TALK ABSTRACTS

2024 ANIMAL BEHAVIOR CONFERENCE

Alphabetical by presenter last name. Presenter last names are shown in **bold**.

CONTROL, CONTEXT & CHOOSINESS: FLIPPING THE LENS TO SEE FEMALE PLASTICITY IN WIDOW SPIDERS

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Adaptive developmental plasticity (ADP) may evolve when traits that confer reproductive success vary with context, and context is indicated by cues available during development. ADP cues trigger developmental changes, resulting in phenotypes matched to the challenges experienced as adults. We have shown that demographic variation (social context) shifts the form of sexual selection on male *Latrodectus* spiders in nature, and that male life history changes in response to demographic cues, conferring higher fitness. For short-lived *Latrodectus* males, this outcome is adaptive. For longer-lived *Latrodectus* females however, ADP of life history seems unlikely. However, for females, social context during development may predict optimal levels of adult choosiness (~the likelihood of expressing a mating preference). We studied ADP in female *Latrodectus hesperus* and *L. hasselti* by simulating natural exposure to cues of future mate availability. Females exposed to cues of high mate availability as juveniles showed increased mechanisms of choosiness in their first mating as adults. This included shifts in mating plug placement, premature cannibalism, and copulation frequency, all of which provide female control over post-copulatory sexual selection. In a related study of *L. geometricus*, we show that females that retain control in their first mating are choosy when remating. Plasticity in female mating preferences for male traits is affected by juvenile social experience in other species. This work extends understanding of ADP shaping female choice to encompass choosiness. We illustrate the complex ways ADP links population characteristics to sexual selection and support *Latrodectus* as a model clade for studies of plasticity.

ASOCIAL TESTING OF SOCIAL SPECIES: STRESS-TESTING THE ECOLOGICAL VALIDITY OF OUR ANIMAL BEHAVIOR ASSAYS

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Presence of groupmates is the ecologically valid condition in virtually every behavioral context for many species. While teasing apart individual differences in noisy group contexts may be difficult–groupmates frequently and dynamically influence each other's behavior –some perhaps overlooked research suggests we may be stunting our understanding of natural individual differences for the sake of convenience. Isolation may even cause an additional and unique stressor in highly social species, placing a new interpretation on old behavioral data. Presented here are repeated calls to consider the effects of isolated contexts from researchers

spanning broad taxa, as well as new empirical data on the results of isolated vs social versions of a novel environment test for Tufted titmice (*Baeolophus bicolor*) and Carolina chickadees (*Poecile carolinensis*).

EYE COLOR IS UNLIKELY TO SERVE AS A STARTLE MECHANISM IN RED-EYED TREE FROGS *AGALYCHNIS CALLIDRYAS*

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Predator avoidance and antipredator mechanisms are ubiquitous in all organisms affected by predation. One such mechanism, startle behavior, uses bright colors or contrasting patterns to intimidate a potential predator in such a way as to provide the prey with a slight escape advantage prior to the subjugation phase of a predation sequence. The extravagant coloration of Red-eyed Treefrogs *Agalychnis callidryas* is commonly explained as functioning in such a startle capacity, yet to our knowledge, this hypothesis remains untested. We assessed the reaction of live Red-eyed Treefrogs to simulated predation events from forceps and a model bird. To determine the role of eye color on the propensity for predator attack, naïve chickens (SN) were exposed to clay frogs with their eyes painted red or green. In a second experiment, naïve chickens were exposed to clay frogs in which the eyes were replaced with red light emitting diodes (LED) that could be turned on, thus stimulating the "flash" of color that a potential predator would experience when disrupting a sleeping frog. Results from the simulated predation event suggest that while eye color could conceivably function as a startle mechanism, the orange hands and blue and yellow flanks of Red-eyed Treefrogs are unlikely to function in this capacity. Experiments with model frogs found that the red eye color increases attack frequency and intensity. Finally, results with model frogs with LED eyes failed to induce a delayed reaction in naïve chickens when turned on. These results suggest that the bright coloration of red-eyed treefrogs is unlikely to function as a startle mechanism, at least in response to avian predators. Hypotheses for the evolution of the Red-eyed treefrogs coloration are reviewed and discussed.

