.
I would also like to thank Terri Natoli, Sara Eddleman, Lori Maine, Dawn Potter, and the rest staff at the Department of Neurobiology and Behavior.

In the Hoy Lab I had the pleasure of working with James Golden, Gil Menda, and Eyal Nitzany. Without this group, the brains of jumping spiders would probably still be uncharted territory. I am also very grateful to Tsevi Beatus, who has always been a joy to work with. Thank you also to Kevin Loope: for his friendship and for so thoughtfully considering so many of the ideas—and half ideas—that we invented and re-invented over the years.

My work has been greatly improved by many conversations with scientists, including: the faculty and graduate students in the Department of Neurobiology and Behavior (especially Lunch Bunch and the Neuro Journal Club), the Society for Integrative and Comparative Biology, the Spence Lab at Temple University, the Structure and Motion Lab at the Royal Veterinary College, London; and especially Lars Chittka, Damian Elias, Beth Jakob, Mathieu Joron, Rob Olberg, Bruno Olshausen, and Bob Wyttenbach.

This work would not have been possible without financial support from the Department of Neurobiology and Behavior, the John S. Knight Institute for Writing in the Disciplines, the Cornell College of Agriculture and Life Sciences Mellon Grant, and the Andrew and Margaret Paul Graduate Fellowship, which allowed me to spend a

year working in Europe. Thank you to Andrew Spence, who arranged for my affiliation with the Royal Veterinary College, London, during my time in England. Thank you to Andrée and Ted Gorton for letting us stay at their place in Bazian; this forever changed my expectations of what a field station should be.

And of course, to my friends and family—particularly my parents, Helen and Alfred, my sister Anna, and my brother Noel, for a lifetime of support. And finally, to my wife, Gene—none of this would have been imaginable, or worth imagining, without you.

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PREFACE

“It is no longer sufficient for biologists to study
organisms at one level alone.”
- Stephen A. Wainwright et al. (1976)

This thesis is about spiders, but at its core it is also about the complexity of small things. It examines systems of mimicry from a novel perspective, using new tools and new analyses, yet also delves into the mind of the spider, peering into the shockingly sophisticated inner workings of a truly tiny brain. This thesis is about the beauty in all eight hairy legs, and two body segments, of a much-maligned creature.

I explore the biology of jumping spiders (Araneae: Salticidae) at three levels: (1) general behavioral ecology, (2) mechanisms of locomotion, and (3) neural correlates of sensory perception. The first two chapters focus on a particular salticid species, *Myrmarachne formicaria*. Unusual even among salticids, *M. formicaria* is a morphological and behavioral Batesian mimic of ants. Native to Europe and northeastern Asia, this mimic was recently introduced to the United States. Although first reported in Ohio only a decade ago (Bradley et al. 2006), it is now one of the most commonly observed jumping spiders in Ithaca, NY, and its environs. A third chapter uses neuroethological methods to understand how a different jumping spider species, *Phiddipus audax*, processes and responds to airborne acoustic stimuli.

Although markedly different in size, shape and overall behavior, both sets of spiders in this study are salticids—web-less active hunters with sensitive eyes and remarkable cognitive prowess.

Previous research had already suggested the exceptional nature of these creatures. We know that salticid eyes have a visual acuity that far exceeds even the most visually adept insects. In fact, they surpass those of mice and rats; certain salticid optics are better than those of chickens, pigeons, and cats (Harland et al. 2012). Such powerful sensory structures make possible spectacularly complex visually guided behaviors. Salticids can navigate three-dimensional mazes (Tarsitano & Jackson 1997), and locate and classify targets 20 to 30 cm away (Harland et al. 1999). Their advanced visual systems have also shaped selection for elaborate conspecific interactions—particularly the dance-like courtships of males (Elias et al. 2012). Here, using new technology (high-speed, 1000-to-4000-frames-per-second videography) and methods recently pioneered by the Hoy Laboratory (extracellular recordings of single neural units in the brains of spiders [Menda et al. 2014]), I was able to study salticids' sensation, perception, and behavior in ways previously inaccessible to scholars.

Chapter 1 examines the relationship between ants and *M. formicaria* over a portion of its native European range. I conducted observations and collections of these species at sites in France and ran behavioral trials between individual spiders and individuals of co-occurring ant species. I conclude that these mimics do not positively associate with ants. I suggest that rather than mimicking a specific ant species, *M. formicaria* is a general mimic of multiple ant species. I discuss these findings in light of recent scholarship regarding the selection and maintenance of imperfect mimicry.

Chapter 2 develops a more mechanistic approach in order to understand *how* these jumping spiders mimic ants. Although previous researchers had often remarked that ant-mimicking spiders move like their models, this observation had not yet been the focus of investigation. My work compared the locomotor behavior of mimics with the behaviors of non-mimetic jumping spiders and multiple ant species. This work adapts techniques pioneered in the field of biomechanics to analyze how these animals use their limbs while in motion. I developed a quantitative framework for understanding the temporal and spatial characteristics of movement patterns across species. My findings refute widely held beliefs that ant-mimicking jumping spiders walk on six legs. However, my results also show that in their overall pattern of movement *M. formicaria* did imitate the locomotion of ants. This work contributes to our understanding of locomotor mimicry—particularly among little-studied terrestrial species—and reveals how natural selection can transform locomotor traits into Batesian signals.

Chapter 3 greatly expands the known sensory space inhabited by jumping spiders by demonstrating that these visual specialists also perceive and rely on airborne sounds. This work shows that the perception of acoustic stimuli occurs over spatial ranges much greater than was previously thought possible. Using the bold jumping spider *Phidippus audax*, this chapter presents behavioral and neurophysiological evidence that these spiders respond to low (< 1000 Hz) frequency sounds—even when meters away from the source. This work continues to advance our understanding of ear-like structures—and their surprising sophistication—in arthropods.

As a whole, this thesis contributes to studies of mimicry, behavior, locomotion, and sensory neurobiology, and hopes to influence how we see and understand the world of jumping spiders.

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CHAPTER 1

ON THE ASSOCIATION BETWEEN ANTS AND THE ANT-MIMICKING JUMPING SPIDER *MYRMARACHNE FORMICARIA*

Abstract

All jumping spiders in the genus *Myrmarachne* (Araneae: Salticidae) are Batesian mimics of ants (Hymenoptera: Formicinae). Most of these species are found in the tropics and are considered to be accurate mimics of a single ant species with which they co-occur. Certain *Myrmarachne* also associate closely with their models. *Myrmarachne formicaria* is one of the few northern temperate species and is generally considered to be a lower accuracy mimic. However, this assertion has been largely anecdotal to date. The current study investigated the relationship between *M. formicaria* and its putative ant models at three distinct levels: species surveys at multiple sites, measures of animal density within a site, and one-on-one behavioral interactions. Based on the presented findings, I suggest that adult *M. formicaria* are general mimics of ants in the genus *Formica* and that as juveniles they may also mimic smaller *Lasius* ants. However, these mimics show no positive association with their models and actively avoid contact with ants. I discuss these findings in light of current theories regarding imperfect mimicry and propose that differing patterns of co-occurrence between mimics, models, and predators may reinforce imperfect mimicry.

Introduction

First proposed nearly one hundred and fifty years ago, the theory of Batesian mimicry describes an evolutionary process through which individuals of a palatable species (the mimics) improve their fitness when a potential predator mistakes them for individuals of an unpalatable species (the models) (Bates 1862). The result is often stunning, seeming to transform harmless flies into stinging wasps, or unprotected grasshoppers into well-defended tiger beetles (Wickler 1968). However, there are also times when results are less than spectacular, resulting in "imperfect" mimicry—ringed snakes, for example, with patterns and colors that only roughly correspond to those of their venomous models (Savage & Slowiksi 1992). Such imperfection seems to fly in the face of a central prediction of mimicry theory—that selection should continuously favor increasingly accurate mimics (Fisher 1958; also see Kikuchi & Pfennig 2013).

Ant-mimicking spiders—along with snakes (Harper & Pfennig 2007; Kikuchi and Pfennig 2010) and hover flies (Golding et al. 2005; Pennet et al. 2012)—have often been used to explore the boundaries and categories of mimetic fidelity (Edmunds 2006; Pekár & Jarab 2011). Much of this work has focused on the jumping spider genus *Myrmarachne* (Aranea: Salticidae)—one of the most species-rich spider genera, with more than 200 described species (Platnick 2014). All *Myrmarachne* species are considered to be Batesian mimics of ants (Edmunds 1974), and the majority are believed to be accurate mimics of a single ant species (Jackson et al. 2008, Edmunds 1978, 2006). Accurate mimicry in these spiders is achieved through modifications of morphological features (including a constriction near the anterior end of the abdomen to appear like an ant petiole, elongated abdomens, and ant-like colorations), as well as behavior (waving of forelegs like antennae [Ceccarelli 2008] and ant-like locomotion [Jackson & Willey 1994; Pekár & Jarab 2011; also see Chapter 2]).

A close physical association with individuals of the putative model species is also an important requisite for establishing accurate mimicry. Sympatry ensures that local predators are educated and wary of models (Edmunds 2006). In other mimetic systems, the degree of overlap between the ranges of models and mimics has been shown to influence accuracy (Harper & Pfennig 2007); in other *Myrmarachne* species, some researchers have reported that mimics may gain additional benefits from associating closely with their ant models (Nelson & Jackson 2009).

Myrmarachne formicaria is one of the few *Myrmarachne* species found outside the tropics. Native to Europe and northeastern Asia (and recently introduced to the United states, Bradley et al. 2006), this species was first described in 1778 by the Swedish entomologist Charles De Geer, but since the late-eighteenth century has been the subject of little focused study (for exceptions see Pekár & Jarab 2011; Pekár & Jiroš 2011). This spider is generally believed to bear a "superficial resemblance" to *Formica* ants (Locket & Milllidge 1951). Although *M. formicaria* lacks the extreme morphological traits characteristic of other species in the genus (e.g., bright colors and a long thin constriction between body segments), it does possess ant-like shape, color (black at its most anterior and posterior while red in the middle), and an ant-like behavioral repertoire (Pekár & Jarab 2011; see also Chapter 2).

The current study examines the relationship between *M. formicaria* and co-occurring ant species. The association between mimics and their putative models was examined at multiple behavioral and ecological scales in order to address the following questions: (1) Do mimics consistently co-occur with a particular model species (or suite of species)? (2) Within a habitat is there a positive correlation between the abundance of models and the abundance of mimics? (3) At the level of individual behavior, how do mimics physically interact with ants?

Firstly, I present evidence that *M. formicaria* co-occurs with several ant species, although with little consistency in ant species present across collection sites. Secondly, the abundance of spiders within a habitat does not positively correlate with the abundance of ants. Finally, at the level of individual behavior, these mimics actively avoid physical contact with ants—even more than non-mimetic jumping spiders do. These results are discussed in light of where *M. formicaria* lies on the spectrum of accurate to imperfect mimicry, with potential implications for advancing our understanding of this continuum.

Methods

Collection sites and basic methods

Collections and observations were made at three sites in France during the summer of 2012. These three sites covered a relatively large latitudinal range (43-48ºN; corresponding to roughly 650 km), as well as a range of ecological habitats. The first site (from 9-14 May 2012) was the Parc de Bercy, Paris (48º 49’ 59.1228”N, 2º 23’ 5.3124”E), a public park built in the 1990s in central Paris. Spiders were found along the southern edge of a pond at the base of reeds on a solid and dry substrate of soil and fallen reed matter. The second site (from 22-25 May 2012) was the Côte Sainte-Catherine park located on a hill above the city of Rouen (49º 26’ 1.8456”N, 1º 6’ 30.477”E), in northern France. Spiders at this site were found in grasses of moderate height (approx. 15-40 cm; with shorter sections that were cut occasionally) near taller grasses and shrubs. The third site (from 2-30 June 2012) was an uncultivated field in the hamlet of Bazian (43º 40’ 14.1996”N, 0º 19’ 18.6054”E), near Auch, in southern France. The grass at this site was short (approx. 2-6 cm) and was kept at this length by periodic machine mowing. Ant and spider species at each location were noted, and all collected specimens were preserved in 70% EtOH. Identifications of species were based on collected voucher specimens. Ants were identified using Bolton (1994) and Casevitz-Weulersse & Galkowski (2015). Spiders were identified following Platnick (2014).

Basic morphological measures were made to enable size comparisons between species. Length measurements were based on calibrated photographs of preserved specimens taken with a digital camera (Leica DC 500; Leica Microsystems GmbH, Wetzlar, Germany) mounted to a stereo microscope (Leica MZFLIII). Measurements were taken using the image processing software Fiji (Schndelin et al. 2012). Preserved ant specimens tend to curl instead of maintaining the postures shown while alive, so these length measurements were based on images of side-views (total length calculated from measures taken of the head, the thorax, and the abdomen). When walking, ants carry their heads at a slightly downward angle (see Chapter 2), thus the addition of these three body measures would have resulted in an overestimation of total length. Head measures were therefore adjusted by the cosine of 30º —a factor based on observations of *Formica fusca* ants viewed in profile with a high speed macro video camera (mean angle ± s.d. = 28.7 ± 5.4º; n = 10; see Chapter 2 for details regarding recording methods). For each ant species the length of a representative worker is reported. Reported length measures for *M. formicaria* are based on an average of multiple adults (n=11).

Color classifications were made by assigning species to one of two color categories based on overall appearance to the human eye: red or black. The main source of bias introduced by even purposefully coarse categorizations of color by human observers such as this are that they overlook contributions from the UV spectrum. However, a study by Pekár and Jarab (2011) which imaged individuals of three myrmecomorphic spider species and their putative models under UV light (390nm) (including an ant of the *Lasius* genus also used in the current study) found no reflectance in the UV range, suggesting that UV reflectance likely plays little part in the coloration of ants. Preliminary investigation of *Myrmarachne formicaria* collected in the United States also found no UV reflectance in this species.

Population density survey

Micro-habitat density measures of *M. formicaria* and common ant species were carried out at the Parc de Bercy site. Here, unlike at the two other sites, the substrate was relatively uncluttered, enabling the observation of a large area from a single location. Parc de Bercy was also the site with the highest density of *M. formicaria* and therefore seemed the most appropriate location for testing for a meaningful correlation between ant and spider density at the micro-habitat level. Surveys were based on observations of 29 distinct (60 x 60 cm) quadrats from 11-12 May 2012. All observations were made in the afternoon (14:00 – 17:30) and each quadrat was observed for one continuous 10 minute interval. Weather conditions over this period were generally typical of the season; daily mean temperature = 16.7 C, 12.2 C; partly cloudy and mostly clear. All identifiable spider species, including any non-mimetic spiders, were counted. Because of the structure of the substrate, foraging ants regularly came in and out of view; ant densities were therefore estimated as the approximate number of ants visible at any given time across the entire quadrat. Although ant densities and spider densities were not directly comparable, this method was sufficient for exploring possible spatial correlations between the two groups.

Behavioral interactions

Lasius and *Myrmica* were the only two ant genera present with *M. formicaria* at all three collection sites and were therefore selected for behavioral interactions. *Myrmica rubra* and *Lasius niger* were used as representatives of these ant genera. As an example of non-mimetic behavior, *Heliophanus sp.,* a jumping spider of the same approximate size as *M. formicaria* and found in the same habitats, was also used in these experiments. All jumping spiders used in these trials were mature adults. Two individuals were placed on a 20 cm diameter ceramic circular arena and allowed to interact for 5 minutes. The arena was surrounded by water to limit animals to the same physical space. This was preferred to a traditional walled arena with a clear cover because salticids are adept at walking on vertical surfaces (even clean glass)—a habit that would have confounded comparisons of interaction frequency between individuals by effectively increasing the arena size for jumping spiders, but not for ants. Thus, one would have expected more interactions between individual ants simply because they were operationally confined to a smaller space. (In the rare case that an animal ventured into the water, it was returned to the arena by the researcher with the aid of a wooden dowel.) Interactions were scored for number of physical contacts between individuals, number of prolonged contacts (contacts longer than 500 ms) and number of avoidance behaviors (when a spider moved so as to avoid impending contact). Three types of trials were conducted: (1) ant paired with conspecific, (2) ant paired with the non-mimetic jumping spider, *Heliophanus sp.,* and (3) ant paired with the mimic *M. formicaria.* Conspecific ant pairings were done in order to establish a baseline for positive associative ant behavior. Pairings between salticids were avoided as many salticids are known to opportunistically prey upon other salticids—a potential result that would have limited subsequent behavioral observations. Thus, the experimental design included a total of six treatments across the three trial types: (1a) *L. niger*: *L. niger;* (1b) *M, rubra*: *M. rubra;* (2a) *L. niger*: *Heliophanus sp.;* (2b) *M. rubra*: *Heliophanus sp.;* (3a) *L. niger: M. formicaria*; (3b) *M. rubra: M. formicaria.*

Statistical analysis

Statistical tests were done using Matlab version R2014B (The Mathworks, Natick, MA, USA). Results are reported as mean ± standard deviation. Because most analyses involved non-normal distributions, nonparametric tests were used: the Kruskal-Wallis test when making comparisons between multiple groups (i.e., occurrences of behaviors in interaction studies) and the Kendall rank correlation test when testing associations between variables (i.e., the relationship between ant and mimic densities).

Results

Ant species

Among the three sites, *Myrmarache formicaria* was found at highest densities in the Parc de Bercy, Paris. Only two ant species were recorded there: the small black garden ant *Lasius niger* and the small red ant *Myrmica rugulosa*. Both were smaller than *M. formicaria* adults. *Lasius niger*, lacking the two segment petiole and postpetiole of *M. rugulosa,* appeared to be the better morphological match to *M. formicaria*, while the red *M. rugulosa*, was the better color match (see Figure 1.1). Multiple ant species were present in the Rouen site, including *Myrmica rubra*, *L. niger*, as well as *Formica fusca*—with the latter most conspicuously abundant. The black *Formica fusca* was a better size match of *M. formicaria* adults, but in color was not an accurate match for the red *M. formicaria.* At the Bazian site, *M. rubra*, *L. niger* were present along with the black *F. fusca* and the much less abundant red *Formica aluilonia*. The presence of the large *Formica rufa* was particularly notable, due to the presence of a nest and the overall abundance of the species. While the best size match for *M. formicaria* was the black medium-sized *Formica* species (*F. fusca* and the less abundant *F. aluilonia*), the spiders were a better color match of the large *F. rufa*.

Morphology and color

Adult *Myrmarachne formicaria* (5.03 ± 0.90 mm, n = 11 [males = 5.19 ± 1.2 mm, n = 6; females = 4.83 ± 0.26 mm, n = 5]) were similar in size to *Formica fusca* (5.63 mm), *Formica aluilonia* (5.20 mm), and *Myrmica rubra* (5.16 mm) but were notably smaller than the larger *Formica rufa* (7.30 mm) (see Figure 1.1). *Myrmarachne formicaria* were also larger than *Myrmica rugulosa* (3.19 mm) and *Lasius niger* (3.22 mm). It should be noted, however, that because myrmecomorphic spiders grow gradually, some are thought to be transformational mimics (Cushing 1997; Cushing 2012 and citations within)—meaning that they have different models at different stages in their lives. Thus, while adult *M. formicaria* may be poorly size matched to these ant species, they may be accurate size mimics of these ants as juvenile spiders.

Myrmica rugulosa, *Myrmica rubra*, *Formica aluilonia*, and *Formica rufa* were all placed in the red color category with *Myrmarachne formicaria*. *Lasius niger* and *Formica fusca* were categorized as black (see Figure 1.1).

