AAS Virtual Summer Symposium
Poster Presentation Abstracts

Arranged by first author's last name
Asterisk (*) indicates contestant in student competition
Presenter underlined

Lethal and sublethal effects of emamectin benzoate on web geometry of *Neoscona theisi*

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Insecticides have disruptive effects on web spiders. When spiders are exposed to insecticides, their web geometry is changed. One of these insecticides is emamectin benzoate applied on spider *Neoscona theisi*. For the experiment, different doses of the insecticide were sprayed on the spiders and their effects on web parameters i.e. mesh height, capture area, capture thread length and number of radii were observed. ANOVA showed significant difference in mesh height, capture area and capture thread length. However, a non-significant difference was found in the number of radii. It is concluded that emamectin benzoate disturbs spider web structure which ultimately affects the insect capturing eficiency of spiders, therefore, it can be recommended for IPM programme. Future researches can assess its sublethal effects on web building behaviour of other spiders to determine the role of the insecticide in agroecosystems.

Parasitism of local and alien widow spiders in Israel by the wasp *Philolema latrodecti*

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Alien invasive species are species that expand beyond their natural range into new locations and become a threat to the native ecosystems. One of the important questions about invasive species is what makes them often more successful than the native

species. The brown widow spider, *Latrodectus geometricus*, is a synanthropic and cosmopolitan species originating from Africa and now invading large parts of the world. Widow spiders are known to be attacked by several parasitic wasps. For example, *Philolema latrodecti*, is a generalist wasp that was first detected in Hawaii, emerging from brown widow egg sac, but it is also known to attack the egg sacs of other widow species around the world. The brown widow constructs unique, spiked silk structures on the surface of its egg sacs—in contrast to the smooth surface of egg sacs of other widow species—which has been suggested to act as a defense against parasites. Hence, defense against parasites is one potential advantage of the brown widow spider over related native species. My goal is to investigate the natural parasitism rate and the oviposition behavior of the wasp, *P. latrodecti*, on egg sacs of the brown widow spider compared to those of native white widow spider, *Latrodectus pallidus.*

How bacterial infection affects locomotor responses in the brush-legged wolf spider, *Schizocosa ocreata* (Hentz)

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Previous studies have shown that infection by the common arthropod pathogen *Pseudomonas aeruginosa* affects the development of physical traits (foreleg tufts) and behavior (courtship signals) of male *Schizocosa ocreata* (Hentz 1844) wolf spiders. In this study, we examined how infection by P. aeruginosa affects the locomotor performance of male and female *S. ocreata* infected at two different life stages: adulthood and juvenile penultimate instar. Previously infected and control spiders were placed onto a quasi-treadmill (a turntable with a movable circular arena) and induced to run until exhaustion. Results show that infection timing (exposure at the penultimate instar vs. adulthood), had no effect on locomotor performance. However, infection status and interaction of status x sex were significant. Control females ran significantly longer than infected females, whereas males showed no difference between infected and control groups. These findings, along with results of other studies, suggest that infection costs impact the sexes differently, and might be explained by the fact that males actively search for females to mate with and therefore have a need for greater stamina and/or resistance to infection.

Genomic determination of reproductive mode in facultatively parthenogenetic harvestmen

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Despite sexual reproduction posing myriad short-term costs to individuals, sex is near ubiquitous in animals. To understand the factors maintaining sex, study of alternative reproductive modes is necessary. Facultative parthenogenogenesis is theorized to mitigate many costs of sex; individuals can participate in occasional sex and obtain genomic benefits. *Leiobunum manubriatum* and *L. globosum* are facultatively parthenogenetic harvestmen well-suited for mating system studies as males vary in frequency and coercive sexual traits across populations. We asked: if populations of facultative parthenogens contain high frequencies of coercive males, will females mate? Will offspring production differ from conspecific, low male frequency populations? We addressed these questions by first collecting females and egg clutches from populations varying in male frequency. In *L. manubriatum*, female fecundity did not significantly vary between populations with high or low male relative frequency, despite the potential release of the latter from sexual conflict. Using three genotyping methods, we revealed *L. manubriatum* offspring from high-male populations were primarily produced asexually, despite sex ratios in these populations approaching equality. Rapid and accurate SNP genotyping will continue to provide inference on sire assignment, allowing us to address broader evolutionary questions regarding the maintenance of sex.

Personality clines across two range expansions of introduced *Cyrtophora citricola* (Araneidae) in Florida

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Animal personalities describe the behavioral phenotypes of individuals that often remain relatively stable over time and contexts. Since they can account for differential dispersal tendencies, understanding how personality types are distributed across the range of an invasive population can lead to important characterization of expanding populations. *Cyrtophora citricola* is a colonial tentweb orbweaver with an Old World native range that is invasive in Florida. As of 2017, two separate expanding populations have spread 450 km northward in 20 years. In this study, we show that in both of these two replicate populations, *C. citricola* spiders were more active and attacked prey faster at the leading edges of their range, although laboratory observations determined these to be plastic behaviors. Surprisingly, boldness and exploration clines across each population's invasion history were found, but the patterns were opposite in each population. The eastern population of Florida *C. citricola* were found to be more dispersive, bold, and exploratory at their invasion front, whereas the western population was increasingly shyer and less exploratory at their invasion front. This study suggests

caution when interpreting phenotypic gradients across expanding ranges, as local adaptation and spatial sorting likely interact and can produce inconsistent patterns.