LONELY AND LISTENING: SEX AND SOCIAL ISOLATION INFLUENCE SOCIABILITY DURING SQUEAK PLAYBACK

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How an individual produces and responds to signals during a social interaction is dependent on contextual factors. During conspecific interactions, house mice employ different vocalizations with highly context-dependent functions. Broadband vocalizations (BBVs, or squeaks) are often negatively-valenced signals produced during distress or female rejection of male courtship signals. In response to BBVs, males decrease courtship effort, by decreasing production of prosocial ultrasonic vocalizations (USVs). As USVs are primarily produced by males, these calls may not be an ideal measure of motivation or response to BBVs in females. However, mice of both sexes exhibit context-dependent social investigation behaviors in

response to social stimuli. While studies have explored how USVs affect social approach behaviors, little is known about how BBVs may affect this behavior. To assess how BBVs influence sociability across interactions consisting of individuals of varying sex and social experience, we altered a classic 3-chamber sociability task by adding a section of BBV playback in the middle of the interaction to gauge baseline sociability, sociability during BBV playback, and sociability after playback ends for socially housed female and male mice, as well as males housed in isolation. We found that BBV playback decreases social investigation in male but not female mice, and that BBV playback alters the trajectory of social behavior in male mice depending on social history. By looking at both same-sex and opposite-sex interactions, we found that the effects of squeak playback are also dependent on sex of both the focal individual and the social partner. These results give insight to how both identity factors and experiences like social isolation may alter both social motivation and response to vocal signals. This also supports that BBVs, a signal traditionally thought to represent distress, may serve different functions depending on the social context of an interaction.

INVESTIGATION OF DIFFERENCES IN MOUSE AND PRAIRIE VOLE STRING-PULLING MOVEMENT ORGANIZATION

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Rodents engage in spontaneous string-pulling behaviors, which can be used to investigate fine motor control. Evolutionary systems and differences in rodent natural habitats have likely shaped the evolution of neural systems that support motor coordination. In the current study, species differences in string-pulling behaviors were investigated in male non-monogamous mice and socially monogamous prairie voles. All animals went through string-pulling trials that started when the rodent was placed in the apparatus and ended when either the baited end of the string entered the apparatus or 20 minutes had elapsed. Male prairie voles (n=6) and mice (n=6) received three trials per day for five days. Approach and pull time behaviors were recorded and analyzed for latency until the string reached a threshold within the apparatus. A significant day effect was observed in latency to approach the string in prairie voles, indicated by a consistent reduction in latency to first interact with the string across test days. Prairie voles also displayed string-pulling behaviors involving a combination of using a front paw and the mouth to reach the end of the string. This strategy differs from previous patterns observed in mice, which are generally quicker to use hand-over-hand coordination. Future research will benefit from evaluating the social structures of mice and prairie voles, which might influence behavioral differences in string-pulling behaviors and help inform our understanding of species differences.

EFFECTS OF NEST PARASITE ABUNDANCE ON BEE-HAVIOR IN CHIMNEY BEES (*ANTHOPHORA ABRUPTA*)

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Understanding the behavior of imperiled species can be critical for guiding effective conservation efforts. Aggregating ground-nesting bees are essential and often understudied pollinators that may be increasingly threatened by anthropogenic change. In this study, we investigated whether parasite load could be an indicator of the health of ground-nesting bee aggregations. Nest parasites (kleptoparasites) often wait at nest aggregations for female hosts to provision their young with pollen before laying their own, faster hatching eggs, on the provision. Kleptoparasites kill the host bee larvae and may also decrease the overall fitness of the aggregation through behavioral changes. To this end, we explored how nest parasitism impacts chimney bee (*Anthophora abupta*) behaviors and overall aggregation health. We used cameras to record host behavior and conducted sweep sampling to provide background nest parasite and host abundance data. We tracked behaviors based on a modified ethogram from Rezkova (2011). Across four different chimney bee aggregations, we found that host behavior was associated with parasite abundance. Our observations suggest that chimney bees may change their nesting behaviors in the presence of nest parasites. Our study provides information on how host-parasite interactions influence bee health for aggregating bees. Understanding the intricacies of bee health and the impact of nest parasites is paramount for native bee conservation.