Density

Over the 29 quadrats surveyed, the mean density of *Myrmarachne formicaria* was 4.2 ± 4.2 individuals/m^2 (present in 23/29 quadrats), while the density of non-mimetic jumping spiders was 0.9 ± 1.3 individuals/m^2 (present in 9/29 quadrats). The two ant species present were *Lasius niger* and *Myrmica rugulosa* —the former much more common than the latter; *L. niger* = 30.6 ± 22.6 simultaneously visible/m^2 (present in 27/29 quadrats); *M. rugulosa* = 1.4 ± 4.7 simultaneously visible/m^2 (present in 3/29 quadrats). Of the 23 quadrats in which *M. formicaria* was present, *L. niger* was also present in all but 2. However, there was not a significant correlation between *L. niger* abundance in any given quadrant and the number of *M. formicaria*

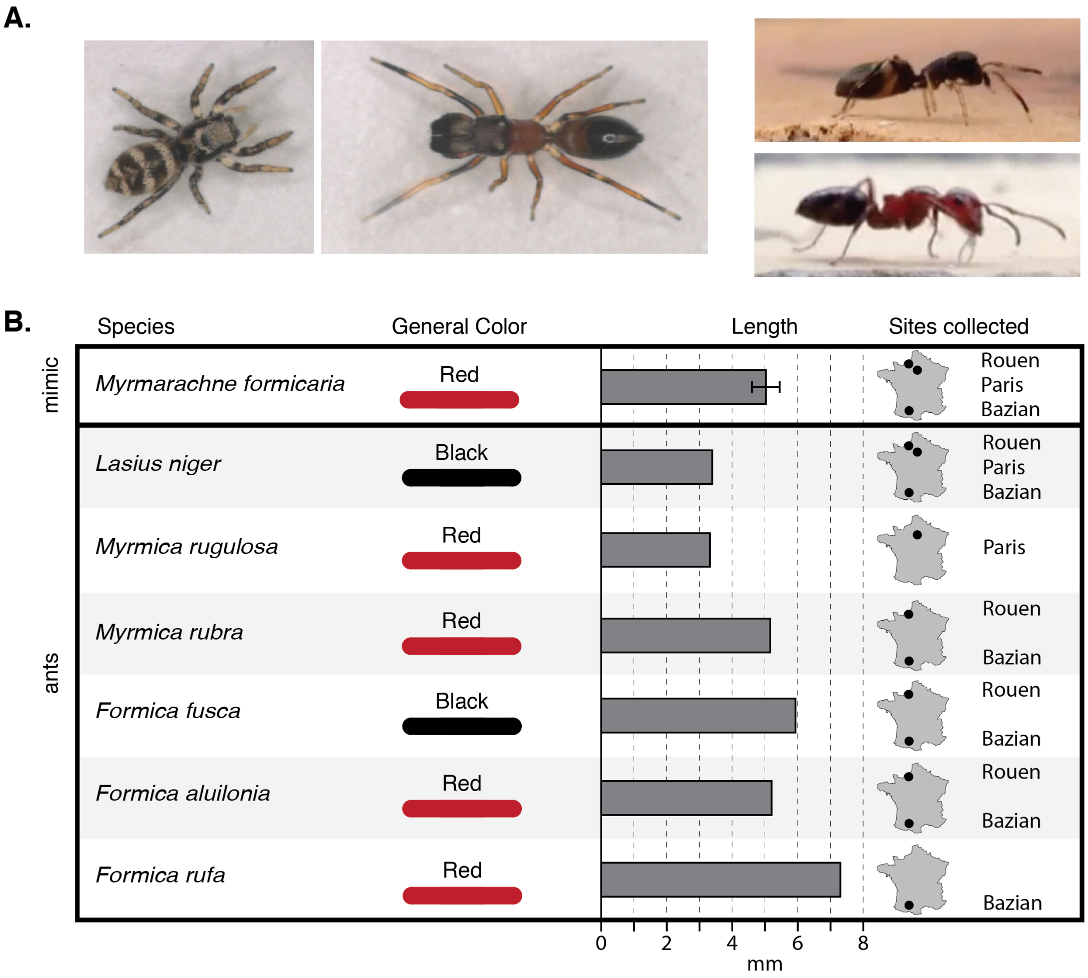

Figure 1.1. Basic morphology of the ant-mimicking jumping spider, *Myrmarachne formicaria*. (A) Example images of the common non-mimetic jumping spider *Salticus senecus* (left), the ant-mimic *M. formicaria* (center, top right), and a *Formica* ant (bottom right). Top-down images are images taken in the lab at similar scales. Side-view images are taken in the field (Bazian) and at similar scales. (B) Collection data and basic morphology of *M. formicaria*, and dominant co-occurring ant species from three sites in France. General color designations are categorical and thus purposefully broad. Ant lengths are based on a representative individual worker while length of *M. formicaria* shows mean and standard deviation (n = 11).

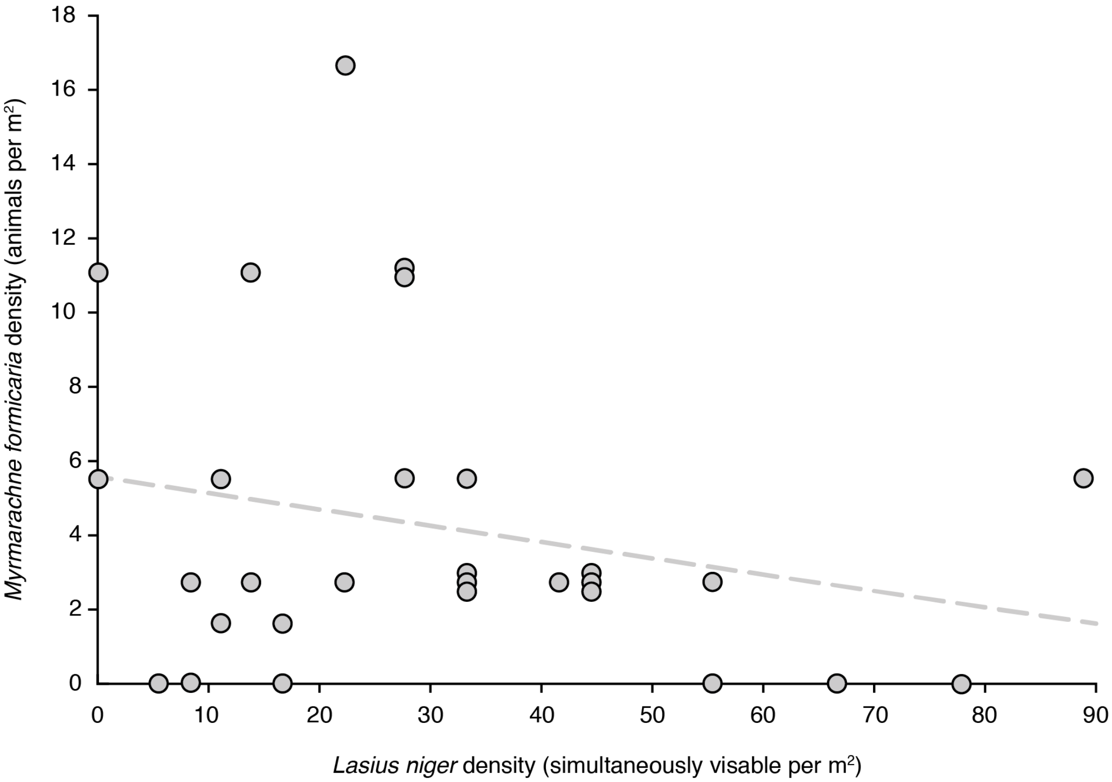

Figure 1.2. Measure of *Myrmarachne formicaria* (mimic) abundance and *Lasius niger* (ant) abundance for 29 quadrats (60cm x 60cm) in Parc de Bercy, France. Units are animals per square meter (for mimics), and estimated number of animals simultaneously visible per meter (for ants). Repeated values are shown as vertically offset, overlapping markers. Dotted line shows a linear fit, though the relationship between the two measures was insignificant (Kendall rank correlation test, p=0.3271, tau = -0.1452).

mimics present (Kendall rank correlation test, p = 0.3271, tau = -0.1452; Figure 1.2) or between *M. rugulosa* abundance and number of *M. formicaria* (Kendall rank correlation test, p = 0.0694, tau = -0.3111). There was also no significant correlation between either ant species and the non-mimetic salticid species (*L. niger*: Kendall rank correlation test, p = 0.8695, tau = -0.0297; *M. rugulosa*: Kendall rank correlation test, p = 0.2497, tau = -0.2188).

Behavioral interactions

No species-specific differences were found in number of short contacts per trial, number of long contacts per trial, or avoidance behaviors per trial between trials involving *Lasius niger* and those involving *Myrmica rubra*. Because of this, data were pooled to form three categories: ant : ant (n=14); non-mimetic salticid : ant (n=13); and mimic : ant (n=17).

Ants showed significantly more long interactions when paired with conspecifics (3.9 ± 2.5 interactions/trial) than when paired with either the non-mimetic salticid *Heliophanus sp.* (0.3 ± 0.6 interactions/trial) or with *M. formicaria* (0 ± 0 interactions/trial; Kruskal-Wallis, p<0.0001) (Figure 1.3a). Ants, regardless of pairing, showed no avoidance behaviors (conspecific ant trials: 0 ± 0 behaviors/trial), while *M. formicaria* showed significantly more avoidance behaviors (5.5 ± 3.0 behaviors/trial) than non-mimic jumping spiders did (1.2 ± 2.4 behaviors/trial; Kruskal-Wallis, p<0.0001) (Figure 1.3b). There were no differences between total number of short contacts across groups (ant : ant = 1.4 ± 2.1 interactions/trial; non-mimetic salticid : ant = 4.7 ± 5.3 interactions/trial; mimic : ant = 2.6 ± 1.9 interactions/trial; Kruskal-Wallis, p=0.0695).

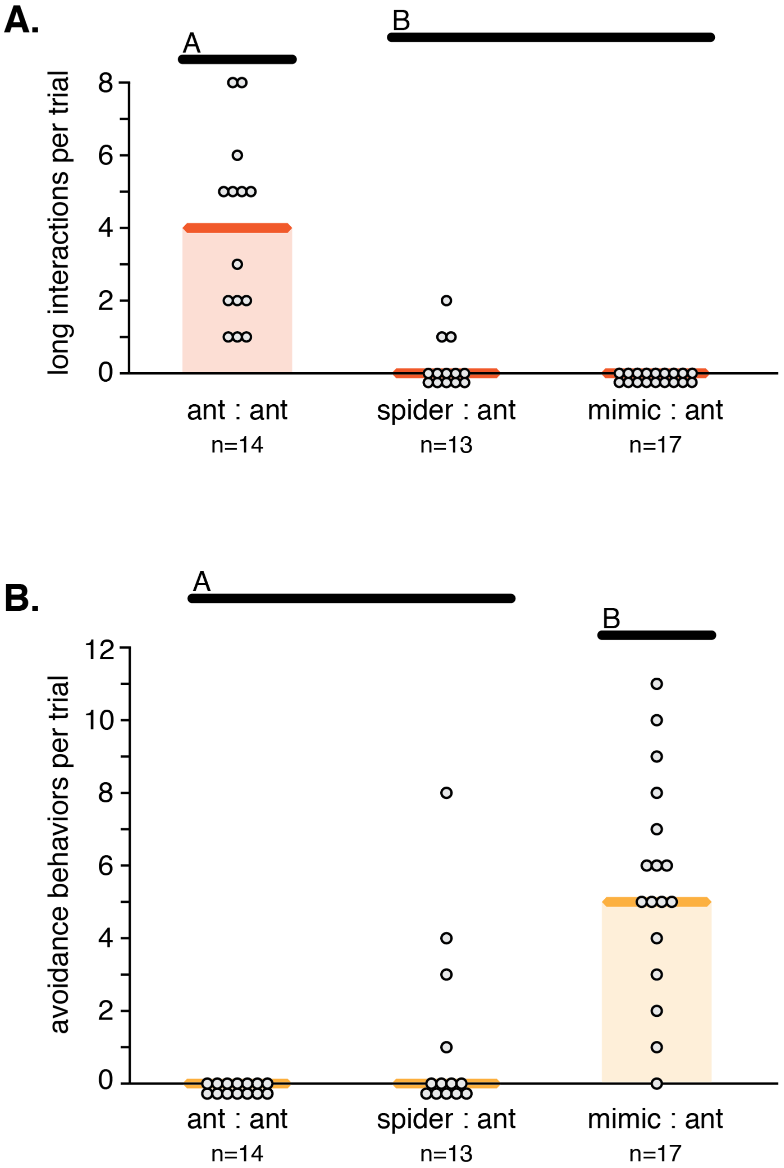

Figure 1.3. Results from one-on-one behavioral interactions for three experimental categories: ants (*Lasius niger* and *Myrmica rubra*) interacting with conspecifics, ants interacting with non-mimetic jumping spiders (*Heliophanus sp.*), and ants interacting with mimetic jumping spiders (*Myrmarachne formicaria*) for 5 minutes in a 20 cm diameter arena. (A) This shows the number of interactions per trial that exceeded 500ms. (B) Shows the number of avoidance behaviors (when an animal moved to prevent impending contact) for each trial—only spiders demonstrated this behavior. Circular markers show results from each trial while the colored bar shows the median of each category. All markers that fall on the zero line, or below, designate trials where none of the corresponding behaviors were observed. Bars and letters above each graph show groupings based on statistical tests (Kruskal-Wallis test, p<0.0001).

Discussion

Based on collections from three geographically distinct locations, measures of species abundance within one site, and behavioral interactions, the results here support the hypothesis that unlike other ant-mimics (Edmunds 2006), *Myrmarachne formicaria* does not positively associate with any particular ant species. On the contrary, these mimics actively avoid contact with ants. While individuals of two ant genera (*Lasius* and *Myrmica*) co-occured with *M. formicaria* at all three sites, these ants appear to be poor models of adult *M. formicaria*; particularly at the Parc de Bercy site where both *M. rugulosa* and *L. niger* were smaller than these mimics (Figure 1.1). Within a site, there was no significant correlation between the abundance of mimics and the abundance of either of these ant species; in fact, the trend (though insignificant) was negative (Figure 1.2). It is worth noting, however, that while densities of models and mimics were not positively correlated, mimics and *L. niger* ants were found together in the vast majority of quadrats—thus supporting the traditional expectation that the ratio of mimics to models should be 1:1 or greater (though see Fisher 1958 for a discussion of possible exceptions). In behavioral trials in which a mimic spider was paired with an individual of either one of the co-occurring ant genera, the mimic actively avoided individual ants (Figure 1.3). The current study investigated potential associations between this ant-mimicking spider and its putative ant models at three levels (between habitats, within a habitat, and at the individual level) and found no positive association between mimics and any ant species at any level. Based on this evidence, I propose that *M. formicaria* is a general mimic of the subfamily Formicinae ants—particularly of species in the genera *Lasius* and *Formica*—and that these mimics show no positive association (obligate or otherwise) with co-occurring ants.

Many ant-mimicking arthropods that live in close proximity to ants benefit from access to the colony's resources and defenses (McIver & Stonedahl 1993). Within the genus *Myrmarachne*, a number of species have been found to associate closely with ants—nesting (Jackson et al. 2008) or foraging (Edmunds 1978, 2006) near their models. Pekár and Jarab (2011) found similarities between the cuticular hydrocarbon profiles of *Myrmarache formicaria* and the ant *Formica rufibarbis* and suggested that *M. formicaria* may infiltrate ant colonies to consume larvae. Based on my observations of the relationship between these mimics and ants, it seems extremely unlikely that such a strategy is part of the biology of these mimics. All observations of predation by *M. formicaria* in the field during this study were of small flies, gnats, and other small winged insects—consistent with previous findings for *Myrmarachne* (Staveley 1866; Jackson & Willey 1994). Although Batesian mimicry theory predicts a frequency-dependent positive relationship between models and mimics (attacks on mimics should be less likely in areas with higher densities of models, or if models are particularly unprofitable [see Harper & Pfennig 2007]), the current study found no positive relationship between the abundance of *M. formicaria* and the abundance of consistently co-occurring ant species.

Spiders grow incrementally throughout their lives, but ants emerge as adults with a stable size. This presents ant-mimicking spiders with a particular problem: they will either be poor size matches for their models as juveniles or as adults. However, certain species known as transformational mimics have evolved to mimic multiple ant species throughout their lives. As they grow, these mimics molt into different colors that match a new model of a more appropriate size (Cushing 1997). *M. formicaria* may be a transformational mimic—as an adult the spider mimics *Formica* ants, but as a juvenile it may mimic different ant species. A likely candidate for such an early-life model would be individuals of the consistently co-occurring *Lasius* species. *Lasius* and *Formica* ants (both of the subfamily Formicinae) share a very similar overall body shape (Bolton 1994) and thus may provide *M. formicaria* with suitable models that differ almost exclusively in size.

One of the most accurate models of *M. formicaria* based on color and shape was the large ant *Formica rufa,* a species only present at the Bazian site in this study but in other studies it is found throughout Europe (Collingwood 1979). These ants were by far the most aggressive of all the ant species encountered, responding threateningly to heterospecific ants, other arthropod species, and larger organisms (including the author). Aggressive or otherwise well-defended species are predicted to be particularly potent models in Batesian systems as the selective pressure for predators to avoid such unprofitable species should be especially strong—thus the benefits of being treated as a model should be especially high (Sherratt 2002). Similarities between *M. formicaria* and *F. rufa*, therefore, present these spiders with clear advantages. However, important morphological differences—namely size (*F. rufa* is ca. 40% longer than *M. formicaria*)—might seem to contradict the hypothesis of *F. rufa* as a model. Scholars, however, have argued that size mismatches in which the model is larger than the mimic may actually benefit imperfect mimics even more than size parity (Penney et al. 2013). In a comprehensive investigation of imperfect mimicry in hoverflies, Penney et al. (2013) found that smaller flies were poorer mimics than larger ones, and yet these small flies benefited despite their "imperfections"—results that matched two previously proposed hypotheses (relaxed-selection: Duncan & Sheppard 1965, Sherratt 2002; and evolutionary constraints: Holen & Johnstone 2004; Speed & Ruxton 2010). Penney et al. (2013) conclude that smaller targets are less profitable and thus not worth the risk for such a small caloric reward; lower predator interest thus results in better protection and subsequently reduced pressure for mimetic accuracy.

One potential concern regarding the current study is that the classification of mimics as perfect or imperfect is subjective, and may not reflect the sensory abilities and perceptual judgments of the predators that act as the agents of selection in these systems (see Dittrich et al. 1993). There is evidence, however, that human and predator categorizations of mimetic similarity can agree (Penney et al. 2012). Color and size, the criteria used in the current study, are widely accepted as important in predator learning, target categorization, and predatory decision-making in a range of predators (vertebrates: Kazemi et al. 2014, Monroy & Nishikawa 2011; invertebrates: Rashed 2005, Nentwig & Wissel 1986). Also, the assertion here that *M. formicaria* is a general mimic is based largely on its lack of a consistently co-occurring model—a measure independent of the perceptual overlap between models and mimics.

Unlike some "inaccurate" ant-mimicking spiders (Pekár & Jarab 2011), *M. formicaria* does possess ant-like features. However, unlike more "accurate" mimics, *M. formicaria* does not possess the extreme ant-like morphologies or close ecological associations that clearly connect certain arachnid mimics to a single formicid model (Edmunds 2006). I therefore suggest that *M. formicaria* is a "general mimic" of Formicinae ants.

Imperfect mimicry

A range of non-mutually exclusive hypotheses have been proposed to explain the existence of imperfect mimics—from misclassification by human researchers to benefits incurred through kin selection (see Kikuchi & Pfennig 2013 for review). A subset of these hypotheses suggests that the overlap between the ecological ranges of mimics and their models may play a role in the evolution and maintenance of this phenomenon. Harper and Pfennig (2007) found that coral snake mimics in the eastern United States were less accurate where the model was most abundant—presumably because in these areas the odds of choosing a poisonous ringed snake were always greater. In a different study, Edmunds (2000) argued that if a mimic inhabited a range containing multiple models, the cost of being an imperfect match in any one location could be outweighed by the benefits of an expanded range. Sherratt (2002) used elements of signal detection theory to expand Edmunds' (2000) concept by making a computational model of the phenomenon. Termed the "jack-of-all-trades" hypothesis, this model predicts that if multiple non-palatable models are present in different areas or at different times, an intermediate mimetic phenotype will be favored.

Myrmarachne formicaria does not co-occur with the same set of potential models at various study sites—a range and distribution that makes this ant-mimic a strong candidate for the application of Sherratt's computational model. An important factor in enabling *M. formicaria* to occupy a range of habitats is that it appears to be a capable disperser—evidenced by its effective colonization of the urban Parc de Bercy, a habitat that has only been present in its current form since the mid-1990s, and its rapid invasive spread throughout the northeastern United States (Bradley et al. 2006).

Because of this dispersal—and the lack of strict habitat preferences that would enforce a tight match between mimics and models—local adaptations to co-occurring models are not likely to provide similar benefits to subsequent generations. The selective landscape encountered by the next generation will not necessarily match that of its parents, thus avoiding the multi-generational, single model theoretically required to generate species-specific mimicry. Further, the sites under study here represent only a fraction of the reported range of *M. formicaria*: published records report the species in Crete (Logunov & Chatzaki 2003), Norway (Aakra 2000), Russia (Logunov et al. 2000), Spain (Carter 1984), and South Korea (Jung et al. 2008), among other locations in Europe and Asia (Logunov et al. 2000). Future surveys of such disparate areas will increase our knowledge of the diversity of co-occurring ant species.

As the evolution of mimetic traits is ultimately a phenomenon driven by predator senses, cognition and selection (Chittka & Osorio 2007), diversity and variability in predators—each with its own sensory and cognitive biases—may result in imprecise mimicry. This would suggest a hypothesis similar to the "multiple predator" hypothesis proposed by Pekár et al. (2011)—yet with one important difference. Pekár et al. (2011) predicted that imperfect mimicry is sometimes the result of selection from two opposing groups: predators with a preference for spiders, and ant-specialists with a preference for ants. The authors argue that the resulting phenotype should be an intermediate between ant and spider. Although a range of potential generalist predators of spiders were observed over the course of this research (birds, lizards, wasps, and other spiders), no predators that appeared to prefer ants were noted. While this does not mean that these specialized predators are not present, it does suggest some limitation to their power as a selective force. Instead, multiple generalist predators could be responsible for maintaining general mimicry in this system—as long as predators' notions of "model-like" are not identical. Imperfect mimicry could be established, and maintained, by a variety of predators acting with different preferences. This could also result in patchworks similar to those described by Edmunds' multi-model hypothesis (2000) and the Sherratt's jack-of-all-trades hypothesis (2002).