Quantitation of post translational modifications of aggregate glue proteins

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Spiders are known to produce silk fibers that serve unique functions with exceptional material properties. The evolutionary transition from dry, mechanically sticky cribellate silk to wet, chemically adhesive aggregate silk improved stickiness and material efficiency, which was a key innovation of the Araneoidea that contributed to their dramatic diversification. However, little is known about molecular attributes of aggregate silk. Post translational modifications (PTMs) of aggregate proteins, especially glycosylation, is thought to be an important contributor to stickiness. The objective of our experiment is to quantitate the PTMs, namely glycosylation and phosphorylation, of adhesive glue proteins in order to better understand the functional implication of PTMs. In this experiment, we initially obtained gumfoot lines (fibers covered with aggregate glue) from the Western black widow, *Latrodectus hesperus*. Solubilized aggregate proteins were digested with trypsin and either received 1) treatment to enzymatically remove phosphates, 2) treatment to enzymatically remove sugars, or 3) no enzymatic treatment. If sugar and phosphate removal succeeds then we would compare peptide abundance of the three treatments to quantitate PTMs. Preliminary mass spectrometry analysis shows enzymatic treatment successfully dephosphorylated peptides, but only partial deglycosylation. Future work calls for chemical removal of the sugar groups from the peptides.

Codon optimization of genes expressed in venom glands

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Venom is a toxic substance that is produced by some animals to assist in capturing prey or self-defense. Due to venom being vital for their survival, these animals must efficiently produce a large amount of venom proteins, often involving specialized glandular tissues. Since venom glands have genes that are expressed at higher rates than many other body tissues, we hypothesized that highly expressed genes in venom glands would exhibit more codon optimization, the evolution of preferential usage of

certain codons to optimize transcription and translation than other body tissues because of their need to be potent and readily available. To test this, RNA sequences from body tissue and venom glands of two spiders (*Latrodectus hesperus, Steatoda grossa*), two robber flies (*Eutolmus rufibaris* and *Machimus arthriticus*), one scorpion (*Mesobuthus martensii*), and one cone snail (Conus consors) were obtained from the NCBI short read archive, assembled into transcripts, and their complete coding sequences were extracted. The extracted coding sequences and gene expression levels were used to infer optimal codons based on the change in relative synonymous codon usage in highly versus lowly expressed genes. The frequency of optimal codons in venom specific genes was then compared to non-venom specific genes using a t-test. Only the cone snail showed significantly increased codon optimization in venom-specific genes. Thus, there is little evidence to support venom optimization occurring in arachnids and robber flies via gene expression optimization.

Endless biomaterials most beautiful: Darwin's bark spider silk project

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Spider silks are renowned for their exceptional toughness and are a rich source of bioinspiration for designing advanced materials. Numerous studies have investigated the molecular basis for spider silk toughness in select species. However, the diversity of different silk types produced by individual spiders and their variation across species at the molecular and mechanical level still remains poorly known, precluding a full understanding of their biomimetic potential. Our research on spider silk is focused on uncovering the molecular composition of spider silks used for different ecological strategies and understanding how silk protein diversity relates to variation in mechanical/material performance. A recent example is our characterization of novel silk proteins in the super-tough dragline of Darwin's bark spider (*Caerostris darwini*). Here we present an overview of our on-going work exploring variation in silk protein diversity within and across species of the bark spider genus *Caerostris*, the underlying genomic mechanisms generating this molecular diversity, as well as their potential relationship to fiber function. As the major proteins of spider silks are long and repetitive, much of our current work focuses on overcoming the challenges of accurately characterizing silk gene transcripts with long-range single molecule sequencing methods.

Phylogeography of *Phidippus johnsoni* in Baja California

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The Baja California peninsula, with a length of approximately 1200 km and 14 ecoregions is an area of great interest for biogeographical studies. In particular, a central mountain range along the north-south axis provides sky-island conditions and acts as a possible barrier to gene flow between eastern and western populations. Furthermore, past events such as marine introgressions have shaped the population structure of the peninsula's flora and fauna. Here, we set out to study population genetics and phylogeographic history of *Phidippus johnsoni* in Baja California. Preliminary results point towards populations with high diversity on the western side of the peninsula.

Effect of invasive Callery Pear on transmission and reception of vibratory courtship signaling of *Schizocosa ocreata* wolf spiders in leaf litter

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Callery Pear (*Pyrus calleryana*) is an invasive species in forests of the US. Callery Pear leaves are smaller and thinner than native species, raising questions about how they affect leaf litter environments they invade. We studied effects of *P. calleryana* leaves on transmission of vibratory courtship signals of the brush-legged wolf spider *Schizocosa ocreata* (Hentz 1844). We recorded spider signal transmission on individual leaves and studied signal active space and mating behavior in leaf litter mesocosms. On individual leaves, the power spectrum of vibratory courtship signals showed higher amplitude across a wide range of frequencies in *P. calleryana* compared to other leaf litter species (Maple, Oak, Sycamore). Additionally, female *S. ocreata* were less likely to orient and show receptivity to male courtship on *P. calleryana* than in a mixed-species mesocosm, although latency to orient was not significantly different. Live mating trials showed no significant difference between the two mesocosm types, despite a trend towards a reduction in mating success on Callery Pear. Analysis of signal attenuation over distance also showed no difference between litter types. Results suggest that differences in vibration spectra of signals on different leaves might account for reduced communication, but impact on mating remains unclear.