WHAT'S THE RUMPUS? THE INTERCONTINENTAL PERCEPTION OF AVIAN ALARM CALLS

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Alarm signals have evolved to communicate imminent threats to conspecifics but animals may also perceive other species' alarm displays to obtain adaptive information. In birds, mixed-species foraging flocks are often structured around a focal sentinel species, which produces reliable alarm calls that inform eavesdropping non-sentinel heterospecifics about predation risk. Ongoing work has revealed that several species can recognize the alarm calls of certain sentinel species even without prior encounters, including when these are from a distant biogeographic region. Similar work has yet to examine whether naive subjects' responses to unfamiliar sentinel calls differ from responses to non-sentinel calls. We played the alarm calls of three subtropical Asian bird species that participate in mixed species flocks, for temperate North American birds. Here birds responded most to the alarm call of a core sentinel and a local positive control species, less so to a secondary sentinel, and least so to a non-sentinel

and a negative control stimulus. These patterns provide evidence that broad phylogenetic and geographic recognition is a pertinent aspect of sentinel alarm calls in general.

WHO CAN TAKE THE HEAT? INVESTIGATING TRANSCRIPTOMIC SIGNATURES OF THERMAL RESILIENCE IN A SONGBIRD.

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There is an urgent need to understand how warming temperatures affect animal behavior. Mating signals may be particularly vulnerable. Even endotherms like birds, are experiencing sublethal heat effects. For example, we know that heat can reduce the rate at which male zebra finches sing. However, some males are more behaviorally resilient than others. Here, we take an organismal approach to identify the mechanisms underlying individual differences in behavioral resilience. Using male zebra finches (*Taeniopygia castanotis*), we measured song production during a standardized heat challenge. We then measured gene expression via RNAseq in both the brain and syrinx (vocal control musculature). We predict behavioral resilience will covary with transcriptional patterning, particularly in neural pathways of reward and syringeal pathways of muscle function. Results will lend insight into how at least some individuals are evading the deleterious effects of heat on adaptive behaviors.

LAND HO! POLARIZED LIGHT SERVES AS A VISUAL SIGNAL FOR LANDWARD ORIENTATION IN DISPLACED SPIDERS

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An organism's ability to identify goals within their environment, orient towards those goals, and successfully navigate to them are critical to all aspects of survival. Long-jawed orb weavers (*Tetragnatha elongata*) occupy riparian zones and perform orientation behaviors when displaced from this habitat onto the water. We conducted a series of investigations to determine the mechanism by which these spiders rapidly achieve zonal recovery. Occlusion experiments indicate that spiders use visual information to identify characteristics of the riparian habitat and navigate to shelter. While environmental characteristics such as color, contrast, and the sun's position do not appear to factor into this orientation behavior, the polarization of light appears critical. We propose that the polarization of light reflecting off the water's surface acts as a water detector and the absence of such at the edges of the pond (or via experimental induction) serves as a visual reference for the closest suitable habitat.