Kazemi et al. (2014) recently proposed a hypothesis for the evolution and maintenance of imperfect mimicry that also may be applicable to the *M. fomicaria* system. By considering how predators learn multiple cues, Kazemi et al. (2014) found that predators disproportionately relied on the cues that they learned the fastest. This strategy was so pronounced that even when multiple learnable cues were present, many were ignored—a phenomenon termed "overshadowing" in the learning literature (Mackintosh 1976). Such a process is especially likely to evolve in systems where predators are more likely to use general rules for classification (see Chittka & Osorio 2007). Ant mimicry by *M. formicaria* seems to provide such a system: the patchy distribution of multiple model species (formicine ants) could favor the learning of general rules for “ant-ness.” The traits presented by *M. formicaria*—color, shape, behavior—could lead predators to quickly identify the mimics as models, leading them to overlook other “imperfections” in their decisions.

Systems in which mimetic accuracy falter, as in *M. formicaria*, are important for understanding the evolution and maintenance of mimicry, and potential limitations of mimicry theory. The patchiness of ant species with which *M. formicaria* co-occur and the mimics’ wide geographic distribution underscore the need for understandings of mimicry that go beyond the traditional single model, single mimic, single predator paradigm.

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CHAPTER 2

WALKING THE WALK: LOCOMOTOR MIMICRY BY THE ANT-MIMICKING JUMPING SPIDER *MYRMARACHNE FORMICARIA*

Abstract

Ant-mimicking species appear to move like their insect models, although a detailed analysis of this observation has thus far been lacking. Here we analyzed the locomotor behavior of the ant-mimicking jumping spider *Myrmarachne formicaria* (Salticidae) and compared it to the movement of non-mimetic spiders and numerous ant species. Using high-speed recording techniques we show that, contrary to previous hypotheses, these mimics do not move on six legs, but instead always walk on eight. Yet mimics and their models, in contrast to non-mimetic spiders, spent a much higher proportion of time moving. Mimics also walked highly curved oscillatory paths—paths unlike the looped paths walked by ants moving across a featureless arena. However, by analyzing the locomotor behavior of ants following artificially drawn pheromone trails, we demonstrate that mimics imitate ants actively engaged in trail-following. We conclude that these spiders do engage in locomotor mimicry, but that they do so by mimicking a subset of ant behaviors.

Introduction

The best known examples of mimicry are overwhelmingly of static phenomena—moths with all the bright colors of a butterfly (Joron & Mallet 1998), cuckoo eggs with the speckled patterns of their diminutive hosts (Stoddard & Stevens 2010), grasshoppers that seem every bit the shape of a tiger beetle (Wickler 1968). Yet the importance of movement has been part of mimicry theory since its inception; Bates noted in his foundational work that mimetic butterflies and their models flew in such similar ways that they were indistinguishable (Bates 1862). More recent works have also included the importance of movement (Wickler 1968), while research concerning the neurophysiological and computational mechanisms that underlie visually guided behaviors stress the important role of motion in object recognition (for review, see Giese & Poggio 2003). That said, researchers have only relatively recently begun to quantitatively investigate locomotor mimicry (Srygley & Ellington 1999). The only cases quantitatively studied to date are two flight-based mimicry systems—passion-vine butterflies (Srygley & Ellington 1999; Srygley 1999; Srygley 2007) and hymenopteran-mimicking hoverflies (Golding & Edmunds 2000; Golding et al. 2005). The study of locomotor mimicry among terrestrial mimics, meanwhile, has been extremely limited.

Mimicry of ants is one of the most common forms of terrestrial mimicry (Wickler 1968). Within this group, spiders make up a large fraction of myrmecomorphic species—animals that have a morphological and/or behavioral resemblance to ants (for a review of myrmecomorphic arthropods see McIver and Stonedahl 1993; for a review of myrmecomorphic spiders, see Cushing 1997, 2012). One genus of jumping spiders in particular, *Myrmarachne* McLeay 1835 (Araneae, Salticidae), provides a spectacular example of ant mimicry with over 217 described species (Platnick 2014). All *Myrmarachne* species are believed to be Batesian mimics (Edmunds 1974)—in which a palatable species avoids predation (and thereby incurs a fitness benefit) by being mistaken for an unpalatable model by would-be predators (Bates 1862; Vane-Wright 1980). Ants seem to be especially worth mimicking because they possess species-specific combinations of powerful defensive traits—general aggressiveness, strong mouthparts, venomous stings, chemical defenses, the ability to recruit similarly armed nest-mates (Hölldobler & Wilson 1990). Ants also tend to be highly conspicuous and abundant when present—further increasing their strength as Batesian models by ensuring that interactions with predators are particularly memorable, if not lethal (Sherratt 2002). Although renowned for their active predatory capabilities, jumping spiders are poorly defended and are common targets for a range of predators—thus fueling the selective advantage for being mistaken for an ant (see McIver & Stonedahl 1993 and citations within).

Batesian mimicry is characterized by the convergence of forms, not simply the expression of similar traits. Whereas most examples of mimicry involve species of the same class or order (i.e. butterflies mimicking butterflies, or snakes mimicking snakes), jumping spiders and ants are members of separate subphyla (Chelicerata and Hexapoda, respectively), with a most recent common ancestor estimated between 600 and 540 Myr ago (Regier et al. 2005; Douzery et al. 2004). Indeed, the morphological changes required to transform a stocky, eight-legged arachnid with two body segments into a thin insect with six legs, two antennae, and three body segments are significant. Resulting mimetic species often possess elongated bodies and thin legs when compared to non-mimetic species (see Figure 2.1). In addition to these morphological changes, ant-mimicking spiders also show behavioral modifications that reinforce their ant-like appearance (Cushing 1997). Notably, some species perform a conspicuous "antennal illusion" behavior during which the spider raises its forelegs and holds them aloft like antennae (Ceccarelli 2008).

Locomotor mimicry has often been indirectly invoked by researchers who report that ant-mimicking species *appear* to move like their models (Edmunds 1978; Jackson & Willey 1994; also see Cushing 1997 and citations within). However, despite some initial attempts at quantification (Pekár & Jarab 2011), this hypothesis has remained observational (Edmunds 2006). We propose that there are two major levels of organization at which such mimicry could exist: (1) similarity of the gait or

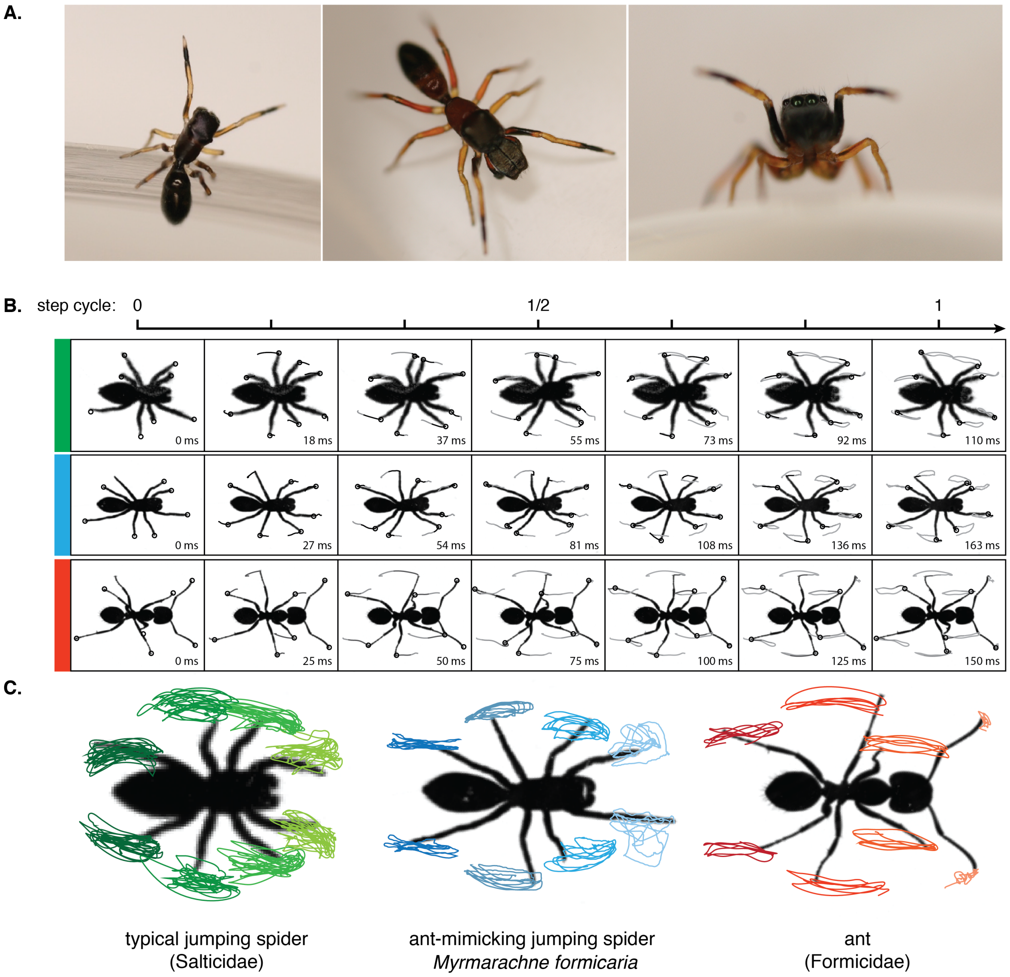

Figure 2.1. Images of the Batesian ant-mimicking jumping spider *Myrmarachne formicaria* as well limb tracings of non-mimetic jumping spiders, *M. formicaria,* and ants while walking. (A) Images of a *M. formicaria* female (left) and a male (center) showing the "antennal illusion" behavior where the forelegs are elevated similarly to the antennae of ants. Also a head-on view (right) showing the large anterior median eyes characteristic of jumping spiders. (B) Original frames from back-lit high-speed video of a non-mimetic spider (*Salticus senicus*) (top), *M. formicaria* (center), and an ant (*Formica fusca*) (bottom) walking. Each sequence shows the complete cycle of a single stride with limb tips marked by an open black circle and the path taken by the limb traced in grey-tones (darker tones show portion of the path covered since previous panel). The animal has been aligned to the center of each frame so limbs in the stance phase (on the ground) appear to move backwards as legs in swing come forwards. Limb tracking was done using the a program written by Revzen (2009). (C) The result of multiple stride cycles superimposed over animal silhouettes.

limb use of the animals, and (2) the overall manner in which the animals move through the environment, including both temporal (stationary or moving) and spatial characteristics (the shape of the route). The current study explores locomotor behavior of ants, mimics, and non-mimetic jumping spiders at both of these levels.

The mimicry of ants' gait poses a particularly interesting challenge for spiders. Spiders could conceivably walk on six legs in an alternating tripod pattern while holding two legs aloft, thus replicating the six legs and two antennae of ants. Many researchers have suggested that mimics do just this (Edmunds 1978; Reiskind 1977; Cushing 1997). While certain arachnids such as amblypygids (Ambylypygi) and vinegaroons (Thelyphonida) walk on six legs—their first pair of legs having evolved into antennae-like sensory structures—spiders propel themselves using all eight legs (Spagna & Peattie 2012). Spiders *can* cope with the loss of forelegs (Taylor et al. 2008) and are often collected from the field with fewer than eight legs (Brueseke et al. 2001). Thus the physical use of eight legs is not strictly necessary for movement or survival in spiders. The modification of the default eight-legged gait of spiders to a six-legged gait, however, would suggest a significant alteration to the underlying neural mechanisms that generate locomotor patterns (Golding & Ennos 2006).

In this study we have undertaken a detailed quantitative investigation of terrestrial locomotor mimicry at the levels of both gait and overall movement in the ant-mimicking jumping spider *Myrmarachne formicaria* (De Geer 1778). *Mymarachne formicaria* is considered to be a mimic of individuals of common temperate zone ant genera—especially ants of the genus *Formica*, though they show no close physical relationship to their models in the field (Chapter 1). These spiders neither follow foraging ants nor closely associate with their models. In order to understand locomotor behaviors in the context of mimicry, we also studied the locomotion of non-mimetic jumping spiders and multiple ant species. We used high-speed cameras to observe freely moving animals, then tracked their appendages to analyze gaits. We then tracked animals as they moved freely across a featureless arena. At the level of gait, our results demonstrate that contrary to previous suggestions, these spiders use all eight legs when walking, just like non-mimetic spiders. However, their forward motion was punctuated by short pauses (~100 ms) during which the forelegs were raised to generate an "antennal illusion." When considering locomotor behavior at the path-level, we found that—like ants—mimics spend a higher proportion of their time moving compared to non-mimetic jumping spiders. When comparing the spatial characteristics of the routes taken, we found that mimics walked extremely curved, sinusoid-like, paths—trajectories unlike those of ants moving across a featureless space. However, when we extracted trail pheromones from two ant species and recorded ants as they followed these artificially drawn trails, we found a similar sinusoid-like path. We conclude that when considering overall locomotor behavior, these mimics are imitating the behavior of ants on a trail.

Methods

All animals were collected within a 9.5 Km15 mile radius of Ithaca, NY from April to August, 2011 (gait observations) and from June 2014 to July 2015 (path-level observations). Spiders were housed in individual plastic containers in the laboratory under a 12:12 light cycle at 23°C ± 2°C, were provided with a constant source of moisture, and were sustained on a diet of fruit flies (*Drosophila melanogaster)* and/or domestic crickets (*Acheta domesticus*). Ants were used in experiments the same day as collection.

All data processing and analysis were done using Matlab (Mathworks Inc., Natick, MA, USA) with the Computer Vision System, Image Processing, Signal

Processing, and Statistics toolboxes. All scripts were written by the authors unless otherwise noted.

Gait analysis

The movement of animals was recorded using a high-speed camera system consisting of three orthogonally oriented cameras (Phantom v. 7.1, Vision Research): one camera was placed directly above the animal, viewing the animal dorsally, and the other two viewed the animal horizontally. Animals were allowed to move freely across an 8 x 10 cm glass plate covered with clear plastic tape (to improve animal traction) and located at the convergence of the three cameras. In addition to typical overhead laboratory lighting, red light-emitting diodes (Diamond Dragon, Osram Opto Semiconductors; peak wavelength ± spectrum width at 50% intensity = 625 ± 10 nm) were focused across the plate and into each camera, creating high-contrast silhouettes of the focal animal (Figure 2.1b-c). Animals showed no obvious aversion to these lights. Video recordings were captured at 1000 to 4000 frames per second, with a spatial resolution of 11.8 pixels/mm; top-down field of view = 9.44 x 7.08 cm; side views = 9.44 x 3.02 cm.

In order to track the location of the animal, we first generated an average body shape template based on the top-down image sequence. This shape was then fit to the original images in the sequence; the center of the shape (the *x,y* location of the animal) was determined as the image-based center of mass (CoM). This shape-fitting process eliminated the noise otherwise generated by the movement of the animal's legs when finding the CoM of raw images—a problem particularly prevalent in tracking non-mimetic jumping spiders whose thick legs tended to "pull" the computed CoM away from the actual center of the animal. The *x* and *y* coordinate data were smoothed using a Savitzky-Golay filter (window = 101, polynomial order = 4), and velocities were determined by taking the derivatives of these functions.

Individual legs were tracked using a modified version of a Matlab program written by Revzen (2009). This semi-automated code library was designed to facilitate the tracking of limb tips from a top-down view to enable leg-phase, or gait, analysis. Images are also rotated and centered on the animal so that the position of legs relative to one another can be easily analyzed, even if the animal does not move across the frame parallel to the axes of the original image. This code-base was further modified to enable tracking of limbs in side views. When indicated in our results, leg height was normalized by the height of the animal (see Figure 2.4), determined as the most dorsal point on the head or cephalothorax for each video sequence.

Results presented here are based on 27 recordings of the ant-mimicking jumping spider *M. formicaria* (3 females, 2 males), 15 recordings of ants (4 *Formica fusca* workers), and 23 recordings of non-mimetic jumping spiders (*Salticus senicus* 2 females; *Sitticus sp.* 1 female; *Phidippus audax* 2 females).

Overall movement

Top-down video was captured of animals moving across an arena (diameter = 75 cm). Animals were introduced to the center of the arena through the floor via a modified plastic syringe (60 ml). The arena floor was white posterboard, with a 57 cm high brown paper visual baffle around the circumference. After each use, the arena floor was wiped down with 70% EtOH to remove any deposited silk or chemical cues. Multiple arena floors were used so that each could dry completely before being reused.

Recordings were made using a GoPro Hero 3+ Black camera (1280 x 720 pixel resolution) capturing 120 frames per second. The camera was located 80 ± 7 cm above the center of the arena floor. In addition to typical laboratory lighting, two LED lamps (Utilitech, 6.5W, 450 lumens, warm white) were placed either side of the camera to more uniformly illuminate the arena. The optical distortions of the camera were corrected using the Matlab Camera Calibration functions; a 15 x 23.5 cm checkerboard was recorded at the level of the arena floor before every trial to allow for further corrections due to potential small changes in camera angle and distance.

Multiple trials were run with each animal, up to a maximum of five. Animals were allowed to acclimate inside the modified syringe for at least 5 min before the first trial, and then for 2 min between trials. Each trial began when the animal first moved off of the entry portal and concluded once it contacted the edge of the arena. By beginning trials only upon portal exit we excluded any time required for an animal to acclimate to the arena before engaging in locomotor activity.

Tracking of animal locations was done using custom written Matlab programs to find the CoM of the image of the animal. The x and y coordinates were then independently smoothed using a Savitzky-Golay filter (window = 41, polynomial order = 5); velocities along these dimensions were determined by taking the derivative of each function. For each time point, animals were determined to be stationary if their velocity was below 4 mm/sec. Stop durations were defined as the sequential number of time samples below this threshold. To explore how velocity profiles compared only when the animals were moving, we generated moving-only trajectories where each continuous stop was replaced by the mean location of the points within that stop.

In order to compare the shape of the paths taken by the animals it was necessary to generate curves that were sampled equally over space as opposed to time. The moving-only trajectories were super sampled by a factor of 100 using a cubic spline interpolation. These super-sampled splines were then subsampled by taking each point at which the animal had traveled 0.2 mm—resulting in a trajectory equally sampled along its length. The angle between each point was calculated as:

$$\theta = \arctan2(x', y')$$

where x' and y' are the derivatives of the equally sampled trajectory. Curvature was defined as the difference between these angles, calculated as the derivative of a Savitzky-Golay function fit to θ (window = 41, polynomial order = 2) (Figure 2.8a). Switchback points—corresponding to when the animal switched from turning in one direction to turning in the opposite direction—were identified along these time-independent trajectories as points where the curvature changed sign (Figure 2.8a). These switchback points were then used to divide each trajectory into sections, a portion of the trajectory bounded by switchback points on either end (Figure 2.9a). Each section was thus a continuous region in which the animal was turning in the same direction. These sections could then be characterized based on the linear distance between switchback points ($\text{Path}_{\text{linear}}$), the distance along the path actually traveled ($\text{Path}_{\text{taken}}$), and the tortuosity of the section where tortuosity = $\text{Path}_{\text{taken}} / \text{Path}_{\text{linear}}$. Finer-scale parameters were also calculated, including the height of the section (the maximum distance of the normal line between each point and the $\text{Path}_{\text{linear}}$ line; akin to the amplitude of a sine wave), and the relative height (height / $\text{Path}_{\text{linear}}$). Analysis of sections was limited to those with at least 25 data points, corresponding to sections in which the animal traveled at least 5 mm.

Trials run using featureless arenas included 50 recordings from 10 *M. formicaria* (5 males, 5 females/juveniles), 45 recordings from 10 non-mimetic *Salticus senicus* (2 males, 8 females/juveniles), 50 recordings from 10 *Lasius niger* ants (all workers), 47 recordings from 10 *Formica fusca* ants (all workers), and 15 recordings from 3 *Camponotus pennsylvanicus* ants (all workers).

Trail following

Trail following behaviors in ants are generated in response to specific chemical pheromones—though compounds and blends vary from species to species, as do the glands in which these chemicals are stored (Morgan 2009). Under normal conditions, upon finding food an ant scout will travel back to the nest, depositing trail pheromone from its abdomen along the way. Colony mates at the nest follow the trail back to the food source. On the return route they too mark the path from food to nest, thus recruiting more nest mates until the resource is exhausted (Wilson 1971).