Exploring mechanisms of tissue-specific gene regulation in the common house spider (*Parasteatoda tepidariorum*)

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The development and maintenance of distinct cell and tissue types with diverse functions in complex organisms depends on the differing composition of the underlying transcriptomes. High-throughput sequencing technologies permit robust characterization of tissue transcriptomes and facilitate identification of the genomic regions which control their expression. In the common house spider, *Parasteatoda tepidariorum*, transcriptomes of four tissues, including silk glands, venom glands and ovaries, each critical to spider functional ecology, were used to define sets of genes with strongly biased expression in each tissue, together with a broadly expressed control set. We explored tissue-biased transcription factors that may contribute to tissue-biased expression of downstream genes. Furthermore, we identified putative promoter regions in the *P. tepidariorum* genome, based on the position of annotated transcription start sites for both tissue-biased and broadly-expressed control sets of genes. Promoter regions so defined were explored for known and novel DNA motifs overrepresented in tissue-biased promoters, which represent candidate cis-regulatory elements controlling tissue-biased expression.

Mass spectrometry reveals components of dragline silk from Darwin's Bark Spider

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Spiders use silk for a variety of functions, most notably the production of webs, and possess up to seven different silk glands. In orb weavers, the major ampullate gland produces dragline silk used to create the supporting web frame and radii, and that also acts as a safety line. Among orb-weaver silks, dragline is noted for its combination of high strength and extensibility and for being tougher (higher energy to break) than steel or Kevlar. *Caerostris darwini*, the Darwin's Bark Spider, is an orb-web weaver endemic to Madagascar that exploits a unique niche with large webs up to two meters in diameter that cross streams and rivers, with bridge lines as long as 25 meters. Such webs are enabled by the exceptional biomechanical properties of *C. darwini* dragline silk, toughest amongst spiders, and thus an intriguing template for biomaterials applications. The production of biologically inspired synthetic materials requires a clear understanding of the physical properties of silk, dependent upon silk protein content,

including spidroins: large, modular and repetitive proteins that are a primary silk constituent. We utilize proteomic data to characterize *C. darwini* dragline silk by directly assaying proteins found in silk fibers, including novel spidroins.

Effect of spider taxa and body size on MeHg concentrations in shoreline spiders: Implications for their use as sentinel species

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Shoreline spiders have been proposed as sentinels to monitor contamination of aquatic food webs by toxic methyl mercury (MeHg). Spiders become contaminated with MeHg by feeding on MeHg-contaminated emergent aquatic insects, but basic information about MeHg bioaccumulation in spiders has not been assessed. In this study, we tested the hypothesis that MeHg concentrations within a spider taxa would increase with spider size. We collected 683 spiders in May-June 2018, from 14 human-made ponds at the LBJ National Grasslands, Texas, USA. As a proxy for spider size, we measured the tibia+patella length (TPL) of leg I for each spider and determined the effect of TPL on MeHg concentrations for 6 taxa of shoreline spiders: Araneidae, Tetragnathidae (Tetragnatha spp.), Salticidae, and Lycosidae (*Pardosa spp., Rabidosa spp*. and *Schizocosa spp*.). Mean MeHg concentrations (mean±SE) in spider taxa ranged from 47.5±2.8 to 148±13.4 ng/g in *Schizocosa spp*. and Araneidae, respectively, and were significantly different between spider taxa. Concentrations of MeHg in spiders increased significantly with TPL for all taxa except *Rabidosa spp*. Our results suggest that measurements of spider size within taxa should be included when shoreline spiders are used as sentinels of MeHg contamination.

Herbicide effects on the feeding behavior of the wolf spider *Pardosa milvina*

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Herbicides can potentially impact spider prey capture in agricultural systems. We measured prey capture of *Pardosa milvina* when exposed to soil with field-relevant concentrations of five commonly used herbicides: atrazine, S-metolachlor, rimsulfuron,

mesotrione, glyphosate, combination of all five herbicides, and a water control. Tested spiders were collected from two adjacent fields: one sprayed with combinations of these herbicides over a twelve-year period while the other received no pesticides over the same twelve years. Non-exposed adult males and females from each plot were exposed to the seven treated soil substrates. Spiders were standardized for hunger then presented a cricket one week and two weeks after initial herbicide treatment exposure. Their prey capture latency of an individual cricket was observed on untreated substrates. We found no significant differences in prey capture latency for spiders across herbicide treatments after one week of exposure, but large differences emerged by the second week. We also found large sex and collecting site differences in prey capture and weight change across treatments. Mesotrione and rimsulfuron-treated spiders showed the greatest weight loss between the first and second week of exposure while atrazine, glyphosate and s-metalachlor treated spiders gained more weight than the control group.

Cold tolerance in the jumping spider *Phidippus audax* from Texas to Michigan

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Phidippus audax (Salticidae) experience different winter conditions across their range. We explored cold-tolerance to understand differences related to climate. From September 2017 to January 2018, we measured Supercooling Points (SCP) of field-caught Michigan spiders. In 2019/20, we maintained spiders from Michigan (MI), Oklahoma (OK), and Texas (TX) in growth chambers, simulating conditions from 2017/18. P. audax were freeze avoidant, suppressing SCP to as low as -15°C, but not freeze tolerant. Freezing was lethal, but chilling to freezing was not. Field-caught Michigan spiders lowered SCPs from -4°C to about -14°C, but there was no evidence of “cold-hardening” in 2019. In 2019, the MI spiders had lower SCPs (Oct: -7.3°C, Dec: -8.0°C, Jan: -8.7°C) than TX spiders (Oct: -5.6, Dec: -5.5°C) but not OK spiders (Oct:-6.1°C, Dec: -6.3°C). In October and December, mortality in the growth chamber was low, and similar, between locations. However, we were unable to estimate SCP for the TX and OK populations in January because all the spiders died in the harsher conditions. The MI spiders continued to experience low mortality. Smaller spiders were somewhat more effective at suppressing their SCP than larger spiders.