EARLY LIFE PAIN ALTERS THE RESPONSE TO AN IMMUNE CHALLENGE IN ADULT MALE AND FEMALE RATS

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Premature infants are more likely to be admitted to the Neonatal Intensive Care Unit (NICU) where they experience upwards of 10-18 painful procedures each day, often without anesthesia or analgesia. Preclinical and clinical studies have shown that neonatal pain disrupts normal CNS development in multiple ways that persist into adulthood. We have previously reported that early life pain results in an exaggerated febrile response to an immune challenge in adulthood. Administration of LPS induces the release of inflammatory cytokines in the periphery to stimulate prostaglandin E2 (PGE2) production. Centrally, PGE2 binding to the EP3 receptor (EP3R) within the hypothalamic median preoptic area (MnPO) induces a pyrogenic (fever) response. Here, we investigate if early life pain (ELP) alters PGE2 signaling within the MnPO. Male and female rats were exposed to a short-term inflammatory insult induced by intraplantar administration of 1% carrageenan on the day of birth (P0). In adulthood (P60-P90), Thermicron iButtons were implanted to monitor core body temperature; 14 days later, lipopolysaccharide (LPS) was injected to elicit an immune response. Rats were sacrificed at one of 3 time points post-LPS: 24 hours, peak fever, or 2 hours. LPS administration resulted in an elevated febrile response in ELP males and females compared to controls and increased sickness behaviors in ELP females. Immunohistological analysis revealed sex and treatment differences in cellular activation in several brain regions and increased receptor and transporter expression in the MnPO in ELP rats. Cox-2 expression is also increased at peak fever in ELP rats. Together, these studies are consistent with clinical studies reporting that children experiencing unresolved pain during the perinatal period show increased severity of sickness behavior and altered immune signaling following exposure to a pathogen and will provide a foundation for future studies examining the biological underpinnings.

EFFECTS OF ADOLESCENT CARDIOVASCULAR AND RESISTANCE TRAINING EXERCISE IN MALE AND FEMALE RATS

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While the beneficial effects of cardiovascular exercise have been well-documented, more recent research has touted the potential benefits of weight training (resistance/hypertrophy model of exercise). However, much of the literature on exercise and its effects on cognition and the brain's physiology observes a cardiovascular model. Cardiovascular exercise positively affects performance in learning and memory tasks and increases hippocampal neurogenesis in rat subjects, but less is understood about the effects of resistance exercise on the brain and behavior. In addition, previous studies only have utilized male rats, ignoring potential sex effects. Lastly, less is known regarding the effects of exercise during the adolescence period on later adult behavior and neuroanatomy. In the present study, adolescent male and female Long Evans rats were divided into three conditions: cardiovascular training (CT) rats with access to a running wheel, resistance training (RT) rats that engage in a ladder climbing

protocol, and a control (sedentary) group of standardly housed rats. After reaching adulthood, these rats were tested for anxiety-like behavior, and spatial memory. We found CT female rats ran significantly longer than male rats on average. RT females hit puberty significantly later than females in cardiovascular and sedentary conditions. In adulthood, anxiety-like behavior was assessed using the elevated plus maze and open field testing (OF). During OF it was found that rats in both CT and RT trended towards taking less time to enter the center of the field and spent more time in the center than sedentary. Spatial memory was tested with an object placement task, with RT rats spent significantly less time with the old and new objects across both trails, this effect was stronger in females. These findings suggest that resistance training exercise affects males and females differently, potentially delaying pubertal onset in females, and negatively impacting spatial memory.

FROM UNDERSTORY TO CANOPY: FROG SIGNAL DEGRADATION IN A TROPICAL FOREST

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The structural complexity of tropical forests imposes challenging conditions for animals who rely on acoustic communication for mating. From the dense foliage of the understory to windier and drier conditions at the canopy, the signals used by animals communicating at different forest heights are expected to be under selection (Acoustic Adaptation Hypothesis). Selection is expected to favor acoustic signals that reduce power loss of the signal and maintain its integrity across distance, increasing the probability of detection by target receivers. Despite how central calling behavior is in the reproductive success of frogs and the broad vertical distribution of calling sites used by frogs in the forest, the influence of forest height on signal transmission efficiency remains unexplored. We investigated signal attenuation across forest height in a Neotropical anuran community. We performed transmission experiments at the understory and canopy height of a degraded forest in Gamboa, Panama, using the 24 most common frog species in this area. To do so, we broadcast the advertisement calls of frogs at 1, 3, 5, 10, and 20 m and recorded the signal and its intensity. We found evidence that frog calls vary in the degree of attenuation experienced between the canopy and understory. Our data also revealed differences in the degree of attenuation among different species in the community. Altogether, these results suggest that height is a factor that influences the transmission of frog acoustic signals, potentially affecting the volume over which target and non-target receivers detect the signal.