In order to analyze the movement of ants along known and reproducible trails in the lab, we extracted chemical compounds from the abdomens of ants—including those that elicit trail-following behaviors. We used these extracts to draw paths of a specific shape. Abdomen extracts were prepared after Akino & Yamaoka (2005). Collected ants were sacrificed in a freezer before the abdomens were removed and placed into solvent at a ratio of 10 abdomens per 100 μL solvent. This mixture was allowed to rest at room temperature for at least 10 minutes. Although the initial extraction step described by Akino & Yamaoka (2005) for exploring the composition and activity of trail pheromone in *Lasius japonicus* used hexane, we found that *Lasius niger* (the *Lasius* species used in the current study) was unresponsive to hexane abdomen extracts. We found acetone to be an effective solvent for this species. In a second ant species, *Tetramorium caespitum*, hexane-based conspecific abdomen extracts did elicit robust trail-following behaviors.

After extraction, the solvent solution was loaded into a 20 μL pipette tip with a piece of cotton wick in the narrow end (similar to a fine felt-tipped pen). The abdomen extract could thus easily and precisely be applied to a substrate, generating a path approximately 2 mm wide. This was then used to draw a 45 x 35 cm rectangle with rounded corners onto a sheet of Canson art paper. Each pattern was drawn using 400µL of extract. A hole was cut at the center of this rectangle to make it compatible with the previously described floor-entry portal. Individual ants were introduced to the arena as previously described. Trials were allowed to continue for approximately 15 minutes, or until the ants left the arena. As a control, solvents without conspecific abdomens were tested in a subset of observations—these did not cause any noticeable reaction from the ants and never elicited trail following behavior. Video and tracking data was collected and analyzed as described previously. Bouts of locomotor activity in which ants had not yet contacted the extract trail or in which ants ignored the trail completely were considered to be "off-trail" and were lumped with the featureless trials previously described.

A total of 17 *Lasius niger* ants and 14 *Tetramorium caespitum* ants (all workers) were used in these trials.

Markov chain analysis

Simple first-order ransition probabilities between "moving" and "stationary" states were determined based on the total data collected for each group. These probabilities were then used to generate a simulated trial of "moving" and "stationary" states with the same total length as the original sample. The duration of stops from this simulated trial were determined and the distribution of simulated stop durations was compared to the distribution of stop durations from the actual data using a Mann-Whitney *U*-test.

Statistics

Given the prevalence of non-parametric data, the Kruskal-Wallis test was used to make multiple comparisons. The Mann-Whitney *U*-test was used when comparing between two groups that were not normally distributed. All p-values reported are after Bonferroni correction.

Results

Gait

The stepping patterns of legged animals when moving (*i.e.*, gait) can be determined by measuring the fore-aft movements of each leg over a step cycle. In the case of many arthropods (including ants and spiders) in which the limbs are splayed out to either side of the body, this is can be studied based on dorsal recordings. When viewed from above (Figure 2.1), the high-speed observations of ants and non-mimetic jumping spiders corroborated relative limb phase patterns previously described in both taxa (for ants see Zollikofer 1994, for spiders see review by Spagna & Peattie 2012). Ants were found to move with an alternating tripod gait in which the middle leg (leg II) on one side of the body moves in phase with the first (leg I) and third legs (III) on the opposite side of the body (Figure 2.2). The antennae were held out in front of the body without any clear phase-relationship in the horizontal plane relative to the movement of the legs. Non-mimetic jumping spiders most often moved with the general alternating tetrapod gait described for other spiders (Wilson 1967; Ward & Humphreys 1981). In this pattern, legs II and IV on one side moved in phase with legs I and III of the opposite side. The set of four legs were then moved in anti-phase with the other set of four (Figure 2.2). The ant-mimicking jumping spider *M. formicaria* was found to move—exclusively—on eight legs. Specifically in regards to the use of its forelegs, over 58 observed forward-moving strides, the forelegs were always out of phase with one another and roughly in phase with the other limbs in their respective tetrapod group. In short—mimics always moved with the alternating tetrapod gait characteristic of other spiders. However, when stationary, both forelegs were typically brought into phase and extended forward—generating the “antennal illusion” behavior characteristic of many ant-mimicking spiders (Edmunds 1978; Cushing 1997). The duration of these stationary episodes was sometimes very short, on the order of 100 ms (see Figure 2.2). All species showed gait patterns that differed from the described alternating-pod gaits, particularly when turning and at low speeds, when gaits appeared to shift to metachronal patterns. These patterns, however, were relatively rare. Throughout the experiment, ants moved on six legs with antennae showing no clear phase relationship with the legs, while all spider species—including mimics—used eight legs when moving forwards.

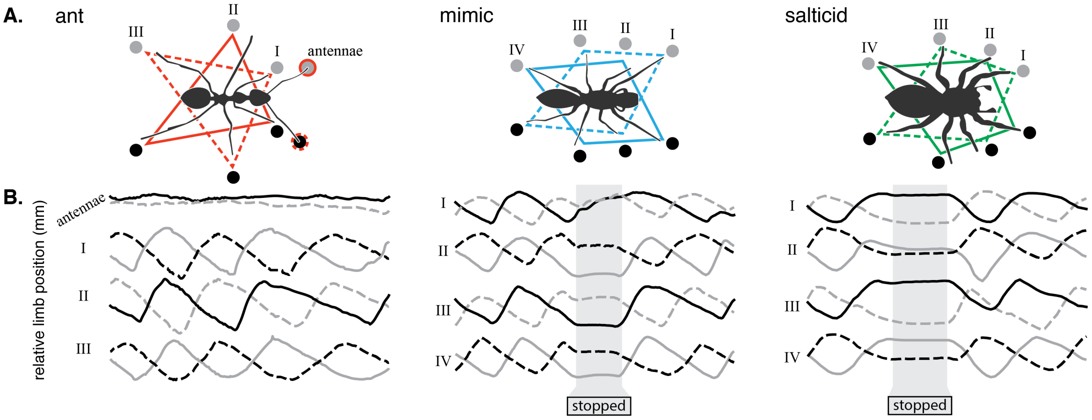

Figure 2.2. Schematic showing the gait patterns of ants (left), mimetic *M. formicaria* jumping spider (center), and non-mimetic jumping spiders (right) most commonly observed in the current study. (A) The alternating tri/tetrapod gait most often displayed by ants and spiders, respectively, are illustrated by the dotted and solid lines. In these common gait patterns, legs of a given position (i.e., the front legs) are moved in anti-phase to one another. There are therefore two sets of legs that work in unison: the dotted group and the solid group. (B) Data from traces of each limb relative to the center of the body over multiple strides with left legs in grey and right in black. Ants clearly conform to the alternating tripod gait and their antennae show no clear phase relationship with the legs in the horizontal plane. Non-mimetic jumping spiders show the typical alternating tetrapod gait and when stationary (zone marked in grey) do not change the position of their legs. The ant-mimic species walks like a typical jumping spider but when stationary its forelegs move into phase with one another—similar to the ant antennae.

Since our dorsal-view analysis of gait did not address height, we used side views to examine the actual height of forelimbs (i.e., antennae in ants and forelegs in spiders) (Figure 2.3). Since it seemed like antennal illusions only occurred when mimics were stationary, we specifically explored the relationship between forelimb height and the speed of the animal (Figure 2.4a). Relative forelimb height differed significantly based on animal type, as well as on whether or not the animal was moving or stationary (animals were deemed stationary if speed < 1 cm/sec). All groups were significantly different (Kruskal-Wallis; $X^2_{(5)} = 2.8 \times 10^5$; $p < 0.001$; see Figure 2.4b). When viewed in terms of how often a limb was likely to be in either an "up" or "down" position (with 0.75 as the relative head height threshold), non-mimetic spiders were unlikely to have raised legs in either cases (up:down ratio $_{\text{moving}} = 7.5 \times 10^{-3}$; ratio $_{\text{stationary}} = 6.0 \times 10^{-4}$), whereas ant antennae were held "up" with similar ratios regardless of whether or not they were moving (ratio $_{\text{moving}} = 0.272$; ratio $_{\text{stationary}} = 0.234$). Mimics, however, rarely raised their legs when moving (ratio $_{\text{moving}} = 0.011$) but when stationary displayed a ratio of "up" to "down" that was similar to ants (ratio $_{\text{stationary}} = 0.266$) (Figure 2.4a).

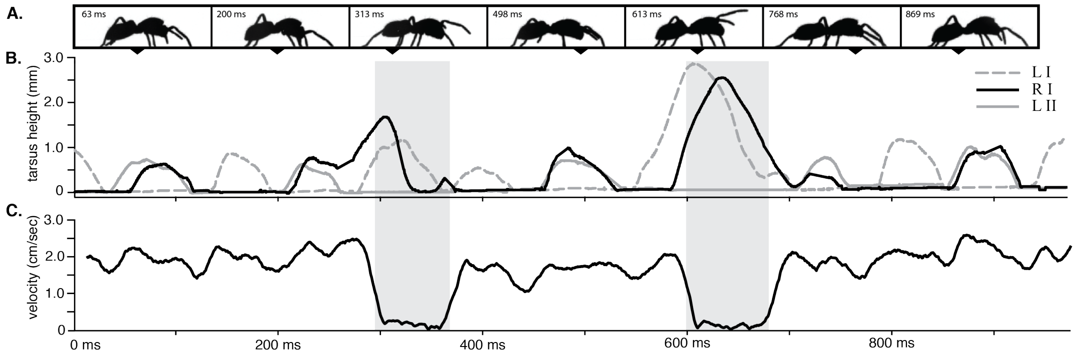

Figure 2.3. Side view of the ant-mimicking jumping spider *Myrmarachne formicaria* during a bout of locomotion recorded at 4000 frames/sec, demonstrating the "antennal illusion" behavior in which the forelegs are raised similarly to ant antennae. However, this behavior only seems to occur when the animal is stationary. (A) Original images from high-speed video. The elevated forelegs can be seen in the third and fifth frames from left. (B) Traces of the vertical position of both forelegs showing an anti-phase relationship when walking, then becoming in-phase when stationary as both forelegs are raised. A trace of the left second leg is shown for reference, demonstrating that when walking the forelegs are raised only to a height comparable to other limbs. (C) The speed of the mimic based on its location as simultaneously observed from a high-speed camera located above the animal. The grey area indicates when the animal is stationary. While the forelegs are simultaneously elevated just before these stationary periods, this occurs during the moments when the animal is decelerating into the stop, not when actively moving forwards.

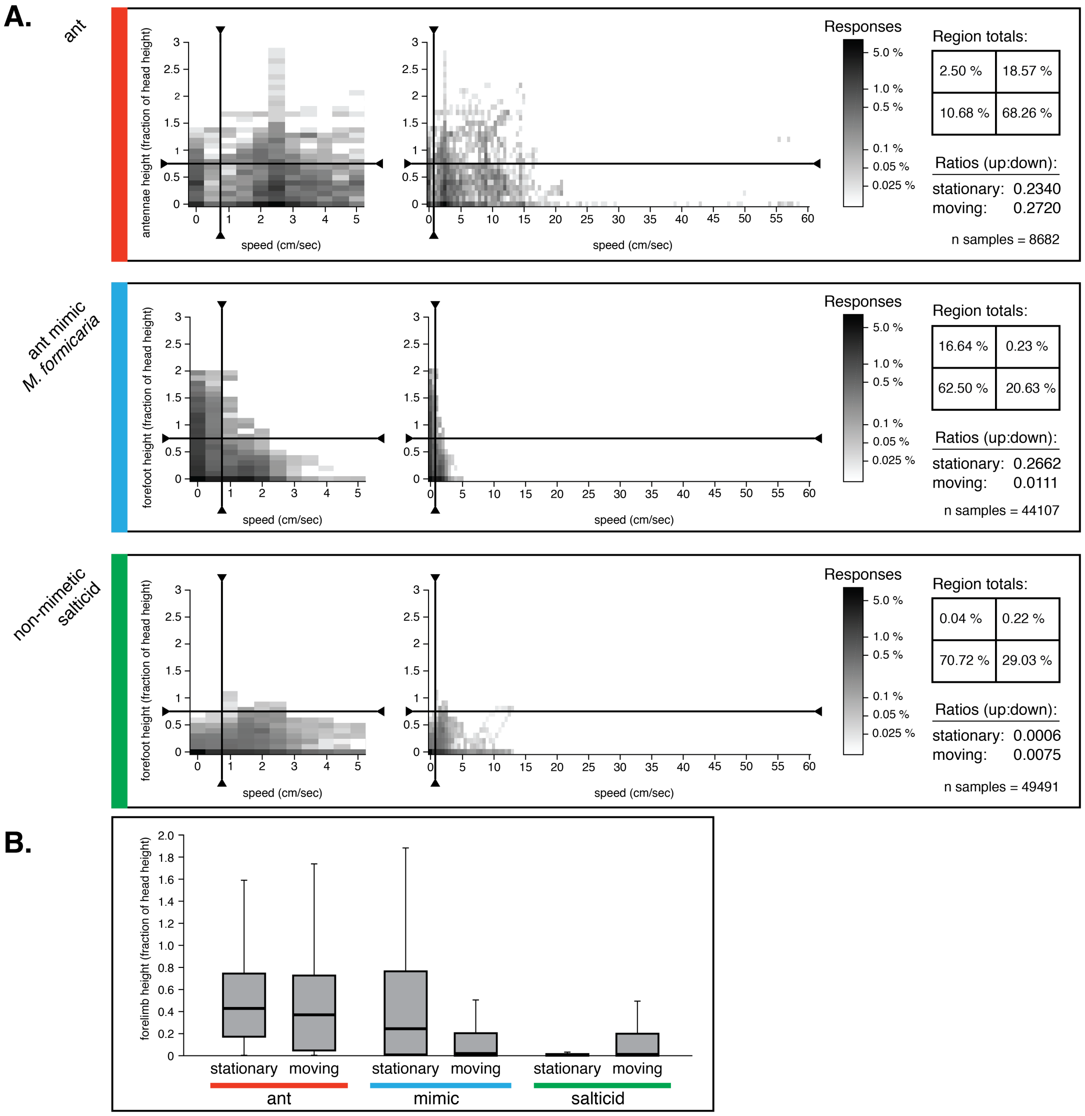

Figure 2.4. A density map showing forelimb height (antennae in ants and forelegs in spiders) based on animal speed from multiple high-speed videos to determine how the ant mimicking spider *M. formicaria* elevates its forelegs, compared with the forelimb behavior of ants and non-mimetic jumping spiders. Forelimb height was normalized by the head-height of each animal. Darker areas show zones with more observations and the density scale has been log transformed to make regions with relatively few counts more visible. (A) The plot at the left shows the same data as at center, only expanded along the x-axis. Lines overlaid on the density map divide each into four regions. Regions are intended to define zones in which the animal is stationary or moving, and in which the forelimbs are either up or down (see text) with region totals displayed at the right. Ratios show the ratio of limb up versus limb down percentages for both stationary and moving states. (B) A boxplot showing relative forelimb height based on whether a given animal was stationary or moving. Thick center lines denote group medians, while grey boxes extend to the 25th and 75th percentiles, and whiskers

to the 99th percentile of a normal distribution. Outliers beyond this range are not shown for clarity. All groups are significantly different from one another (Kruskal-Wallis; $X^2_{(5)} = 2.8 \times 10^5$; $p < 0.001$ after Bonferroni correction).

Overall movement

Example traces of overall movement are shown in Figure 2.5. The non-mimetic spider *Salticus senicus* spent a much higher percentage of time stationary (60.2 %; total time analyzed = 47 min 27 sec) than did the mimic *Myrmarachne formicaria* (22.3 %; total time analyzed = 54 min 21 sec), or any of the ants (*Camponotus*: 4.5 % [time analyzed = 6 min 29 sec]; *Formica*: 28.6 % [time analyzed = 45 min 8 sec]; *Lasius*: 16.6 % [time analyzed = 75 min 44 sec]; *Tetramorium*: 13.3 % [time analyzed = 5 min 25 sec]; *Lasisus* on trail: 2.3 % [time analyzed = 32 min 15 sec]; *Tetramorium* on trail: 4.5 % [time analyzed = 146 min 5 sec]) (Figure 2.6b). The stops made by *Salticus senicus* were significantly longer than *Formica* and *Lasius* ants, and longer than *Lasius* on trails, *Tetramorium*, and *Tetramorium* on trail, and stops made by mimics were the second shortest, with only *Camponotus* displaying shorter stops (Kruskal-Wallis; $X^2_{(7)} = 1964$; $p < 0.001$) (see distributions in Figure 2.7). We also examined how the overall time spent stationary changed as stops of a given duration were excluded—revealing that if very short stops are ignored, the percent time mimics spent moving rapidly falls to the levels of even the most stop-averse ants (Figure 2.6c).

Markov analysis revealed that with the exception of the mimic *M. formicaria* and *Lasius* ants on trails, there was a significant difference between the observed stop durations and those generated by the Markov simulation (Mann-Whitney *U*-test, $p < 0.001$). Transition probabilities for all species groups are shown in Figure 2.7. However, when using the Kolmogorov-Smirnov test to compare the distributions, themselves, observed and simulated stop durations for *M. formicaria* were found to be significantly different ($p < 0.001$) while those of the *Lasius* ants were still statistically indistinguishable (K-S test, $p = 0.376$). We were unable to reject this null hypothesis for the activity patterns of *Lasius* ants on trails, which suggests that transitions between states for this group are not based on the events leading up to that time point—the major condition of a first order Markov chain process.

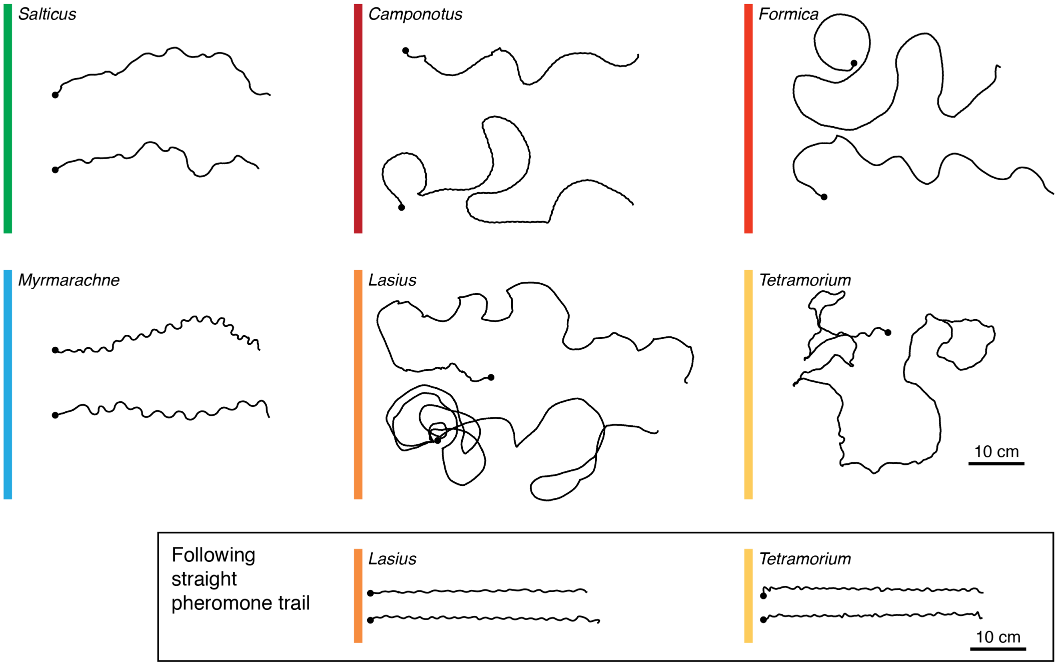

Figure 2.5. Example traces of the non-mimetic jumping spider *Salticus senicus*, the ant-mimicking jumping spider *Myrmarachne formicaria*, and four ant species, viewed from above. Black dots mark the starting position of each animal. Traces in the bottom row show ants following trails that were artificially drawn (see text); all other trials were done on flat surfaces lacking directional chemical or visual cues. All traces are shown at the same scale.