Socially-facilitated and chemically-mediated antipredator responses in the wolf spider *Pardosa milvina*

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Animals may use direct cues from predators to show defensive behavior or may use indirect sources of information such as observing antipredator responses of nearby conspecifics. The relative value of direct predator cues and indirect social information about predation risk is unclear, particularly when these information sources conflict. *Pardosa milvina* freeze when detecting silk from the larger predatory wolf spider, *Tigrosa helluo*. We measured *Pardosa* activity under six different social and predator cue conditions including treatments when the subject does or does not have access to predator silk cues directly, and/or is able to observe conspecifics with or without access to these same predator cues. Spiders walked significantly less in the presence of direct predator cues independent of social cues, however mean speed and freezing responses were both significantly influenced by direct predator cues and social cues. When nearby conspecifics had access to predator silk but the subject did not, the subject increased antipredator responses. When the subject had direct exposure to predator silk but nearby subjects did not, the subject reduced antipredator responses. Although *Pardosa* mediate some defensive behavior based on social cues, direct predator information had a stronger antipredator effect. Behaviors of nearby conspecifics can influence antipredator behaviors in complex ways.

Preliminary observations of the schizomid exoskeleton

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Short-tailed whip scorpions (Schizomida) are small arachnids in the clade Pedipalpi, which also contains whip scorpions (Thelyphonida) and whip spiders (Amblypygi). Despite advancements in schizomid systematics over the past decade, there is still a paucity of data on their fine structure, which might otherwise provide insights into schizomid evolution and ecology. Here, we provide preliminary observations on the histology, fluorescence, and elemental profile of the exoskeleton of *Stenochrus portoricensis* Chamberlin, 1922. Results reveal the exoskeleton to be 4–9 μm thick and include a thin epicuticle (< 1 μm), a hyaline exocuticle (2–7 μm), and a meso- and endocuticle (< 3.5 μm). Excitation of histological sections with a mercury lamp revealed fluorescence at 346 nm (UV), 488 nm, and 546 nm in the exocuticle and possibly meso- and endocuticles. Elemental analyses revealed much of the exoskeleton to contain trace levels of sodium and chlorine, with calcium present in the antenniform appendages, and zinc present in the palpal and tarsal claws, and chelicerae. We intend

to increase the resolution of these studies by examining additional specimens as well as using CLSM to insure the accuracy of our fluorescence observations.

Revision and cladistic analysis of subfamily Nothopuginae (Solifugae, Ammotrechidae)

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The solifuge family Ammotrechidae is widely distributed through America and comprises 99 species. Currently, Ammotrechidae includes five subfamilies: Ammotrechinae, Saronominae, Oltacolinae, Mortolinae and Nothopuginae. This subfamiliar classification has been questioned by different authors. Here, we present a cladistic analysis of Ammotrechidae to test the monophyly and the relationships within the subfamily Nothopuginae. A morphological data matrix of 144 characters and 23 terminals was performed and analyzed under parsimony. The outer group consists of one species of Eremobatidae, three species of Mummuciidae, three species of South American Daesiidae, and 11 species of Ammotrechidae representing the subfamilies Ammotrechinae, Saronominae and Oltacolinae. Traditional characters are reevaluated and other, poorly studied characters, such as arolium and tracheal system, are explored. The results support the monophyly of Nothopuginae as a sister group of Oltacolinae. Two new genera and species of Nothopuginae are herein proposed as well as a new species of *Nothopuga*. We discuss about geographic distribution patterns of Ammotrechidae and characteristics of major clades within Ammotrechidae.

Communicating knowledge and respect for spiders and their functions in ecosystems for audiences such as elementary students

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Ecologist Stephen Johnson joined me to collaborate with literacy coach and fifth-grade language arts teacher, Liza Zylstra, in teaching a unit on E.B. White’s Charlotte’s Web and literacy about spiders. Since White fosters respect and wonder about his spider protagonist, we wanted to underscore the necessity for spiders and how they fit in

ecosystems. Lisa and I created a plan for close reading of the literature and Dr. Johnson presented a close reading of White's information on spiders and drew the parts of a spider's leg that Charlotte delineates when Wilbur tries to weave a web in chapter nine. Stephen gave a copy of the artwork to each student in the class to accompany White's written description.

Stephen described the diversity of spiders found in Pella, Iowa, and the students adopted one of his photographs. The fifth-graders researched information about habitat and hunting styles as well as why spiders should be respected. The students included their own written description of their adopted spider. The fifth graders presented their Power Points to third graders and kindergarten students.