HOW SKIN COLOR MODULATES BEHAVIORAL FEVER IN NORTHERN LEOPARD FROGS

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With increasing rates of infection and subsequent mortality among amphibians globally, further understanding of how amphibians cope with infection is critical to orchestrating conservation. In particular, *Batrachochytrium dendrobatidis* (*Bd*) is responsible for many of these disease-related mortalities. One partially investigated method of clearing infections,

including *Bd*, is behavioral fever, where amphibians will preferentially seek warmer temperatures to help clear infection. One factor that may modulate behavioral fever is skin coloration, which may differentially absorb and reflect light's radiation, heating amphibians' bodies at different rates. This may modulate time length needed for increasing body temperature, which also has negative consequences like potential exposure to predators and dehydration. We investigated whether Northern leopard frogs (*Lithobates pipiens*) exhibited behavioral fever when inoculated with *Bd* and whether their skin coloration may modulate potential behavioral fever. First, we collected Northern leopard frog eggs and raised them to metamorphs in the field and lab. Then, we constructed in-lab temperature gradients for 20 metamorphs and monitored their temperature preference in these gradients while inoculated with *Bd*. While we are still analyzing results, we predict that Northern leopard frogs inoculated with *Bd* preferred higher temperatures on average than uninfected frogs, and that frogs with darker skin coloration on average spent less time in higher temperature microclimates increasing their body temperature. These potential results lend further insight into how some frogs may cope with high-mortality diseases like *Bd* and inform future management and investigation of the dynamic between disease and amphibians.

MICROPLASTICS ARE PRESENT IN EPHEMERAL POOLS AND ELEPHANT DIGESTIVE TRACTS IN KRUGER NATIONAL PARK

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Past studies conducted in Kruger National Park (KNP), South Africa found high levels of microplastic pollution in perennial rivers, and suggest that this results from anthropogenic water contamination outside the park that travels within the flowing water into the protected areas. Building upon previous research concerning the prevalence of microplastic pollution in urban areas and marine environments throughout the world which largely focus on abiotic microplastic cycling, we sought to investigate microplastic concentrations and movement in a conserved terrestrial area. We examined the presence of microplastics in ephemeral pools and elephant fecal matter within the Sabie River catchment. The minimum microplastic concentration in sediment was 71±18 NMP/200g and the maximum was 180±18, whereas in dung we found a minimum of 320±189 NMP/200g and a maximum of 1493±189 NMP/200g. Altogether, we found higher concentrations of microplastics in three of the sediment samples and all six dung samples, compared to their respective controls. In five of the six sites, microplastics were more concentrated in dung than sediment. Overall, elephants could serve as a vector for microplastic dispersal throughout the landscape, suggesting that other terrestrial organisms may fulfill the same role. Additionally, while microplastics are present in ephemeral pools, these water bodies are less contaminated by microplastics than perennial rivers within KNP. Future studies should scrutinize additional biotic mechanisms for microplastic cycling, as well as the consequences of its concentration in sediments.

BEHAVIORAL AND HORMONAL EVALUATION OF FOUR METHODS OF REINFORCEMENT TRAINING IN DONKEYS *EQUUS ASINUS*

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Donkeys learn best with positive (POS) or neutral reinforcement training but are often handled like horses, which learn best via negative (NEG) reinforcement. No research has evaluated combined (POS/NEG) reinforcement training in donkeys. The study objective was to assess behaviors and hormonal responses of donkeys undergoing reinforcement training during daily 10-min sessions over 6 sessions. Donkeys were asked to cross a walkway with a rubber mat (mimicking a floor scale) placed between Points A and B. Incremental advancement in the walkway was reinforced as follows: POS (n=6) food reward; POS/NEG (n=6) releasing lead rope pull-pressure plus food reward; NEG1 (n=6) releasing lead rope pull-pressure; NEG2 (n=6) releasing push-pressure across the rump. Control (CON) donkeys (n=6) moved freely within the walkway without reinforcement. For all experimental groups, number of *crossings* (traversing from Point A to B) and ethogram data (with behaviors classified into affiliative, fearful, or explosive) were collated. Pre-/post-training salivary samples were collected before animal entry and egress at Points A and Point B, respectively, for salivary cortisol (s_{CORT}) and oxytocin (s_{OXT}) analysis. Negative control (CON-) donkeys (n=6) were not exposed to the walkway or reinforcements. Results revealed significant interactions between session:reinforcement ($P<0.0001$), and session:sex ($P=0.03$) on *crossings*. Average *crossings* over all sessions were: POS/NEG (14.9), NEG1 (11.1), NEG2 (10.2), CON (8.8), and POS (7.3). Johns showed higher average *crossings* ($P<0.05$) than jennies. CON donkeys demonstrated most affiliative (68%) behaviors. Johns had higher pre-/post-training s_{CORT} across sessions and reinforcements ($P<0.0001$). Post-training s_{OXT} was highest in POS/NEG donkeys irrespective of sex ($P<0.0009$). POS/NEG reinforcement training applied in short daily intervals seemed best at facilitating acclimation to novel stimuli and retention of learned skills.