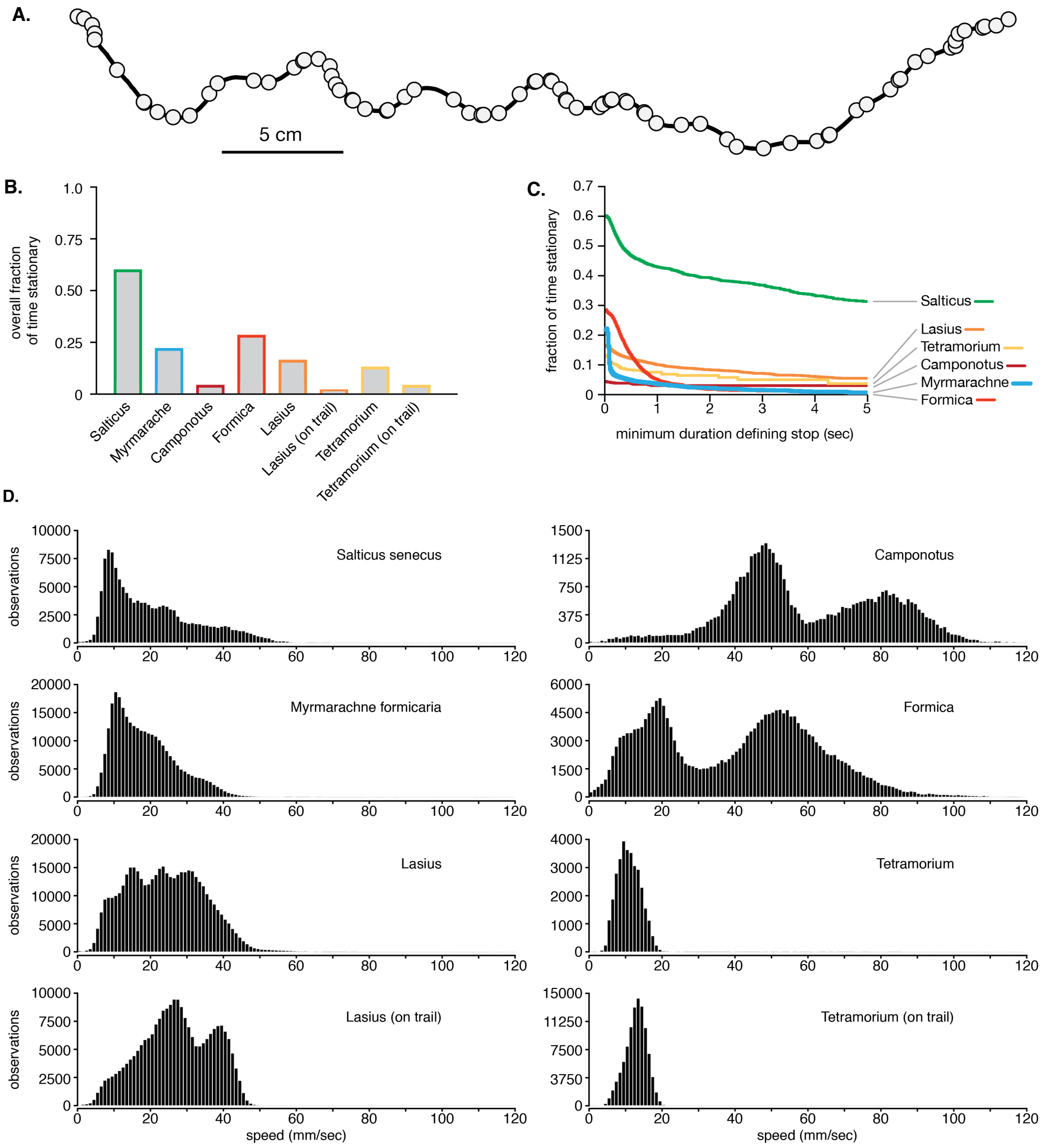

Figure 2.6. Distributions of speeds and measures of the percent of time spent moving for the non-mimetic jumping spider *S. senicus*, the ant-mimicking spider *M. formicaria*, and four ant species when allowed to walk freely across a flat surface. (A) An example trace from a single trial of a mimic walking—as in Figure 2.5—with circles indicating stops (speed < 4 mm/sec). (B) The overall percent of time spent stationary for each animal group, after having left the initial starting location in the arena. (C) The overall percent of time spent stationary as the amount of time required to define a "stop" increases. Thus for a given time value on the x-axis, all stops with a duration less than that value are not included in the calculation of overall percent time stationary. For example, this shows that while mimics were stationary roughly a quarter of the time, overall these stops were almost exclusively of very short duration.

This is unlike non-mimetic jumping spiders who retained high stationary percentages even when short stops were ignored. (D) Distributions of speeds for all species groups used in the current study (excluding high-speed video trials). All groups are statistically distinct at the $p = 0.05$ level with the exception of the non-mimetic jumping spider *S. senicus* and the ant-mimicking jumping spider *M. formicaria* (Kruskal-Wallis; $X^2_{(7)} = 8.9 \times 10^5$; $p < 0.001$, after Bonferroni correction). Of particular note is that ants tended to increase their speeds when following trails and that most ants traveled faster than the jumping spiders.

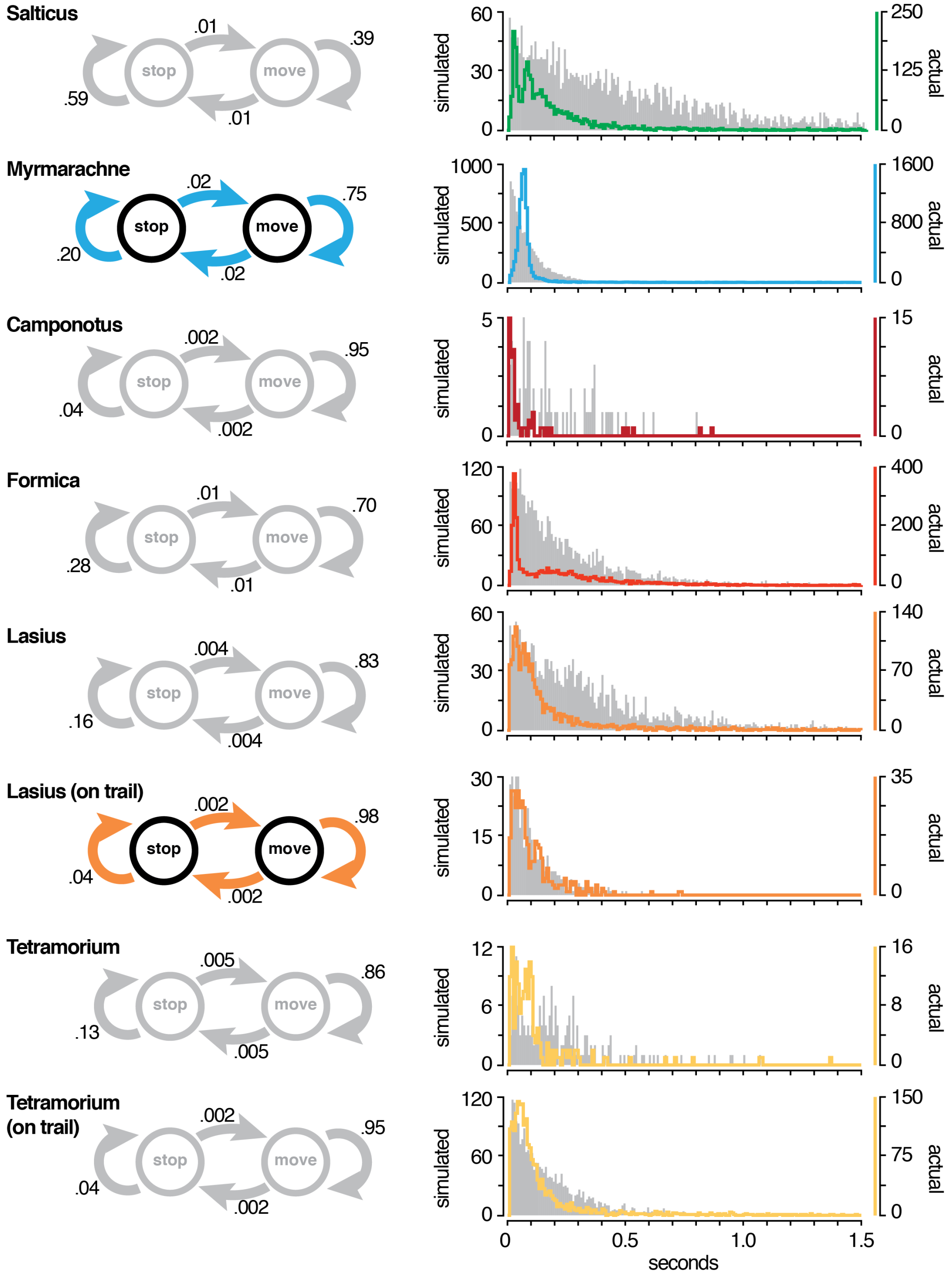

Figure 2.7. Transition probabilities between moving and stationary states for all species groups based on the movement of animals across a flat surface. Based on each set of transition probabilities, a first order Markov chain model was used to generate a simulated sequence of states. The durations of stops present in the simulated sequence (histograms shown in grey) were then compared to the actual distributions of stop durations (colored-line histograms) using a Mann-Whitney *U-test*. Markov chains

shown in grey on the left indicate species groups where there was a significant difference between the simulated and actual data ($p < 0.05$), thus cases where the simulation did not reproduce the observed behavior. The stop durations for the ant-mimicking jumping spider *M. formicaria* and for the ant *Lasius* when following a chemical trail were not significantly different from that generated by the simulation. Distributions of observed stop durations of *M. formicaria* were, however, found to be significantly different from the simulated distribution when compared using the Kolmogorov-Smirnov test ($p < 0.001$), while the observed and simulated distributions of stop durations for the ant *Lasius* when following trails remained statistically indistinguishable (K-S test, $p = 0.376$).

With the exception of *Tetramorium* sp., all ant species examined had broader walking velocity distributions than either of the jumping spiders, which were quite similar to one another (Figure 2.6d). Median speed values were: *S. senicus* = 1.7 cm/sec; *M. formicaria* = 1.7 cm/sec; *Camponotus* = 5.4 cm/sec; *Formica* = 4.4 cm/sec; *Lasius* = 2.4 cm/sec; *Lasius* on trail = 2.7 cm/sec; *Tetramorium* = 1.1 cm/sec; *Tetramorium* on trail = 1.3 cm/sec. Aside from *S. senicus* and *M. formicaria*, the velocity distributions of all groups were significantly different from one another (Kruskal-Wallis; $X^2_{(7)} = 8.9 \times 10^5$; $p < 0.001$). The bimodal distributions of *Camponotus*, *Formica*, and *Lasius* ants are particularly noteworthy compared with the relatively unimodal distributions of the other species. This may correspond to distinct gaits, with peaks representing preferred speeds for a given gait (Blickhan & Full 1987). The unimodal speed distributions of the other species are also interesting, particularly in spiders which are known to use at least two gaits—a metachronal "walking" gait at low speeds and a "trotting" alternating tetrapod gait when moving more rapidly (Spagna & Peattie 2012). However, multiple authors have noted that unlike insects (in which the coordination between limbs is tightly locked temporally [Full and Tu 1990]), the alternating tetrapod gait of spiders is much less precise (Wilson 1967). Thus it may be that when moving unperturbed at a range of speeds, the strict timing of gait in insects produces distinctly preferred speeds, while the less precise movement of spiders results in a broader distribution within each gait—though this hypothesis remains to be tested.

Curvature distributions are shown in Figure 2.8b. Mimics had the highest absolute curvature values (considering left-ward and right-ward curves together), and all groups were found to be significantly different from one another (Kruskal-Wallis; $X^2_{(7)} = 4.3 \times 10^5$; $p < 0.001$) with the exception of *Tetramorium* off-trail and *Lasius* on-trail which were statistically indistinguishable at the $p = 0.05$ level (Figure 2.8c).

The absolute curvature of both *Tetramorium* and *Lasius* increased when comparing off-trail trials with trail following trials. A comparison of tortuosity scores across all sections above the minimum length of 5 mm are shown in Figure 2.9b**,** with mimics showing the highest values and with scores for *Tetramorium* and *Lasius* increasing when on trails versus when off of trails (Kruskal-Wallis; $X^2_{(7)} = 6381$; $p < 0.001$). Relative section heights were also significantly different, as shown in Figure 2.9c-d, with mimics again showing the greatest values and *Tetramorium* and *Lasius* showing increases when on trails (Kruskal-Wallis; $X^2_{(7)} = 6426$; $p < 0.001$).

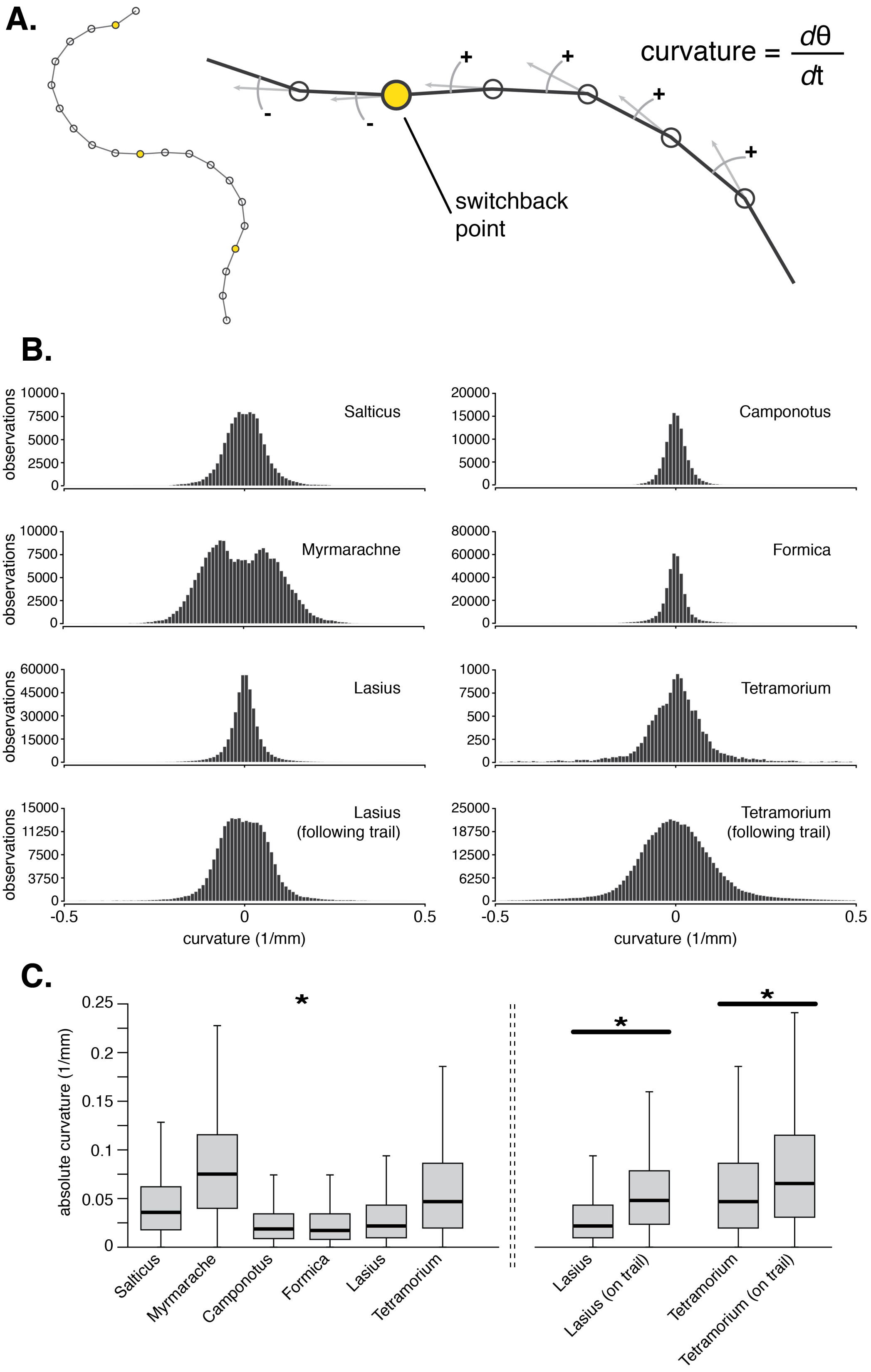
A.
curvature = $\frac{d\theta}{dt}$
+
+
+
+
-
-
switchback point
B.
observations
10000
7500
5000
2500
0
Salticus
20000
15000
10000
5000
0
Camponotus
10000
7500
5000
2500
0
Myrmarachne
80000
60000
40000
20000
0
Formica
60000
45000
30000
15000
0
Lasius
1000
7500
500
250
0
Tetramorium
15000
11250
7500
3750
0
Lasius (following trail)
25000
18750
12500
6250
0
Tetramorium (following trail)
-0.5
0
0.5
curvature (1/mm)
C.
absolute curvature (1/mm)
0.25
0.2
0.15
0.1
0.05
0
*
*
*
Salticus
Myrmarache
Camponotus
Formica
Lasius
Tetramorium
Lasius
Lasius (on trail)
Tetramorium
Tetramorium (on trail)

Figure 2.8. Basic analysis of the shape of trajectories traveled by the non-mimetic jumping spider *Salticus senicus*, the ant-mimicking jumping spider *Myrmarachne formicaria*, and four ant species moving freely across a flat surface. (A) Curvature is defined as the difference between sequential angles along an equally sampled path (see text). Thus values near 0 represent straighter portions of the trajectory, while larger curvature values are representative of more tightly curved trajectories. In the current study, each sample was separated by 0.2 mm. Switchback points were identified as points where the curvature changed sign (i.e. from positive to negative), corresponding to points where the animal switched from turning left to turning right. (B) Histograms of the curvature for each animal group. Note the unique "M" shaped distribution of curvature displayed by mimics. Also note that for the ant species with on-trail and off-trail data the distributions are broader when following trails. (C) Boxplots comparing the absolute curvature (thus treating leftward and rightward turns together) across species groups. Mimics walked paths with highest curvature. All species groups on the left were statistically distinct, and the differences between off-trail and on-trail trajectories were also significant (Kruskal-Wallis; $X^2_{(7)} = 4.3 \times 10^5$; $p < 0.001$). Boxplot parameters are as described in Figure 2.4B.

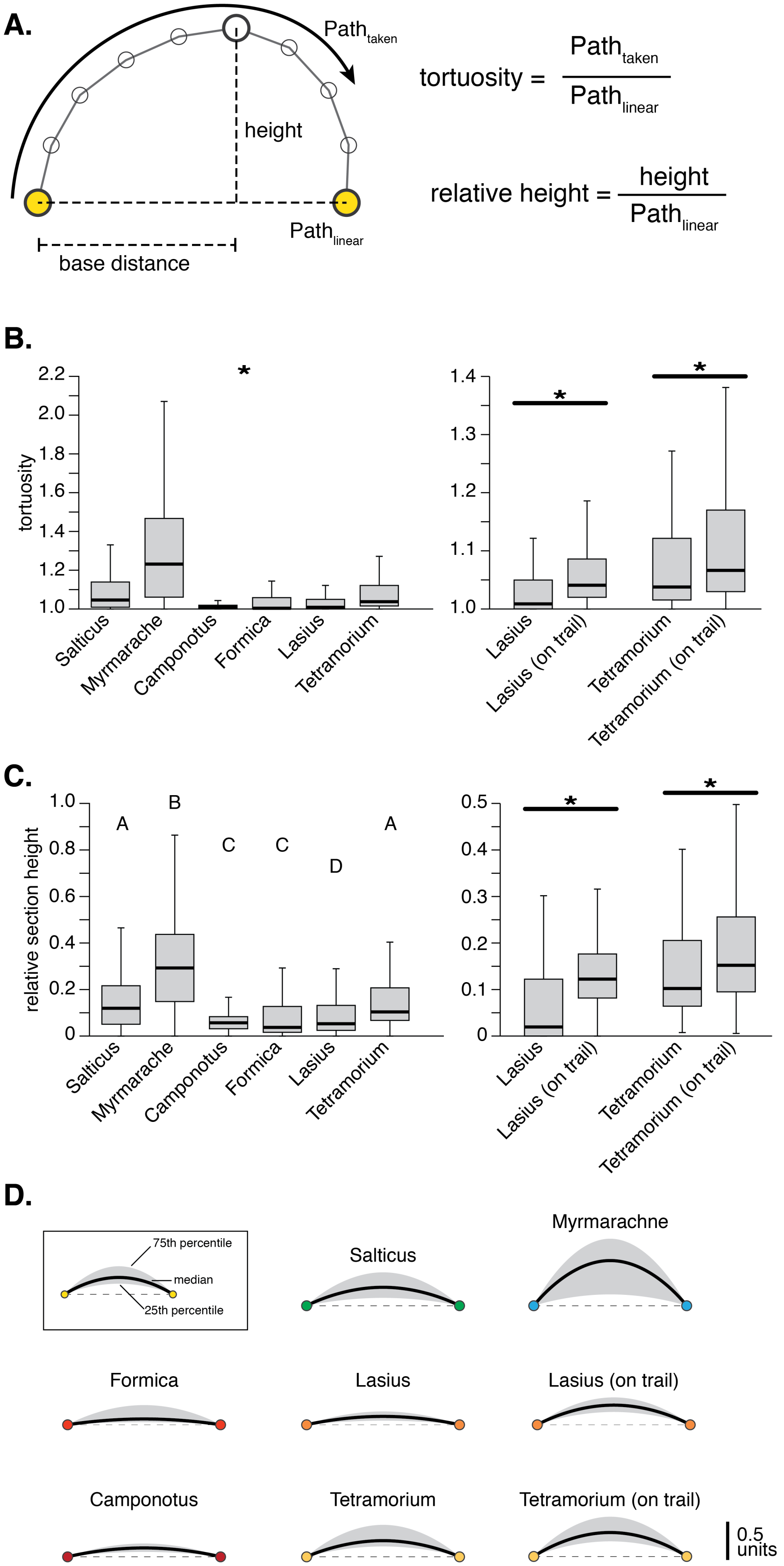
A.
Path$_{taken}$
height
Path$_{linear}$
base distance
tortuosity = $\frac{Path_{taken}}{Path_{linear}}$
relative height = $\frac{height}{Path_{linear}}$
B.
tortuosity
2.2
2.0
1.8
1.6
1.4
1.2
1.0
*
Salticus
Myrmarache
Camponotus
Formica
Lasius
Tetramorium
1.4
1.3
1.2
1.1
1.0
*
*
Lasius
Lasius (on trail)
Tetramorium
Tetramorium (on trail)
C.
relative section height
1.0
0.8
0.6
0.4
0.2
0
A
B
C
C
D
A
Salticus
Myrmarache
Camponotus
Formica
Lasius
Tetramorium
0.5
0.4
0.3
0.2
0.1
0
*
*
Lasius
Lasius (on trail)
Tetramorium
Tetramorium (on trail)
D.
75th percentile
median
25th percentile
Salticus
Myrmarachne
Formica
Lasius
Lasius (on trail)
Camponotus
Tetramorium
Tetramorium (on trail)
0.5 units

Figure 2.9. Detailed shape analysis of trajectories walked by freely moving non-mimetic spiders (*Salticus senicus*), ant mimicking spiders (*Myrmarachne formicaria*), and four ant species. Trajectories were subdivided into sections of curvature with a continuous sign, i.e. points between sequential switchback points (see Figure 2.8A). All boxplot parameters are as previously described in Figure 2.4B. (A) Schematic diagram showing basic measures including tortuosity and relative section height. Analysis excluded all sections with fewer than 25 points, corresponding to sections shorter than 5 mm. (B) A boxplot showing section tortuosity across species groups when moving in the absence of trail cues (left). Mimics showed the highest values, and all groups were significantly different from one another, including differences between ant species when off trails and when following trails (Kruskal-Wallis; $X^2_{(7)} = 6381$; $p < 0.001$). Note the change in axis for boxplots on the right. (C) Boxplot showing a comparison of relative section heights across species groups. Significance groupings at the $p = 0.05$ level are as indicated by letters above each category (Kruskal-Wallis; $X^2_{(7)} = 6426$; $p < 0.001$). Relative heights were greatest among mimics (left), while ants tested on and off of trails showed increases in relative section height when trail following. (D) A graphical depiction of the relative section height data presented in C. The grey arc region shows the range bounded by the 25th and 75th percentiles while the dark arc shows the median value.