Effects of chronic exposure to the herbicide, mesotrione on seven species of spider

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Mesotrione is a widely used herbicide but its effects on spiders is largely untested. Mesotrione toxicity may vary with spider size, sex, and microhabitat. The spiders *Frontinella pyramitela, Tetragnatha laboriosa, Mecaphesa asperata, Pisaurina mira, Hogna lenta, Tigrosa helluo*, and *Trochosa ruricola* were exposed to field-relevant concentrations of mesotrione-treated soil over a 55-day period. All seven of these species occur locally in agricultural systems. We found that the web-building spider Frontinella but not Tetragnatha showed increased mortality compared to control treatments. *Mecaphesa, Pisaurina, Trochosa*, and *Tigrosa* all showed significant increases in mortality when exposed to mesotrione-treated soil whereas the wolf spider *Hogna lenta* was unaffected. We found no significant interaction between mesotrione and the sex of the spider among *Pisaurina, Trochosa, Tigrosa*, or *Hogna*; however, several species showed significant shifts in space use when exposed to mesotrione and we found a significant interaction between species and mesotrione treatment with respect to spider weight gain. Mesotrione was toxic for five out of the seven tested spider species but we found no toxicity pattern based on spider size, space use, web-building, or sex. Alternatives to mesotrione should be considered to minimize the negative biocontrol impact on beneficial spiders within integrated pest management systems.

Differences in characteristics of eggsacs of *Metepeira sp* in two environments of the Monte Desert, Mendoza, Argentina

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The guild of weaver spiders uses vegetation as an anchor for their webs. Therefore, an environment that has vegetation with greater structural complexity provides a greater number of anchor points, protection against predators, greater number of sites available for oviposition. I compare eggsac characteristics and oviposition sites of *Metepeira cf. calamuchita* (Araneidae) at two different habitat types, creosote bush scrubs and Prosopis sp. forest patches in the Ñacuñan Biosphere Reserve located in the Monte Desert of Mendoza, Argentina. In each site selected, eggsacs were collected by hand between November to April. I found a total of 27 eggsacs (scrub n=13; forest n=14). At the scrub site, there was a greater number of eggsac anchored to the vegetation, (62%) while in forests sites most of them were suspended by web threads (79%). Characteristics of eggsac recorded were: presence of detritus (most common in scrub sites) and color of eggsac (brown/dark brown). There was an association between the presence of detritus and the oviposition site. Most of the eggsacs anchored to the vegetation contained detritus, while the egg sacs that were suspended from the web had no detritus. These characteristics associated with the environment could be defense strategies against predators and parasitoids.

Inland and coastal sites show multi-decade declines in spider abundance and diversity

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Many taxa show alarming declines in diversity and abundance. Although charismatic megafauna and plants are the primary focus of such studies, a growing body of research documents drastic changes among terrestrial arthropods. These latter studies emphasize insects. We examined changes in spider abundance and diversity at two sites in New Jersey, USA, for which historic data were available: Hutcheson Memorial Forest (HMF - inland area of mixed field and forest) and Great Bay Boulevard Wildlife Management Area (GBB - coastal salt marsh). Survey methods used in 2019 mirrored those of the original 1985 (GGB) and 1993 (HMF) studies. At both sites, spider abundance had declined significantly by 2019: spider densities at GBB were 3-to-7-fold

lower in 2019; at HMF, total spider abundance was 4-fold lower in 2019. Family-level diversity also declined at the two sites. Only 9 of the 12 families present at GBB in 1985 were found in 2019 (25% loss). HMF experienced a 26% decrease, dropping from 19 to 14 families. Due to the protected nature of the two sites, the observed declines in spider abundance and diversity are likely to be direct or indirect results of regional climate change, rather than consequences of land use changes.

A review of the Malagasy endemic genera *Anaceros*, *Hovianoceros* and *Malgacieros* (Opiliones: Laniatores: Biantidae)

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Anaceros Lawrence, 1959, Hovanoceros Lawrence, 1959 and Malgaceros Lawrence, 1959, are three biantid genera endemic to Madagascar. *Anaceros* was originally described to accommodate four species, whereas *Hovanoceros* and *Malgaceros* were described as monotypic. Before now, species in these genera were known only from the original descriptions, in which important taxonomic characters (e.g. male genital morphology) were not described. After examining 250 specimens from the California Academy of Sciences collection, we provide a redescription of all previously known species and describe six new species. As well as describing the exomorphology, we provide a detailed description of the male genitalia, and of microdetails documented through Scanning Electron Microscopy. Our results show that the characters previously used to define the genera (e.g. ocularium size) are highly variable among species and have no utility for generic delimitation. We propose *Hovanoceros* and *Malgaceros* as junior synonyms of *Anaceros* and consequently propose their new combinations respectively. Also, we emend the diagnosis of the genus *Anaceros*. This study reveals just a small part of the rich and uncharted diversity of litter-dwelling Malagasy opilionids. Because of the high level of micro-endemicity observed, we predict that many new species of this group remain to be discovered on the island.

Evidence for positive selection on spider developmental genes. Molecular evolution of spider developmental genes

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The ability of producing and using silk-like threads has been described for many metazoan taxa. However, no animal spins like spiders do. These arthropods possess opisthosomal appendages called spinnerets, which allow them to finely manipulate the threads synthesized in the silk glands. Spiders use silk for numerous activities that pervade virtually all ecological aspects of their life history. Yet, questions on how spinnerets came about and evolved have been neglected; despite a few proposed hypotheses, there is still much to learn. Hence, we selected six genes that had been previously described as participants in opisthosoma and spinneret development, searched for their orthologs in a wide range of spider and other arachnids and looked for molecular evolution patterns. We aimed to test whether modifications in the evolutionary regimen guiding the evolution of such genes could be at least partially responsible for the origins of spinnerets, structures that have no clear homology to any known arthropod morphological feature. Our results indicate no great differences in the selective pressures on those genes when comparing spiders to other arachnids, but some site-specific positive selection evidence was found in Araneae. These findings lead us to new insights on spider evolution to be further tested.