Discussion

In order to understand how the movement of the ant-mimicking jumping spider *Myrmarachne formicaria* is—and is not—similar to their models, we have quantified the locomotor behavior of the mimics, non-mimetic jumping spiders, and multiple ant sympatric species at two distinct levels of organization. At the level of limb coordination and gait, our high-speed camera analysis revealed that contrary to previous reports about locomotion in ant-mimicking spiders (Reiskind 1977; Edmunds 1978; Cushing 1997), these mimics do not walk on six legs. Instead, they move on eight legs as is typical of non-mimetic spiders (Spagna & Peattie 2012) (Figure 2.2). We did find that, when stationary, mimics raised their forelegs and held them aloft in a manner quantitatively similar to ants (Figure 2.4)—thus producing the "antennal illusion" behavior described in other ant-mimicking spider species (Cushing 1997; Ceccarelli 2008). In terms of overall movement, mimics spent more time moving than non-mimics, and the pauses that mimics made were very short, similar to the movement patterns of ants. The shapes of mimic trajectories were unlike those of ants moving across a featureless arena as measured by curvature as well as more detailed measures of section shape. Instead, mimics were found to walk paths that were more similar to those of ants following conspecific chemical trails. Our data demonstrate that *M. formicaria* imitates ants that are actively engaged in trail following.

Our high-speed analysis revealed that contrary to previous speculation, these mimics are every bit the jumping spider when walking. They move on all eight legs, predominantly with the general alternating tetrapod gait common to other spiders (Spagna & Peattie 2012). Mimics were observed to raise their forelegs simultaneously off the substrate, as has been described in other *Myrmarachne* species (Ceccarelli 2008). It is now clear that this "antennal illusion" only occurs when the animal is stationary. Thus it may be that whenever a mimic is sufficiently stationary for an observer to examine it (be that observer human or a predator in the field), it is standing on six legs with two appendages raised in the air. This strategy would be especially effective if the visual system of the observer was unable to accurately resolve single limbs while the mimic was walking, either due to limitations in spatial resolution and/or the critical flicker fusion frequency (i.e. the maximum capture rate). Given the size of these mimics—and the fact that through a typical stride cycle each leg is in contact with the ground for less than 100 ms—it is understandable that human observers have thought that these animals move on six legs. The task of determining that the forelegs of these mimics do actually touch the ground during locomotion is further challenged by the coloration of the legs themselves. While most of the legs are uniformly colored, the second-to-most distal segment of the foreleg (the metatarsus) is heavily pigmented while the distal segment (the tarsus) is exceedingly pale (see Figure 2.1a). Thus, even when the leg is in contact with the ground, due to its coloration it may appear as though its terminus "hovers" just above the surface, a trick employed by other mimics (Reiskind 1977), but one that was ignored by the silhouette method we used in collecting our high-speed data. The functional role of this coloration would be an interesting avenue of future research.

When considering overall movement, one of the most striking differences between non-mimetic jumping spiders and ants was the percent of time that the former spent stationary (Figure 2.6). Indeed, over the course of our experimental observations, non-mimetic jumping spiders were stationary approximately 60 % of the time—over twice as long as the most stationary ant species. While mimics moved much more than non-mimetic jumping spiders, this difference was accentuated when short stops (~100-200 ms) were ignored, making the movement profiles of the mimics comparable to even the most active ants. This suggests that frequent, long pauses are a hallmark of jumping spider movement. This trait may therefore have been selected against in mimics in order to increase their similarity to ants. However, this selection for increased movement is directly at odds with the limb based mimetic adaptations that they employ. If all stops were eliminated, the antennal illusion behavior would also be lost. This simultaneous pressure could explain the prevalence of short stops. For example, predators (such as birds and some mammals [Healy et al. 2013]) with visual systems rapid and acute enough to detect that such an animal is stationary would see that the target appears to have 6 legs and antennae, and is therefore likely to be an ant—not an 8-legged spider. Predators with slower and less acute vision (such reptiles or amphibians [Healy et al. 2013]) would be unable to resolve such short stops and would therefore perceive an animal whose movement is essentially continuous—again, a trait characteristic of ants, not spiders.

When considering the shape of the routes taken by animals, mimics walked sinusoid-like paths (see Figure 2.5). This preference was so dominant that it even appeared that mimics avoided walking straight paths with low curvature—demonstrated by the bimodal “M” shaped curvature distribution (Figure 2.8b). The paths taken by mimics were also characterized by high tortuosity and high relative section height values (Figure 2.9). However, none of these features were demonstrated by ants moving across a featureless arena. Instead, these ants tended to move in looping paths (Figure 2.5) with low curvature (Figure 2.8b). In fact, when moving in the absence of significant visual or chemical cues, it was the shape-based characteristics of non-mimetic jumping spider routes that were more similar to those of mimics (Figure 2.8). However, once ants began to follow artificially applied linear pheromone trails, the shape of their routes became more similar to those of the mimics in every shape metric—even though the predetermined chemical trail path was overwhelmingly straight. Although no ant species reached curvature or section-based values as high as those of the mimics, both species saw significant shifts in all metrics towards the scores of the mimics when following trails. This change in locomotor behavior represented a transition away from long loops and towards highly curved, oscillating patterns in which ants tacked back and forth across the chemical trail. Indeed, this oscillatory-like movement matches the description of trail following in *Lasius fuliginosus* ants described by Hangartner (1967) and communicated by Wilson (1971). Although not tested here directly, it appeared that ants walked across the chemical trails until the trailing antennae passed over the edge of the trail—at which moment they would have lost contact with the signal, and therefore would tack back across the trail, repeating the process with the other antennae (an observation again matching the descriptions provided by Hangartner [1967]).

However, while ants walking on trails had shape-based scores similar to those of mimics, in no cases were the scores of the mimics identical to those of ants. Indeed, mimic scores were higher than ants in all path shape categories. This would appear to go against the traditional concept of mimicry, as one might expect selection to continuously favor mimics that resemble their models with increasing accuracy (Fisher 1958). There are a number of possible explanations for this seeming imperfection. First, it may be that the lack of curvature in the drawn trail was responsible for lowering the overall curvature scores of the ants following trails. The artificial paths draw here were almost certainly much straighter than trails would be if laid down by nest mates returning with food; thus our experimental design may have effectively acted as a high-pass filter, eliminating additional curvature that would have otherwise been present due to a gently undulating chemical trail. Second, it is possible that the ant species observed following trails in the current study are not the species that have served as the model for these mimics. Indeed, while *M. formicaria* is considered to be a generalist mimic of multiple ant species, one of the main putative models are ants in the genus *Formica* (see Chapter 1), a group represented in the current study but only in the featureless trials. It may be that when following trails these ants (which are much larger than the *Lasius* and *Tetramorium* species used in trail following trials) travel more curved paths that match the behaviors observed in the mimics. A third possibility is that the mimics actually exhibit traits that are more extreme than those of their models. Along with other hypotheses concerning so-called “imperfect mimicry,” Kikuchi & Pfennig (2013) describe a perceptual exploitation hypothesis. Based on work with flowers that attract pollinators through sexually deceptive signals (Scheistl 2004; Vereecken & Scheistl 2008), this theory is similar to concepts used in studies of sexual selection to explain the evolution and maintenance of highly exaggerated traits and preferences (Ryan et al. 1990; Endler & Basolo 1998). This hypothesis proposes that traits can be favored even if they lie far outside the usually encountered phenotypic range—as long as the selective force has an open-ended preference for these traits. In the context of the current study, if would-be-predators use path curvature as a graded metric for differentiating ants from non-ants, the seeming “inaccuracy” of mimics may instead be an example of a supernormal trait.

It should be noted that while ants required a very specific chemical signal in order to elicit this highly curved locomotor behavior, the mimic *M. formicaria* carried out these maneuvers in the absence of such cues. While in ants this tacking behavior seems to serves a mechanistic function enabling them to effectively follow chemical trails (Hangartner 1967), there is no apparent mechanistic pressure for this behavior in ant-mimicking spiders. Instead, it seems that this trait has instead been favored by natural selection due to its effect on predators—i.e., due to its power as a Batesian signal. This presents a dramatic departure from how basic locomotor behaviors—such as how an animal navigates its world—are generally considered by researchers. These behaviors are usually viewed in a time-budgeted, optimal foraging context (Charnov 1976) in which an animal is expected to maximize its chances of discovering resources while minimizing its exposure to predators and limiting its metabolic costs. Locomotor mimicry, however, provides a striking example of how even behaviors as basic as the movements of an undisturbed individual through its environment can evolve into signals (albeit dishonest signals in the case of Batesian mimicry)—shaped by the perceptual and cognitive processes of a receiver.

The fact that mimics walk trail-following-like paths in the absence of cues is also interesting because—as our data show—this means that their behavior is very different from ants that are not actively engaged in this behavior. Indeed, given that trail-mediated foraging in ants is dependent on various factors such as the proximity and quality of food sources, only some fraction of ants in a given colony are likely to be engaged in trail-following at any given time (Wilson 1971). In cases in which ant mimics associate closely with their models this seems less likely to be problematic since numerous ants in close proximity to one another are more likely to be following a trail than ants that are alone. However, since *M. formicaria* actively avoids close contact with ants (see Chapter 1), one might expect these mimics to have evolved to imitate the behaviors of solo scout ants, as opposed to ants engaging in a behavior emblematic of sociality. Despite this expectation, it appears that *M. formicaria* has instead been selected to mimic a locomotor trait that is most likely to be expressed by a large fraction of observable ants at any given time. The repeated characteristics of the tacking behavior may further reinforce the efficiency of this trait as a signal—providing observers with multiple examples of the trait within a relatively small time and space. Indeed, it seems feasible that a predator might use such often observed signals when classifying a target as ant-like.

An interesting potential consequence of the behavioral modifications required to enable locomotor mimicry is the impact that such changes may have on the lifestyle of the mimics. This is particularly relevant in systems involving locomotor mimicry because, unlike in other examples of behavioral mimicry, the expression of these behaviors is highly independent of context. While some animals produce signals to falsely convey their status in a specific context (e.g., some impending threat [Adams & Caldwell 1990; Barber & Conner 2007]), ant-like locomotor behavior is the default movement pattern for these mimics. Jumping spiders are famous for their visual prowess, enabling them to solve 3-dimensional mazes (Hill 1979; Tarsitano & Jackson 1997), to identify prey at ranges of tens of centimeters (Harland et al. 1999), and to leap onto prey from a distance (Nagata et al. 2012)—all behaviors that are seemingly incompatible with the nonstop back-and-forth movements of ant locomotion, and therefore seemingly incompatible with ant mimicry. Indeed, *M. formicaria* does not forage like other jumping spiders by stalking and pouncing on their prey from multiple body lengths away. Instead, they lunge at prey from close range, a strategy has that also been reported in other ant mimicking jumping spider species (Jackson & Willey 1994). Excellent work has been done on the details of visually guided behavior in non-mimetic jumping spiders (Land 1969; Zurek and Nelson 2012), and recent developments have allowed for the investigation of visual processing in the CNS of these animals (Menda et al. 2014). It would be fascinating to extend these lines of research to explore whether the visual systems of ant-mimicking jumping spiders have been altered by their novel behavioral adaptations.

One potential concern involving the current study might be that the observed behavior of animals introduced to an unknown and featureless arena may not be representative of their behavior in the field. This seems especially relevant when considering ants, who do not share the wandering, vagabond-like lifestyle of jumping spiders. Indeed, many ant species have been found to use navigation strategies such as path integration that rely heavily on some prior experience with an environment—especially of a “home” location (Müller & Wehner 1994). However, we contend that the behaviors that we observed in the current study fit well with previous work on ants searching for the unknown location of a target (i.e., a nest)—particularly the prominence of large loops with low-curvature (see Figure 2.5), features that have been suggested as part of a strategy used to optimally search an unknown area (Müller & Wehner 1994). Further, in both species where we were able to observe ants on and off of trails, ants increased their speed when trail-following—suggesting that animals in the featureless condition were not simply attempting to flee.

Future directions

A major challenge in studies of mimicry is the identification of the operator—the predator(s) responsible for the selection on and maintenance of mimetic traits. Indeed, the role of the operator as the selective pressure makes it central to understanding the evolution of these systems. However, field observations of predation involving specific species are rare and, in the case of mimicry, the likelihood of observing such an event is further hampered by the central tenet of the phenomenon—that predation of a mimetic species should be even more rare than if the focal species was not a mimic. In studies of invertebrate mimetic systems, the most commonly proposed predators are birds (Brower 1960; Bowers et al. 1985; Kassarov 2003) and invertebrate predators (Cutler 1991; Nelson et al. 2006; Durkee et al. 2001). We believe that our findings provide evidence that both of these groups may have a role in the evolution and maintenance of mimicry in *M. formicaria*, and that particular traits may have been under selection by different suites of predators. Appreciating the curved trajectories taken by mimics requires a visually unobstructed (and therefore likely elevated) vantage point and some minimal viewing time before the path of a target can be classified as curved (ant-like), or not. This is suggestive of the type of view that a bird might have if viewing the ground from a perch, or while walking along the ground. Further, the use of motion cues—especially speed and direction—in classifying objects has been well studied in birds, and is known to be a salient cue in informing decisions (Dittrich & Lea 1993; Hebranson et al. 2002). On the other hand, as previously suggested, it seems likely that predators with slower visual systems may be more attentive to whether or not the target appears to make long pauses. Importantly, having now identified and quantified a suite of characters likely to be important as mimetic signals, it should now be possible to directly test hypotheses such as these. Indeed, we hope that the quantitative nature of our approach will encourage and enable future researchers to use artificially manipulated stimuli to experimentally test the impact of traits independently. By presenting such stimuli to putative predators, we may be able to explore how specific cues influence predator decisions regarding the categorization of prey—the sensory-cognitive phenomena that lay at the heart of mimicry in theory and in practice (Chittka & Osorio 2007), but which are rarely explored experimentally (though see Bain et al. 2007).

It will also be interesting to examine how other ant mimics have evolved to accomplish similar feats, and to what extent the traits identified here may be considered general principles of locomotor mimicry of ants. Most *Myrmarachne* species are high fidelity mimics of specific ant species as opposed to the general mimicry of *M. formicaria*—perhaps resulting in different evolutionary outcomes. In addition to the *Myrmarachne,* ant-mimicry in spiders is thought to have evolved approximately 13 times independently (Cushing 1997), while ant mimicry across all arthropods has also evolved independently in multiple lineages (McIver & Stonedahl 1993) providing many opportunities for natural selection to have shaped numerous solutions to the challenge of ant mimicry. We also hope that this quantitative approach to terrestrial locomotor mimicry will be useful beyond ant mimics. Other classic examples of terrestrial mimicry such as cockroaches that mimic ladybird beetles

(Wickler 1968) or even the spectacular juvenile *Eremias lugubris* lizard that mimics well defended “oogpister” beetles (Carabidae: *Anthia* spp.) in southern Africa (Huey & Pianka, 1977), would be fascinating candidates. These approaches may even prove useful in exploring mimicry systems beyond terrestrial habitats—for example in marine environments where there are numerous anecdotal observations of locomotor mimicry (see Randall 2005 for a review). Here we have shown how the quantitative study of a spider’s motion reveals a system as rich and intricate as the famous static traits characteristic of passion-vine butterflies and ringed snakes, underscoring the importance of considering locomotion and behavior in studies of mimicry.

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CHAPTER 3

NEURAL AND BEHAVIORAL EVIDENCE FOR AIRBORNE ACOUSTIC PERCEPTION IN A JUMPING SPIDER

Abstract

Spiders are famous for their extraordinary sensory capabilities. Particularly remarkable is their ability to perceive mechanical disturbances, including minute changes in the movement of air particles, via the deflection of specialized hairs called trichobothria. While previous work has explored the neurobiology and mechanics of sensory hairs in the context of prey capture by the large wandering spider *Cupiennius salei* (Ctenidae), little has been done outside of this system. Here we investigated airborne acoustic mechanoreception by the jumping spider *Phidippus audax* (Salticidae) using behavioral trials and neurophysiological recordings. Behaviorally, spiders responded to 80 Hz tones (55 dB SPL) by freezing, but did not respond to a higher tone (2 kHz). Extracellular recordings of electrical activity from units in the brain showed responses to low-frequency airborne stimuli (16 to 2048 Hz) and to direct mechanical stimulation of single sensory hairs over a similar frequency range. All airborne stimuli originated more than 2 meters from the animals—over twice the functional distance reported in other arthropods with similar sensory systems. Our findings show that these common spiders can "hear" over large distances, greatly expanding the previously assumed sensory range of these animals.

Introduction

The hairiness of spiders is well known, even to the casual observer of arachnids. However, that many of these hairs are sensory organs is less obvious and less well known. Spider hairs, seemingly simple structures, have a number of highly specific roles: some hairs have pores that aid in chemoreception, other thicker hairs enable tactile perception (Foelix 2010). Hundreds of other thin hairs are arranged in arrays across the bodies and legs of these animals, acting as detectors of even the most minute changes in air movements (Barth et al. 1993), and enabling spiders to respond to airborne acoustic stimuli.

Humans usually associate the perception of airborne stimuli with our own sense of hearing, and therefore with structures like our own ears. However, ears (and similar structures that have independently evolved numerous times in other groups such as insects [Hoy & Robert 1996]) specialize in detecting just one aspect of airborne acoustic information: rapid changes in air pressure. Yet the mechanical disturbances that generate sounds also cause changes in the velocity of the air particles themselves—a phenomenon coupled to changes in air pressure but not its equivalent (Robert & Hoy 2007). The differences between these two forms of sound are often referred to "far-field" and "near-field" because the former (air pressure) retains power more efficiently over distance than the latter (particle velocity) (Robert & Hoy 2007; Jacobsen 2007).

The filiform hairs of insects and the analogous trichobothria hairs of arachnids are unable to access information contained in the pressure component of a sound (see Barth 2014 for a discussion of the likely independent evolutions of these structures in arthropods). Since they can register changes in the flow of air particles around them, they are part of a class of structures known as medium-flow sensors (Casas and Dangles 2010; Barth 2014). These structures should not be seen as "second-rate" ears—to the contrary, these mechanoreceptors are astonishingly sensitive; estimates place their thresholds for response near the very limits imposed by Brownian motion (Shimozawa et al. 2003; Bathellier et al. 2012). The behaviors enabled by these structures are no less impressive: for example, the large spider *Cupiennius* (Ctenidae) can, without any visual cues, capture flying prey even at a distance of 20 cm away (Barth et al. 1995). Analogous hairs enable crickets and cockroaches to escape from danger by detecting the wave of air preceding an on-rushing predator (Dangles et al. 2006, Casas et al. 2008, Dupuy et al. 2012).