Phylogeography of the spider *Sicarius thomisoides*: intraspecific adverse response to Pleistocene rainfall increase in Chilean xeric zones

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Central-North Chile is characterized by a rainfall gradient with less than 1mm/yr northward to 900mm/yr southward. Climatic oscillations of the Quaternary caused changes in atmospheric circulation systems and rainfall regimes. *Sicarius thomisoides* is a spider strictly adapted to dry conditions occurring throughout this gradient. We tested if the southern populations were extinct in areas of increased rainfall during the last glacial maximum (LGM). We modeled the climatic niche using 171 records, and projected this model in Mid-Holocene, LGM and last interglacial. We sequenced COI, ITS2 and H3 of 127 specimens from 48 localities. We analyzed genetic structure, phylogenetic relationships and divergence times. We found two major clades, one occurring in northern Chile and the other in central Chile, each divided in geographically structured subclades that diverged during the Plio-Pleistocene. The climatic model showed reduced adequability during the LGM, caused by increased rainfall in the south. The southern subclades showed higher genetic structure and long branches among their populations, compared to the northern haplogroups. The long branches could indicate a significant populational reduction locally, with few lineages probably persisting in small refuges. Thus, *S. thomisoides* southern populations might have suffered population bottlenecks, but were not completely extinct during the LGM.

Effect of Thiamethoxam and Trichlorfon on web building behavior of *Neoscona theisi* (Araneae; Araneidae)

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The field collected spiders were divided into control and experimental groups. Spiders were kept in specially designed boxes and allowed to build webs under laboratory conditions. Spiders of experimental group were exposed to different concentrations of insecticides, Thiamethoxam and Trichlorfon, while control group were treated only with distilled water. Observations were made after every 24, 48 and 72 hours of application of insecticides. Pictures of webs constructed by both control and experimental group spiders were taken by digital camera and web parameters (no. of spirals, no. of radii, mesh height, web diameter, radius, capture area and anchoring thread length) were recorded and compared. Results showed that both insecticides effect spiders and their web building behavior. Trichlorfon proved highly lethal for spider survival as it caused 100 % mortality at recommended field rate concentration, while Thiamethoxam caused only 20 % mortality at field rate. Statistical analysis revealed that higher concentrations affected web building more significantly while lower concentration (half field rate) did not affect web parameter significantly and normal web building was observed. Trichlorfon caused more lethal effects while Thiamethoxam had sub lethal effects on web building.

Coexisting in sympatry: evaluating the phenotypic variation of multiple morphological traits in two species of mountain scorpions

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In sympatric areas, the correct sexual recognition among conspecifics is critical to ensure reproductive isolation. However, the recognition can fail resulting in processes like reproductive interference (RI). For this, certain reproductive traits can suffer reproductive character displacement (RCD), allowing species' coexistence. Two species of closely related scorpions (*Urophonius brachycentrus, U. achalensis*) have sympatric populations with synchronic reproduction (Argentina). Antecedents in these species reveal the occurrence of heterospecific mating and chemical communication overlapping, which allows us to test if certain traits experiment RCD in response to RI.

We perform linear and geometric morphometrics of several non-sexual and sexual traits (involved in different mating stages and sperm transfer). We observed a highly asymmetric RCD in both sexes of *U. brachyentrus* in the pedipalp' shape, a key trait in courtship behavior. However, most of the structures size experiment convergence towards sympatry, which would evidence some ecological processes. These results are extremely interesting in terms of the occurrence of asymmetric RCD and the possible competitive exploitation of mates by *U. achalensis*. Furthermore, a scenario with scramble competition, a short reproductive period and the existence of strong sperm competition make our findings an important piece in the puzzle of the reproductive biology of these species.

A candidate gene approach to elucidating *Parasteatoda tepidariorum* circadian clock genetics

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Circadian rhythms are daily patterns in behavior and physiology. Molecular timekeepers within cells dictate circadian rhythms and can be entrained to environmental time cues such as light. In the absence of time cues, biological rhythms in most animals maintain timing very close to 24 hours. Arachnids deviate from this norm, exhibiting rhythms as short as ~18 hours in constant darkness. Transcription-translation feedback loops (TTFL) that regulate circadian processes in *D. melanogaster* are well-studied, and many components are conserved in other invertebrates. Our lab is using the common house spider, *Parasteatoda tepidariorum*, to construct the spider TTFL using D. melanogaster clock gene orthologs as candidates. Despite the existence of an ancient genome duplication in arachnid lineage, we identified single *P. tepidarorum* orthologs of core clock genes and most clock gene regulators. Putative core clock genes are expressed throughout the full life cycle of *P. tepidarorum* and transcript levels oscillate within a single light/dark cycle, like their insect counterparts. Two insect clock regulators that have three orthologs in *P. tepidarorum* are Cryptochrome and Doubletime, proteins that target core clock components for proteolysis. Together these findings show conservation of major insect clock genes in *P. tepidariorum*. Additional orthologs of regulatory proteins may add complexity to the arachnid TTFL. Future studies will study demonstrate functionality of core clock proteins and their regulators to determine how they relate to the unique circadian rhythms of spiders.

Beauty under the mud: soil crypsis in new species of the Malagasy genus *Ankaratrix* (Opiliones: Triaenonychidae: Triaenobuninae)

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Camouflaging with crypsis is a known strategy in several invertebrates such as sea urchins, gastropods, crabs and insects. In harvestmen, soil crypsis are used as a defense strategy. The soil particles are attached to their bodies using secretions. We investigate this kind of camouflage in the Malagasy endemic genus *Ankaratrix*. Members of this genus produce a glue layer covering the cerotegument. This glue layer seems to play an important role in soil crypsis, together with the long tubercles. These long tubercles could help to catch and retain the soil particles bringing stability to the camouflage layer. The specimens are covered by a very thick soil layer, determined by the tubercle sizes, mainly on the dorsal body surface. Only the body parts involved in feeding, reproduction, excretion and sensory functions are clean of mud. Legs I exhibit a very long longitudinal row of spiniform apophyses that will be completely covered by soil, forming a kind of wall that protect the chelicerae and pedipalp of the dirt and could be adopted as camouflage strategy in thanatosis or resting periods. During our investigation, we discovered four new species, which are described. Finally, we discuss the sexual dimorphism in pedipalps of the genus *Ankaratrix*.

***Agalenocosa pirity*, an evenly sized wolf spider without courtship**

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Agalenocosa pirity is a middle-sized lycosid that inhabits the shores of lagoons and ponds of Argentinian north east. In order to study their sexual behavior, live juvenile specimens were collected and bred to maturity to conduct mating trials. No complex courtship was displayed at any moment of the mating, and in 107 trials we recorded only one case of post-copulatory cannibalism. In spiders, the outcome of sexual conflicts is related to sexual size dimorphism (SSD), this led us to hypothesize that the lack of a complex courtship in *A. pirity* could be related to a lack of SSD, and thus males would not need to engage in costly displays to reduce the risk of being cannibalized. We assessed the significance of SSD by measuring length and width of prosoma (n = 27 females, 37 males). Both parameters were non-significantly different between sexes, supporting the lack of SSD. Generally in Lycosidae females are bigger than males and there are reports of males being cannibalized during the sexual encounters with only one report with inverse pattern, males bigger and cannibalistic. In this regard, *A. pirity* could represent a middle point, were the equal vulnerability to attacks during mating context might have led to a loss of complex courtships.

Sex and age-based differences in sericophily among subadult and adults of the wolf spider *Pardosa milvina*

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Adult male *Pardosa milvina* are attracted to silk (sericophily) from unmated adult females but sericophily may also occur among adult females or penultimate instar spiders. We tested whether subadult male and female *P. milvina* were attracted to silk from conspecific subadult or adult males and females. We also tested adult male and female responses to silk from adult and subadults of the same or opposite sex. We measured subadult and adult male and female attraction or repellency to seven different pairs of silk substrates or no silk (7x4 design). For each spider, we measured time on each substrate within each pair over one hour. We found that adult male spiders were attracted to adult female silk and avoided male silk while adult females were indifferent to silk from adult males and avoided silk from subadult males. Subadult females showed no responses to silk from males or females of any age but subadult males showed attraction to silk from adult females and avoidance of silk from adult males. Silk-mediated communication can occur prior to sexual maturity and silk can serve as a repellent as well as an attractant depending on the sex and age of the spider.

Put on your sexy genes: Examining potential sex determining gene expression in the common house spider*, Parasteatoda tepidariorum*

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Sex determination is the mechanism by which organisms develop as males or females. This process differs in many species, and nothing is known about sex determination in spiders. Spiders are interesting organisms because of their complex genome. An ancient genome duplication has led to the presence of many new genes which have driven their evolution. The purpose of this study was to determine whether several Drosophila melanogaster genes involved in sexual development are present in the common house spider (*Parasteatoda tepidariorum*), and if so, whether there is sex specific expression of these genes. We found that SETDB1, a gene that functions to trigger cell-type specific methylation and gene silencing in post-embryonic cells, is expressed in early embryos but is restricted to just females in adulthood. This was

expected because SETDB1 was found to regulate the differentiation of the female Drosophila germ line. Future studies will determine the role of SETDB1 in spider germ line development.

Modified orb webs in the cave spider *Meta menardi* (Tetragnathidae)

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The nutrient-poor subterranean environment has resulted in a spectacular morphological and anatomical adaptations, but behavioral adaptations remain poorly studied. Orb spiders are good model organisms for the study of behavior as their webs can be viewed as records of foraging behavior frozen in time. Surprisingly, given the perpetual darkness, orb spiders in the genus Meta are common in caves. We compared the web structure between the European cave orb spider, *Meta menardi*, and two related aboveground species; *Metellina mengei* and *Tetragnatha montana*. We found that the webs of the cave spider differed significantly from the two surface-dwelling species. The most dramatic difference was the lack of frame threads with the radii instead attaching directly to the surrounding rock, but other differences in relative web size, web asymmetry and number of capture spiral threads were also found. The fact that radii connect directly to the cave wall could be a potential adaptation that allow prey capture off the web by alerting the spider walking prey. More data on prey capture and the web geometry of other Meta spiders, including the North American *M. ovalis* and *M. dolloffs* is needed.