These small and sensitive sensors have fascinated biologists, physicists, and engineers alike. Years of study have resulted in a rich and interdisciplinary understanding of the behavior, neurophysiology, and physics of many of these systems (see Barth 2004 and citations within). However, among arachnids, the vast majority of work has focused on the previously mentioned banana spider, genus *Cupiennius* (Barth 2002; though also see Santer & Hebets 2008 and Santer & Hebets 2011 for work in amblypigids [Arachnida, Amblypygi]). These very large (adult leg-spans exceed 10 cm) tropical wandering spiders do not rely on capture webs, nor do they possess acute vision (Barth 2002). Instead, they are mechanoreception specialists—relying on air and substrate-borne vibrations for most of their predatory and mating decisions (Barth 2002). In much the same way that other sensory specialists—such as bats (Moss & Sinha 2003; Connor & Corcoran 2012) and electric fish (Caputi & Budelli 2006; Nelson & MacIver 2006)—have served as model systems for the study of unique sensory systems, *Cupiennius* has become fundamental to our understanding of trichobothria.

Trichobothria, are not unique to *Cupiennius* (Barth 2014). Yet, few recent studies have explored medium-flow sensory systems in other spider species or in behavioral contexts outside of prey capture (for an exception see Klärner & Barth 1982; also see Friedel and Barth 1997 for response of *Cupiennius* to wind). Indeed, our current understanding of the neurophysiology and physics of airborne mechanoreception in spiders—and the dependent behaviors of sound reception—is almost entirely based on airborne mechanoreception in *Cupiennius* (Foelix 2010). The work that has been done outside of this model system suggests that the reception of airborne information is important in other species and contexts. For example, behavioral studies have explored the role of this sensory system in short range predator detection and avoidance by fishing spiders (Pisauridae) (Suter 2003) and in mate choice among wolf spiders (Lycosidae) (Rundus et al. 2010).

In the current study we sought to explore the perception of airborne stimuli by the jumping spider *Phidippus audax* (Salticidae). The most speciose family of spiders (Platnick 2014), jumping spiders are best known for their high resolution principal eyes (Harland et al. 2012). Behaviorally, they are renowned for their active pursuit of prey (Nelson & Jackson 2011) and for elaborate courtship rituals that combine substrate vibrations with striking visual displays (Elias et al. 2006). Like *Cupiennius*, salticids are also covered with sensory hairs. Unlike *Cupiennius*, however, jumping spiders are widely distributed geographically and are much smaller (on the order of millimeters to two centimeters) than their banana spider cousins.

Using behavioral assays and neurophysiological recordings, our work examined the responses of *P. audax* to airborne acoustic cues. Thanks to recent methodological advances (Menda et al. 2014), we were able to made extracellular neurophysiological recordings from neurons in the brains of these spiders; the majority of units responded to stimulus frequencies between 16 and 512 Hz—similar to recordings reported from more peripheral neurons in *Cupiennius* (Barth & Höller 1999). We were also able to stimulate single trichobothria hairs on the legs of spiders using a mechanical microshaker; we found that this was sufficient to generate action potentials in neurons that also responded to airborne cues—suggesting that these trichobothria are implicated in processing airborne stimuli. Behaviorally, we found that these spiders responded to low frequency (80 Hz) tones by freezing by freezing, but did not respond to tones a higher frequency (2000 Hz). While work in *Cupiennius* has shown responses to sources at maximum ranges between 20 and 70 cm (Barth et al. 1995), we obtained responses to stimuli at ranges in excess of 2 m. We discuss the importance of these findings for our understanding of the sensory ecology of jumping spiders, as well as for our broader understanding of airborne acoustic reception.

Methods

Adult and penultimate *Phidippus audax* were collected from areas in and around Ithaca, NY, USA, between November 2012 and December 2014. Spiders were housed individually in the laboratory on a 12:12 L:D light cycle, were provided a constant source of moisture, and were sustained on a diet of domestic crickets (*Acheta domesticus*).

Neurophysiology

Only adult females were used for electrophysiological experiments. Spiders were cold anesthetized and held in place using a specifically designed 3D-printed holder (see Menda et al. 2014) and Kerr dental sticky wax (58ºC melting point; Syborn Kerr, Emeryville, CA, USA). Extracellular recordings were made using a 4MΩ Parylene-insulated tungsten electrode (part #WE10014.0F5; MicroProbes Inc., Gaithersburg, MD, USA), passed through a small hole in the cuticle (following Menda et al. 2014) and directly into the brain. Recordings were made from a portion of the brain near the arcuate body—a structure thought to be one of the main cites of multisensory integration in spiders (Hill 1975; Babu & Barth 1984; Homberg 2008). However, while previous work reported visual responses from neurons in this structure (Menda et al. 2014), the current study targeted an area just below this visual sensitive region. Recording location was based on external morphological features readily identified under a stereomicroscope (Wild M3Z Leica Microsystems GmbH, Wetzlar, Germany; maximum magnification of 800x), with electrode placement guided by stereotactic micromanipulators (MM-3, Narishige International USA, Inc., East Meadow, NY, USA). Once in place, the electrode was advanced using a digital hydraulic microdrive (Model 607W, David Kopf Instruments, Tujunga, CA, USA). A second sharp tungsten electrode was inserted into the opisthosoma (homologous to the abdomen in insects) to serve as a ground. Electrical activity was amplified by a differential AC microelectrode amplifier (amplified 10,000x; bandpass filtered 100Hz-5,000Hz with a 60Hz notch; fitted with associated headstage; Model 1800 A-M Systems, Sequim, WA, USA). This analog signal was then converted into a digital signal (NI PCI-MIO-16E-1, National Instruments, National Instruments, Austin, TX, USA) and recorded on a PC (Windows 7; 64-bit; Microsoft Corporation, Redmond, WA, USA) using the data acquisition software Spike Hound (previously g-Prime, Lott et al. 2009) at 15000 or 20000 samples per second per channel (a maximum of 4 channels were simultaneously recorded for a maximum overall recording rate of 80000 Hz). Recordings were done on a vibration-isolating air table (Micro-G, Technical Manufacturing Corporation, Woburn, MA, USA) fitted with a 5-sided custom built wire-mesh Faraday cage and acoustic grid foam. Typical recordings yielded one or two clearly distinguishable spiking units and the spike sorting program Wave_Clus (Quiroga et al. 2004) was used to isolate responses of individual neural units by grouping candidate spike waveforms based on amplitude and coefficients of a wavelet decomposition.

Acoustic stimuli

Acoustic stimuli were generated using custom-written Matlab programs and were played via a studio monitor speaker (Mackie HR824) driven by a stereo amplifier (Nikko NA-790). Tones were 500 ms long, with 1.5 sec between tones and were presented in randomized order. Amplitude thresholds were tested using a frequency sweep (linear ramp from 50 Hz to 400 Hz) with a duration of 500 ms and 1 sec between each presentation; different amplitude values were presented in randomized order. The speaker was located 2 m and 50 cm below the level of the animal. A calibrated ¼ inch microphone (4135 microphone with 5935 amplifier, and 4420 piston phone; Brüel & Kjær, Denmark) was oriented towards the sound source and placed within 5 cm of the animal such that the distance between the source and the microphone was equal to the distance between the source and the animal. Microphone signals were converted from analog to digital and recorded in the same manner as the electrophysiological recordings.

Verifying isolation of airborne cues

A Doppler laser vibrometer (Polytec) was used to verify that acoustic signals were being received via airborne cues and not via substrate. Vibratory recordings were made by focusing the Doppler laser vibrometer on the leg of the spider while playing acoustic stimuli at a range of amplitudes. Signals from the vibrometer were recorded as described for acoustic and neurological recordings. Laser vibrometry recordings showed no stimulus-induced vibrations for stimuli amplitudes of 89 dB and below (amplitudes as measured at the location of the animal). When amplitudes reached 94 dB and above there was evidence of stimulus-induced vibrations—we therefore limited our test stimuli to amplitudes below this 89 dB threshold.

Stimulating single trichobothria

In a subset of experiments a Piezo microshaker system (E-500, Physik Instrumente GmbH & Co. KG, Karlsruhe, Germany) was used to drive individual hairs. The shaker was mounted to a micromanipulator and positioned using the stereoscope so that it contacted a single sensory hair on the patella of one of the forelegs. Stimuli played through this shaker were identical to the airborne stimuli in terms of signal design (i.e. frequency composition and duration). However, these direct mechanical stimuli were not verifiably comparable in amplitude to airborne stimuli since the physical displacements of single hairs as driven by airborne signals are not known. Control trials were run in which the shaker was activated while out of contact from the spider (~ 1 cm away). These failed to generate a response in acoustically sensitive neurons—confirming that responses found using this apparatus were due to the physical displacement of the hair by the actuator and not airborne by-products of the microshaker's activity. This microshaker contains a reference sensor to measure actual displacements of the actuator. Signals were passed through the analog to digital converter and recorded in parallel with other data.

Neural response statistics

In order to enable statistical validation of neural responses, each tone was presented multiple times. In experiments investigating frequency sensitivity, each tone was repeated 30 times. In experiments exploring amplitude thresholds, each amplitude was repeated 40 times. To determine if neural responses were significant, a paired t-test was used to compare firing rate (spikes per second) during the 500 ms stimulus period with firing rate for a post-stimuli interval of similar duration.

All data processing and statistical analysis were done using Matlab.

Behavior

When studying the perception of airborne signals, particularly in invertebrates, precautions must be taken to ensure that the signal is actually being received by the animal via the intended medium. This is especially true in spiders, whose seismic vibratory sense is so acute that even acoustic stimuli originating in the air (i.e., from a speaker) can cause substrate vibrations of sufficient amplitude to elicit a behavioral response (see Lohrey et al. 2009). This can make it difficult to determine which sensory system is responsible for signal reception. Behavioral trials were therefore carried out on a 25 x 25 x 2 cm, 14.3 kg, stone block to ensure that the airborne stimuli did not cause the substrate to vibrate (Hebets 2005). An arena was constructed from this block by surrounding it with a wire mesh screen (2 mm openings). A soft paintbrush was used to discourage spiders from climbing the walls of the arena. Trials were excluded if any part of the animal was in contact with the arena wall when the stimulus was presented. After each trial, the arena was wiped down with 70% EtOH to prevent accumulation of silk and chemical cues.

Two stimulus frequencies were used: 80 Hz and 2000 Hz (sample rate 44100 Hz). These frequencies were chosen because our own recordings of neural units in the CNS and previous work with trichobothria in other spider species has demonstrated that these systems are typically sensitive to relatively low frequencies (< 1000 Hz) (Barth et al. 1993). We also hypothesized that the lower (80 Hz) frequency was likely to be behaviorally relevant to *P. audax* as this is in the range of fundamental frequencies produced by flying insects (both predators of jumping spiders such as wasps [Sudo et al. 2001], as well as potential prey such as flies [Klopsch et al. 2013]). Stimuli tones with a duration of 2 sec were constructed using Matlab (Mathworks Inc., Natick, MA, USA). A 2 sec silent period was also used a control to quantify any spontaneous occurrences of the behaviors measured—thus aside from frequency the protocol for administering these controls was identical to that of the high and low frequency treatments. Tones were played through a studio monitor speaker (Mackie HR824, LOUD Technologies, Woodinville, WA, USA) located 2 m away and 0.5 m below the arena, driven by a stereo amplifier (Harman/Kardon HK6100, Stamford, CT, USA). Stimulus amplitude at the center of the arena was set to 55 dB SPL using a calibrated ¼ inch pressure microphone (model 4135, amplifier model 5935 Brüel & Kjær; calibrated as stated above). To ensure that spiders were in comparable positions from trial to trial (i.e., near the center of the arena) when the stimuli were administered, tones were triggered by a researcher via a PC. Individual spiders were assigned to a given stimulus frequency (either 80 Hz, 2000 Hz, or the silent condition) and experienced 4 presentations of that treatment. Trials were omitted form our analysis if the animal was later determined to have been in contact with the side of the arena at stimulus onset. However, for every animal the final analysis included at least 3 responses. There was a minimum of 15 seconds between the presentation of each tone. All trials were filmed from above using a digital camcorder recording at 60 Hz (Panasonic HC-X920, Japan) and all responses were scored based on these recordings. "Responses" were defined as any change in behavior during a 2 sec window following tone onset. Thus trials in which the spider continued any previously ongoing behavior (such as walking) for the entire 2 sec period were scored as "no response." Positive responses were further categorized as one of four behavioral events: crouch (in which the legs were pulled closer towards its body), freeze (in which the spider halted its current behavior), run (in which the spider began to move), and turn (in which the spider preformed a reorienting rotation). The time elapsed between stimulus onset and response was recorded. Statistical tests were carried out using the statistical software JMP Pro v11 (SAS Institute Inc., Cary, NC, USA).

Results

Neurophysiology

Single units showed responses to both airborne and mechanical stimulation of single trichobothria (Figure 3.1). When presented with airborne stimuli with

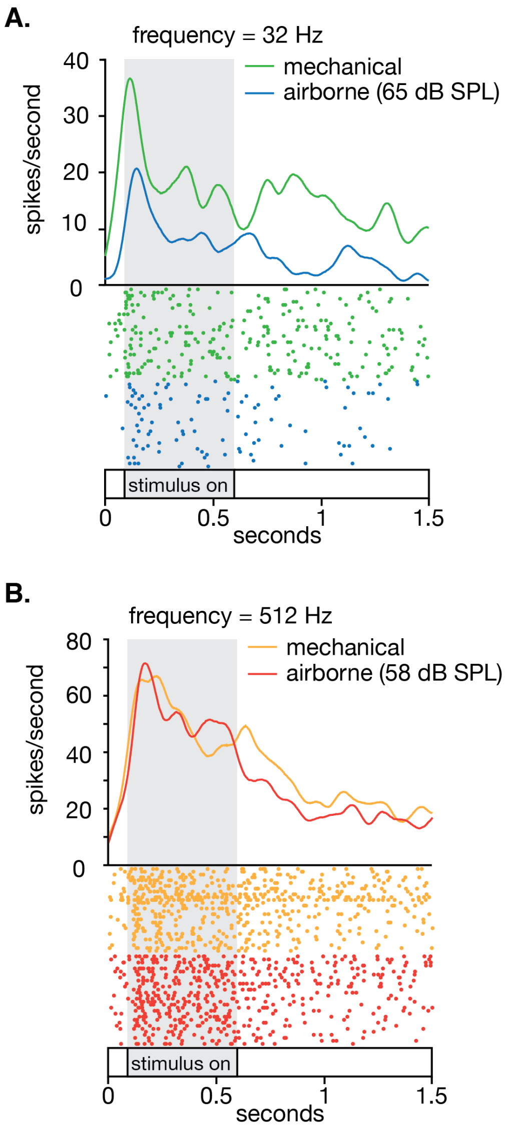

Figure 3.1. Examples of neural activity from extracellular recordings of two independently recorded neural units in the brain of *Phidippus audax.* Both single neural units responded to airborne stimuli and to the mechanical stimulation of a single trichobothrium hair on the patella of the foreleg. Rasters show spike times and line histograms (smoothed with a Gaussian filter, SD = 36 ms) show a summary of all 30 trials for each stimulus set. The grey bar denotes the 500 ms time interval when the stimulus was present and stimulus frequency is as stated for each recording. Example shown in (A) is the same as the unit summarized in Figure 3.2a, while (B) is the unit summarized in Figure 3.2b.

amplitudes between 45 and 80 dB, units ($n = 3$) showed significant responses to frequencies between 16 Hz and 2048 Hz (t-test, $p < 0.01$, repeats per tone, $n = 30$), though only one cell responded to tones above 512 Hz (Figure 3.2). However, because stimuli of different frequencies were tested at different amplitudes, we were unable to directly explore the relationship between frequency, amplitude, and neural response. Thus while our results do show whether or not a response to a given frequency was significant, this data should not be interpreted as the minimum amplitude thresholds for each frequency. Overall airborne amplitude thresholds were determined based on the sweep stimuli and showed variation between cells ($n = 4$), with response thresholds of 48.62 dB, 54.21 dB, 63.39 dB, 77.84 dB (determined as mean lowest stimulus amplitude with a significant response; t-test, $p < 0.01$, repeats per amplitude, $n = 40$) (Figure 3.3).

The activity patterns of neural responses to airborne stimuli versus mechanical stimuli were similar (Figure 3.1), but because the deflection of hairs in response to airborne stimuli was unknown, direct comparisons between firing rates in response to these two stimuli types could not be made. Single units that responded to both airborne signals and the mechanical stimulation of a single trichobothrium hair showed similar though not identical frequency tunings; the majority of responses were in the 32 and 512 Hz range (Figure 3.2a, 2b).

All units were active in the absence of stimuli (see Figure 3.1). However, because all experiments were carried out with levels of background noise typical of a laboratory setting (approximately 45 dB, due to building ventilation, etc.), it is unclear whether this represented spontaneous neural activity or simply a response to these extraneous airborne disturbances.

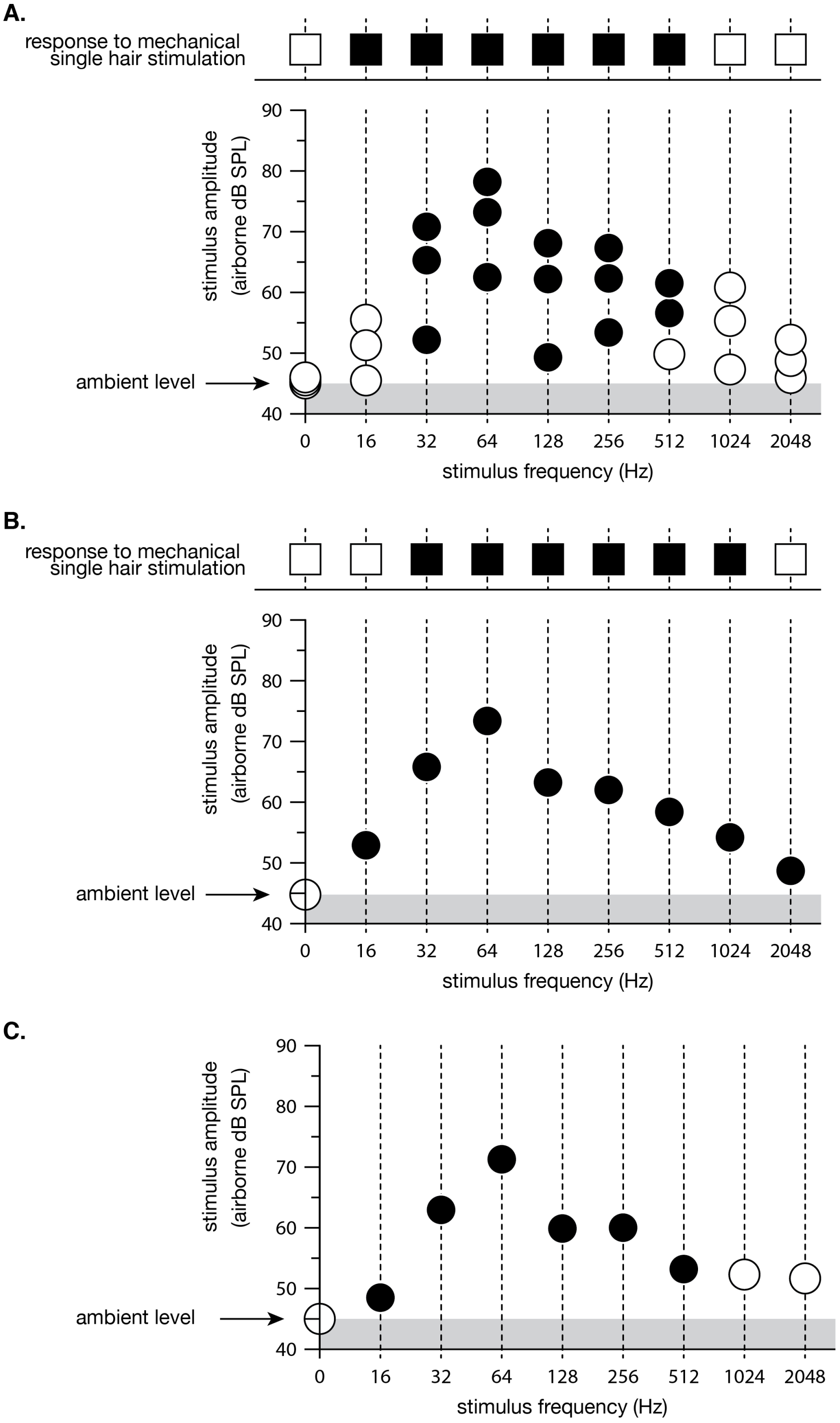

Figure 3.2. A summary of extracellular recordings of three independent single units in the brain of *Phidippus audax* in response to tones of various frequencies and amplitudes. Black shapes indicate responses in which the unit fired significantly more during the stimulus than in the post-stimulus interval (each tone repeated 30 times, t-test, $p < 0.05$) and white shapes indicate trials in which there was no significant

difference in response. All amplitudes were measured at the location of the animal. In (A) and (B) squares at the top of the figure indicate responses of these units to the mechanical stimulation of a single trichobothrium hair on the patella of the foreleg of the animal. The ambient sound level for each set is shown in grey.