Sequencing the mixed ploidy genomes of facultatively parthenogenetic Japanese harvester *Leiobunum manubriatum*

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Japanese harvester species *Leiobunum manubriatum* exhibits facultative parthenogenesis and intra-population mixed ploidy, characteristics found rarely in animals. Polyploidy, a condition in which an organism has three or more times the normal haploid chromosome number, frequently co-occurs with parthenogenesis, an asexual reproduction mode in which ova develop without fertilization. Both diploid and tetraploid *L. manubriatum* individuals occur within the same populations, and females of both ploidies are able to reproduce asexually. As a reference genome for any opilionid

is currently lacking, we used Oxford Nanopore to sequence and assemble the genome of the *L. manubriatum* diploid race to explore hypotheses about the origin, consequences, and relationship of polyploidy to facultative parthenogenesis. BUSCO scores indicate excellent completeness metrics of the assembled and annotated first draft of diploid females, and we are currently sequencing the genome of the tetraploid race. These *L. manubriatum* genomes will facilitate efforts to understand evolutionary mechanisms for the maintenance of sex, cytological processes of parthenogenesis, and genetic associations of polyploidy and sexual strategy.

Leaf litter moisture impacts efficacy of vibratory courtship signals and detection of predator cues in *Schizocosa ocreata* (Hentz) wolf spiders

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Male *Schizocosa ocreata* (Hentz 1844) wolf spiders use multimodal (vibratory, visual) signals in courtship, and variation in the leaf litter environment can affect signal efficacy and mating success. Additionally, courting males show anti-predator behavior in response to airborne cues from avian predators, conducted as vibration through leaf surfaces. We examined the effect of environmental variation - wet vs. dry leaves - on transmission of courtship signals and bird calls, as well as responses of spiders to each. Laser Doppler vibrometry showed that dry leaves transmit spider signals across all frequencies at greater amplitudes than wet leaves, but with bird calls wet leaves had greater noise in lower frequencies. Males courted females on wet and dry leaves equally, but mating success was significantly greater on dry leaf litter. In response to predatory bird calls, spiders on dry leaves responded with anti-predator “freeze” behaviors more often and with longer duration than those on wet leaves, and latency of return to courtship after a bird call was greater on dry leaves. Results suggest that environmental substrate moisture conditions can influence efficacy of spider vibratory signaling, as well as detection of substrate vibration from airborne cues indicating potential predators.

Silk physico-chemical variability and mechanical robustness facilitates intercontinental invasibility of a spider

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There are substantive problems associated with invasive species, including threats to endemic organisms and biodiversity. Understanding the mechanisms driving invasions is thus critical. Variable extended phenotypes may enable animals to invade into novel environments. We explored here the proposition that silk variability is a facilitator of invasive success for the highly invasive Australian house spider, *Badumna longinqua*. We compared the physico-chemical and mechanical properties and underlying gene expressions of its major ampullate (MA) silk between a native Sydney population and an invasive counterpart from Montevideo, Uruguay. We found that while differential gene expressions might explain the differences in silk amino acid compositions and protein nanostructures, we did not find any significant differences in silk mechanical properties across the populations. Our results accordingly suggest that B. longinqua's silk remains functionally robust despite underlying physicochemical and genetic variability as the spider expands its range across continents. They also imply that a combination of silk physico-chemical plasticity combined with mechanical robustness might contribute more broadly to spider invasibilities.

Lethal effects of common herbicides on an agriculturally abundant wolf spider *Pardosa milvina*

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The effects of chronic exposure to single or multiple herbicides on spiders have been understudied. We tested the lethal effects of field-relevant dosages of five commonly used herbicides on the wolf spider, *Pardosa milvina*. Tested herbicides included atrazine, S-metolachlor, rimsulfuron, mesotrione, glyphosate, a mixture of all five herbicides, and a distilled water control. Spiders were collected from two nearby fields; one sprayed with combinations of these herbicides (conventional field) while the other site was pesticide free (no herbicide field) (N=1,214, n= 43 spiders across 28 treatments). Spiders were maintained on treated soil substrates for 52 days, fed weekly, and checked for mortality daily. We found significant herbicide treatment effects, with mesotrione being particularly lethal to wolf spiders while atrazine and S-metolachlor had modest, but significantly higher survival than the control. We also found significant differences and treatment interactions by sex and collecting site. Spiders from the no-herbicide field had longer survival than spiders collected from the conventional field. The mesotrione-treated spiders had significantly poorer survival than even the combined herbicide treatment suggesting a complex antagonistic interaction of some of these herbicides on wolf spider survival.

Total-evidence analysis of an undescribed fauna: resolving the evolution and classification of Australia's golden trapdoor spiders (Idiopidae: Arbanitinae: Euoplini)

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In the trapdoor spider genus *Euoplos* Rainbow & Pulleine, it was recently discovered that two divergent lineages occur in sympatry in Australia, challenging the monogeneric classification of the tribe Euoplini. To resolve this, we conducted a total-evidence cladistic analysis on a largely undescribed continental fauna – the first such analysis on a group of Australian Mygalomorphae. We combined molecular and morphological/behavioral data from all species from eastern Australia (described and undescribed), plus a subset of Western Australian species, to produce a phylogeny. We mapped morphological/behavioral characters onto this to identify diagnostic characters and applied these data to a generic reclassification of the tribe. We recovered two sympatric lineages ('wafer-door' and 'plug-door/palisade') in the Euoplini and revealed the phylogenetic position of all known eastern Australian species within these. Character mapping revealed morphological and behavioral (burrow architecture) diagnostic features of the lineages and clades within them. Accordingly, we erected a new genus, *Cryptoforis* gen. nov, to represent the wafer-door lineage, described the type species, and transferred two species from *Euoplos* to *Cryptoforis*. This study resolved phylogenetic structure within the Euoplini, characterized clades within the tribe to facilitate future taxonomic revisions, and demonstrated the subtle yet informative nature of female characters in mygalomorph spiders.