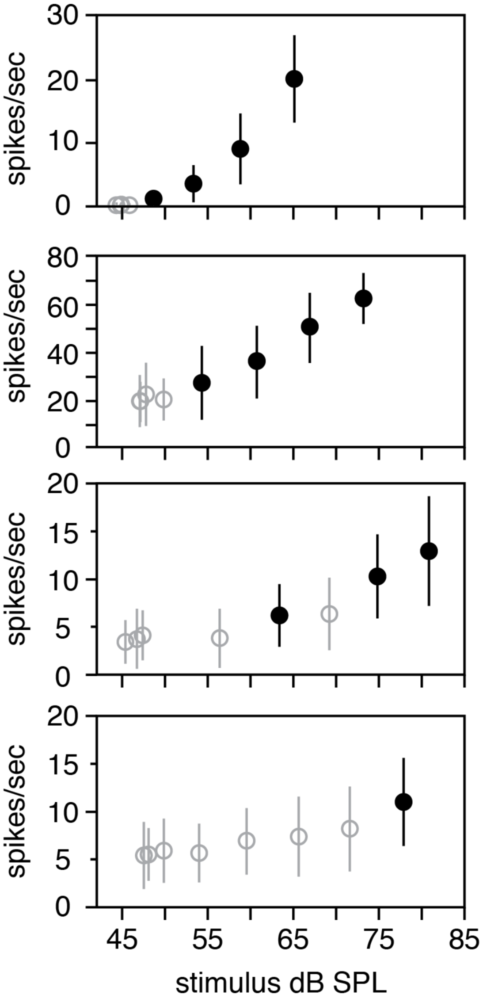

Figure 3.3. Difference neurons have different amplitude thresholds. Extracellular activity of four independent neural units in the brain of *Phidippus audax* in response to airborne acoustic stimuli (500 ms linear sweeps, 50 Hz – 400 Hz) at a range of amplitudes. Black markers indicate responses that were significantly greater (increased activity during stimuli compared to the post-stimulus interval, repeats per amplitude n = 40, t-test, $p < 0.05$), while grey markers show responses that were not significantly greater. Stimulus amplitude was measured at the location of the animal.

Behavior

We calculated the probability of response for each spider (number of responses/number of trials), and compared these individual-based scores across treatments to determine whether responses to each condition were significantly different (ANOVA: $F_{2,52} = 20.8832$, $p < 0.0001$). Using the post hoc Tukey HSD test to compare means, we determined that the response to the low (80Hz) tone (mean probability of response ± standard deviation: 0.62 ± 0.32; $n_{animals} = 23$, $n_{tone\ presentations} = 92$) was statistically greater than the response to the high frequency (2000Hz) tone (0.22 ± 0.22; $n_{animals} = 22$, $n_{tone\ presentations} = 84$; $p < 0.0001$) and to the negative control (0.08 ± 0.14; $n_{animals} = 10$, $n_{tone\ presentations} = 38$; $p < 0.0001$). There was no significant difference between the frequency of responses to the high tone and the silent control ($p = 0.3288$) (Figure 3.4a). Since each spider was exposed to a tone repeatedly, we also tested for an effect of trial number on response for each frequency using a nominal logistic regression; we found no significant effect of trial in any treatment ($p > 0.05$). In order to model the probability of response for our results, we used a standard logistic regression with parameters for frequency, trial number, and an interaction term (frequency × trial). This model showed a significant effect of both the 2000 Hz and 80 Hz treatments when compared with the control tone ($p < 0.001$; see Table 3.1 for report of all model parameters).

Within the responses to the 80 Hz tone, the most common response type was freezing ($n = 47$), followed by crouching ($n = 11$), running ($n = 7$), and turning ($n = 4$) (Figure 3.4b). Response latency varied significantly with behavior (ANOVA: $F_{3,65} = 20.9968$, $p < 0.0001$). A post hoc Tukey HSD test revealed that response latency for freezing (mean ± standard deviation; 118 ± 129 ms) was significantly shorter than for crouching (779 ± 512 ms; $p < 0.0001$), running (525 ± 319 ms; $p = 0.0062$), and turning (686 ± 628 ms; $p = 0.0024$) (Figure 3.4c). There was no significant difference

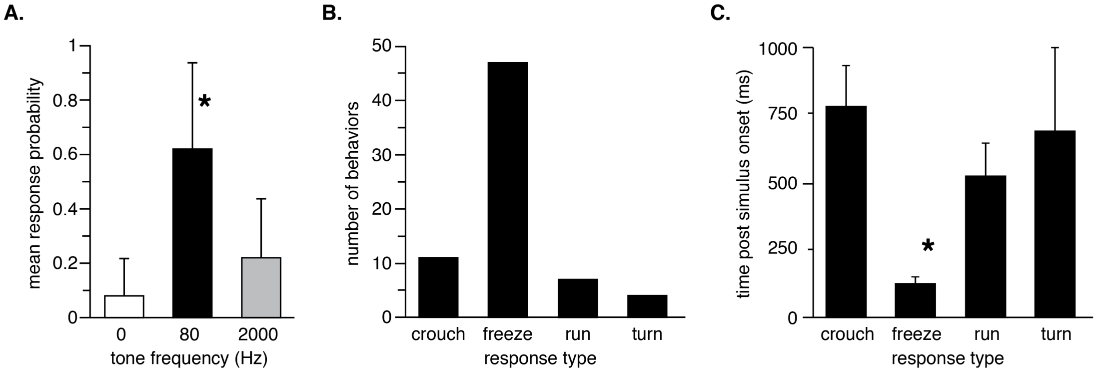

Figure 3.4. Results from behavioral tests in which unrestrained jumping spiders, *Phidippus audax*, were presented with 4 presentations of a given airborne tone: 0 Hz ($n_{spiders} = 10$), 80 Hz ($n_{spiders} = 23$), or 2000 Hz ($n_{spiders} = 22$) from a speaker source 2 m away and 0.5 m below the level of the animal. (A) Spiders responded significantly more to the low frequency 80 Hz tone than to the control tone or to the 2000 Hz tone (ANOVA, Tukey HSD test, $p < 0.05$), but showed no significant difference between the control tone and the 2000 Hz tone ($p > 0.05$). Values plotted are mean ± 1 s.d. (B) Four types of behaviors were observed in response to the 80 Hz tone. (C) The mean time elapsed between stimuli onset and each observed behavior. The onset of freezing responses was significantly more rapid than for other behavior types (ANOVA, Tukey HSD test, $p < 0.05$). All error bars represent one standard deviation.

Table 3.1. Parameter report of the nominal logistic model based on the presence or absence of a behavioral response due to airborne acoustic stimuli. Significant terms are shown in bold. The model uses the response to the 2000 Hz treatment as a point of comparison—thus a significant effect of the 0 Hz frequency term (as shown) implies a difference between the 2000 Hz treatment and the 0 Hz treatment, not that there was a significant behavioral response to 0 Hz, *per se*.

Term	**Estimate**	**Std. Error**	**ChiSquare**	**Prob > ChiSq**
Intercept	0.7484	0.5455	1.88	0.1701
Trial	0.1354	0.2056	0.43	0.5102
Freq. [0 Hz]	**1.3807**	**0.4207**	**10.77**	**0.0010**
Freq. [80 Hz]	**-1.5958**	**0.2652**	**36.22**	**< 0.001**
F. [0 Hz]*Trial	-0.0175	0.3697	0.00	0.9622
F. [80Hz]*Trial	0.2230	0.2354	0.90	0.3425

between the response times for crouching, running, and turning ($p > 0.05$). Although rare ($n = 5$), freezing behaviors were recorded in some high frequency trials. However, the mean latencies and stand deviation of these events (337 ± 365 ms) were much greater. This suggests that while the freezing responses to the 80 Hz tone were likely indicative of a discrete and repeatable behavior, freezing responses in other trials were likely due to random behavioral occurrences.

Discussion

Through behavioral trials and neurophysiological recordings from units in the brain of the jumping spider *Phidippus audax*, we demonstrate that these animals perceive and respond to airborne acoustic stimuli. Extracellular neural recordings from the brain typically showed responses to airborne stimuli for frequencies between 16 and 512 Hz, with one cell responding up to 2048 Hz (Figure 3.2). Amplitude thresholds in response to FM sweeps were ranged from 47 dB to 77dB SPL (Figure 3.3). Neural units that responded to airborne stimuli also fired when single trichobothrium hairs on the legs of the animals were stimulated, suggesting that these hairs are sufficient to transduce airborne acoustic signals and may be involved in their perception (Figure 3.1). In behavioral trials, spiders responded significantly more often to 80 Hz tones than to the silent control or the higher frequency tone (2000 Hz); spiders showed no significant response to high tones compared to the control (Figure 3.4). Interestingly, all stimuli originated from a sound source over 2 m away from the test animals, greatly expanding the known spatial range over which airborne acoustic stimuli can influence the behavior of these organisms.

Neural responses

Single neural units in the brains of these jumping spiders were shown to respond to a range of relatively low frequency tones (between 16 Hz and 2048 Hz), with the majority of cells responding to frequencies between 16 Hz and 512 Hz. Frequency sensitivities below 1000 Hz have been reported from more peripheral stages of sensory processing in the spider *Cupiennus* (Barth & Höller 1999), while experiments in cockroaches (Rinberg & Davidoxitz 2003), crickets (Shimozawa & Kanou 1984), and other arthropods (Santer & Hebets 2008) have focused on even lower frequencies. This general sub-1000 Hz, low-pass tuning seems to be typical of medium-flow sensors among land-dwelling arthropods (Barth 2014). This is likely because responses to higher frequencies are limited by the basic properties of the sensors themselves (i.e., length, restoring force of the joint, and mass) and constraints due to boundary-layer effects (Barth 2014)—both of which limit the ability of hairs to respond to high-frequency acoustic stimuli. Neural responses to frequencies up to 2048 Hz, as reported here, are therefore especially interesting and are certainly worthy of future study. Beyond responses to higher frequencies at the neural level, our behavioral work suggests spiders may be responsive to these higher frequency cues. Although we found no statistically significant response to the 2000 Hz tone in our behavioral tests, our results' statistical model suggests that there may be a difference between responses to control and high 2000 Hz tones.

The amplitude thresholds to FM sweeps reported here varied widely (roughly 47 to 77 dB SPL), but are generally in line with other studies of airborne acoustic perception in arthropods—including anti-predatory responses in systems where the receivers possess pressure-sensitive tympanic membranes (Hoy et al. 1989). In all cases where there were multiple significant responses, firing rate increased with increased amplitude, suggesting that these neural units are responsive to amplitude (Figure 3.3).

In the subset of neural recordings where we were able to record responses while administering both airborne and mechanical stimuli, neural units responded similarly to both stimuli types. However, direct comparisons of neural activity in response to mechanical and airborne stimuli were limited by the difference in amplitude of these signals. It is important to note that although the responses to airborne stimuli and those generated when driving single trichobothria were similar in terms of overall frequency receptivity, this does not necessarily mean that the trichobothria stimulated were the sensors solely responsible for the reception of the airborne signal. However, the fact that the same neural units responded to both sets of stimuli demonstrates that both inputs are relevant to a single network high up in the central nervous system (CNS); this suggests that they have some shared behavioral salience. Given the demonstrated importance of these hairs in processing airborne acoustic signals in other spiders (Barth 2002), we believe it is likely that trichobothria are indeed responsible for the observed response to airborne stimuli in jumping spiders.

Although recordings have been made from neurons associated with trichobothria in other spiders, extensive recordings from the brains of spiders had not been possible until recently (Menda et al. 2014). The recordings presented here represent an initial step towards understanding the role of these signals in the CNS. One interesting avenue of research now possible will be to determine how signals are integrated across the hundreds of trichobothria that cover spiders' bodies. Work in *Cupiennius salei* has provided anatomical evidence for the somatotopic organization of projections in the suboesophageal gangelion (SOG) based on the location (distal or proximal) of trichobothria along the leg (Anton & Barth 1993). There is also evidence for leg-specific organization in the SOG (Anton & Barth 1993). Intracellular recordings from a set of trichobothria-related interneurons in the SOG of *Cupiennius salei* have found direction-sensitive cells—suggesting that even at this earlier stage in the CNS, information from trichobothria is meaningfully integrated (Friedel and Barth 1997). Given this integration in the SOG, it will be interesting in future work to investigate what type of spatial and/or spectral integration across hairs is demonstrated by neurons in the brain. The question of integration is particularly relevant to neurons in the region recorded from here (the area just below the arcuate body—a structure analogous to the central complex of insects) since this area is believed to be the site of broad sensory integration across numerous sensory systems (Hill 1975; Babu & Barth 1984; Homberg 2008).

Behavior

Freely moving spiders most often responded to the 80 Hz airborne acoustic tone by abruptly halting their forward movement (freezing) (Figure 3.4). Freezing is a common anti-predatory response, demonstrated by a wide range of organisms including rodents (Hendrie et al. 1998), fish (Giaquinto & Volpato 2001), and other arachnids (Chelini et al. 2009). This behavior is believed to allow prey to minimize the chances of alerting a potential predator (Eilam 2005). The reaction times reported here (approx. 100 to 200 ms) were marginally slower than have been reported for other arthropod anti-predatory behaviors (circa 80 ms; see Suter 2003; Hoy et al. 1989). It should be noted, however, that in these other systems the relevant acoustic signals are typically generated by predators in very close proximity—likely necessitating particularly rapid counter-measures; the current study explored longer-range cues.

There are two major categories of potential threats that seem most capable of generating long-distance airborne acoustic signals. The first is the acoustic signal generated by dipteran parasitoids (notably small-headed flies, Insecta: Diptera: Orthorrhapha: Acroceridae) and flying predatory wasps (Insecta: Hymenoptera), many of which specialize on spiders and are a major cause of spider mortality (Finch 2005; Schlinger 1987; Blackledge et al. 2003; McQueen 1979). The dominant acoustic frequencies of flying hymenopterans are around 100 Hz (depending on the species) with powerful harmonics, though the majority of the acoustic power is below 1000 Hz (Sudo et al. 2001). These signal properties match well with the frequency sensitivities measured in the current study. Indeed, hairs sensitive to medium flow have been shown to be important in detecting airborne wasp predators in other systems to a range of 70 cm (Tautz & Markl 1978).

The second possible category of potential threat is general disturbances—such as the rustle of leaves or breaking of twigs—that might signal the presence of a large predator. Environmental disturbances like these produce broadband signals (Magrath et al. 2007; Lewicki 2002) and therefore carry substantial acoustic power in frequencies that these spiders are sensitive to. Although not dangerous in and of themselves, such signals might have a priming, or attention-altering effect, allowing a spider to rapidly respond to any subsequent cues (Hoy 1989).

Medium-flow sensors tuned to lower frequencies have also been shown to be important in receiving short-range signals, particularly in the context of anti-predatory responses. Elegant experiments using crickets (Dangles et al. 2006), cockroaches (Camhi et al. 1978), and fishing spiders (Suter 2003) have shown that the air-currents produced by predators lunging towards prey are sufficient to generate a response in the target—this detection often still gives would-be prey enough time to increase their chances of escape. Although, to our knowledge, a similar role in jumping spiders has not yet been demonstrated, we believe such behavior would be likely and that the trichobothria system studied here would play an important role in mediating such escape responses.

From an ecological perspective, however, the detection of airborne cues could benefit these spiders in any situation where information is at a premium, including aiding in predation and/or conspecific signaling, as well as predator avoidance. Indeed, the role of trichobothria has been demonstrated or suggested in conspecific signaling contexts (Santer & Hebets 2008; Rundus et al. 2010) and for detecting and capturing prey (Klopsch et al. 2013) in other arachnids. Based on the similarities between the freezing behavior reported here and those of other systems we believe that the response to the 80 Hz tone that we observed is most likely to be an anti-predatory response. That said, future experiments using different experimental designs and stimuli will be necessary to confirm this hypothesis and may reveal other contexts in which jumping spiders make use of airborne acoustic cues.

Hearing at a distance

In both behavioral and neurophysiological trials, the distance between the sound source and the animal was much greater than is typical of studies on medium-flow systems. Across a wide range of previously studied taxa, responses seem to cease beyond the 70 cm mark (Barth 2014). This is noteworthy because the so-called “near-field” particle velocity component of sound (received by medium-flow sensors) does not vary over distance in the same manner as the “far-field” pressure component. While the amplitude of the pressure component of a sound drops by 6 dB with every doubling of the distance, the particle velocity component decays more rapidly (Robert & Hoy 2007). It is therefore generally thought to only be relevant over a distance equivalent to some fraction of the wavelength of the signal (Michelsen 1992). Although some researchers stress that stimulus amplitude is crucial for determining the relationship between particle velocity and detection distance (Robert & Hoy 2007), most common references to the “near-field” adhere to these wavelength-based estimates. Some researchers place the threshold at the half-wavelength mark (Jacobsen 2007), others at one-wavelength (Michelsen 1992) (for reference: under standard conditions the wavelength of a 100 Hz tone is 3.4 m, for 500 Hz the wavelength is 0.7 m, and for 1000 Hz the wavelength is 0.3 m). This has lead to a general assumption in the biological community that medium-flow sensors are only effective when located relatively close to a sound source—usually within tens of centimeters. However, our work suggests that this traditional understanding of these sensors—and their potential use by organisms—is incomplete. Indeed, it appears that given sufficient stimulus amplitude, these hairs are capable of registering airborne stimuli over spatial scales at least double what has typically been believed and far in excess of wavelength-based limits. At 500 Hz, for example, the animals in our study were roughly three wavelengths away from the sound source. This greatly expands the known sensory space over which airborne stimuli can affect the behavior of jumping spiders. While these findings may redefine how we conceive experiments aimed at exploring the mechanisms of medium-flow sensory systems, they also suggest that airborne cues may serve a previously unappreciated role in informing jumping spider behavior. Indeed, it may be that airborne sound plays an important role across a range of behavioral contexts, even in these visual specialists.

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CONCLUSIONS

If one chooses to look closely at a jumping spider—even for the smallest amount of time—one might be struck by the sense that there must be a tremendous amount happening in their tiny worlds. It is not that their lives seem particularly fast or chaotic. Instead, this feeling stems from the degree of planning, consideration, and care that appears to inform their actions. One might notice it in the way that they peer at objects with their oversized eyes; or in their punctuated, stochastic, carefully deliberate movements. This thesis was, in part, an effort to understand the jumping spider's world through the scientific exploration of the behaviors, habits, and abilities of these creatures, as well as through the study of the selective forces that have shaped them.

Mimicry provides a particularly interesting lens through which to study the biology of species, since by definition mimicry requires comparison. The reward for difficult work is potentially triple: studies in mimicry can reveal as much about non-mimics and models as they do about the mimics themselves. I discovered this firsthand during the work for Chapters 1 and 2 on the ant-mimicking jumping spider *Myrmarachne formicaria*. The work presented in Chapter 1 found that these mimics are even more ant-averse than typical jumping spiders, while the quantification of locomotor behavior for Chapter 2 revealed not only how mimics move, but broadened our understanding of ant and typical jumping spider movement as well.

There is enormous potential in future work concerning locomotor mimicry, both in ant-mimicking spiders and in other systems. Locomotor behavior is often only discussed from a purely functional perspective (e.g. the stability of a particular gait pattern, the efficiency of a given type of movement, the cost/benefit tradeoffs of searching for hidden resources…). My work here argues that in some cases, locomotor traits may be more usefully considered as signals—signals shaped by the evolutionary dynamics of a signaler-receiver system. There is still a great deal of potential in explicitly applying concepts pioneered in the fields of sexual selection and animal communication to studies of mimicry—by combining mimicry with cognitive-based exploitation hypotheses or with understandings of multi-modality, for example.

Conversely, I hope that quantitative approaches to studying dynamic behaviors—like those those developed in Chapter 2—will increasingly be utilized in a range of animal behavior studies. The importance of animal movement is certainly not a new human passion, but the tools necessary to enable the quantification of such traits (e.g. high-speed cameras and powerful computers) have only recently become readily available. Biologists have long noted that movement is important in sexual selection and predator-prey interactions, but we are increasingly in a position to turn general observations into tractable systems for investigation at several level of analysis.

The exploration of acoustic perception in jumping spiders in Chapter 3 underscores the importance of recognizing multi-modal excellence even in animals famous for sensory specialization. This work provides evidence that spiders can detect sounds over several meters; how these signals are received at the level of single hairs, how signals are integrated across the body, and the behavioral significance of such signals are all questions that require future study. This research also quietly underscores the wonderful amount of complexity present in small creatures. Several decades ago, pioneering studies revealed that spiders communicate through vibratory cues; those findings fundamentally altered the way we considered the lives of these animals. Today, we continue to be impressed by the jumping spider; it is now clear that these small arachnids live in a multi-modal, hi-definition, low-frequency world full of sights, shakes, and sounds.