THE ROLE OF ISOTOCIN IN THE DIVERGENT PARENTAL CARE BEHAVIOR OF TWO STICKLEBACK POPULATIONS

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Parental care has evolved repeatedly but is rarely lost. The neural and hormonal mechanisms associated with the loss of care are not well understood. When care is lost, are mechanisms that promote caregiving lost? For example, oxytocin, and its homolog isotocin, are neuropeptide hormones with a conserved role in promoting caregiving in vertebrates. How might the role of isotocin change when care is lost? To address this question, we used the threespine stickleback (*Gasterosteus aculeatus*). These fish typically provide obligate, male-only parental care. However, an atypical population (the “white” ecotype) has evolutionarily lost care. Instead of caring for their embryos, males of the white ecotype spit them out of their

nests soon after fertilization. We investigated the role of isotocin in the loss of care by comparing males of the non-caregiving ("white") and caregiving ("common") ecotypes in two experiments. In the first experiment, we found that commons show greater activation of preoptic isotocin neurons as they become fathers compared to whites. In the second experiment, we show that injection of isotocin has different effects on the behavior of whites and commons, consistent with the idea that isotocin plays different roles in regulating behavior in these two populations. Our results shed light on how the role of a conserved neuropeptide, isotocin, may be tweaked by evolution when caregiving is lost.

DIVERSE HOST-FEEDING STRATEGIES IN AN UNDERSTUDIED TRIBE OF MOSQUITOES

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Blood-sucking insects use different strategies to detect, locate, and feed on their hosts. Among mosquitoes, host-seeking behavior and specificity ultimately modulate disease dynamics and have relevant ecological and evolutionary implications. We synthesize current knowledge on the diversity of host-seeking strategies and host interactions in an understudied tribe of mosquitoes, Uranotaeniini (Diptera: Culicidae). We performed a systematic review which revealed that mosquitoes from this clade have a broad host range, including both invertebrates and vertebrates, and implement specialist and generalist blood-feeding strategies. To detect and localize their host, these mosquitoes are expected to use diverse host-emitted cues, but little is known about their host-seeking behavior. About half of the species in this clade, however, are specialized on biting frogs and exploiting the communication system of their host. Since several species harbor pathogens and are potential disease vectors, generalist species are expected to affect disease dynamics. Overall, interest in this group continues to grow as the number of studies has grown exponentially in the last two decades addressing a growing number of topics about the diverse and intricate interactions between these mosquitoes and their hosts. By consolidating the information on Uranotaeniini, we provide valuable insights into the feeding strategies used by mosquitoes and identify fertile gaps for future research.

CRYPTIC GENETIC VARIATION IN BRAIN GENE EXPRESSION PRECEDES THE EVOLUTION OF CANNIBALISM

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Although behavior is often assumed to play a critical role in the evolution of novel morphologies, much less is known about the evolution of novel behaviors, themselves. Here, we focus on a behavioral novelty among frogs: tadpole cannibalism. Members of the genus *Spea* possess tadpoles that, when exposed to high population density or a live prey diet consisting of fairy shrimp, can develop as a discrete and alternative carnivore morph (*i.e.*, a polyphenism) that is aggressive and capable of capturing and consuming live macroscopic prey, including other conspecific tadpoles. Using *Scaphiopus holbrookii*, a closely related species that does not exhibit this striking polyphenism, we tested and found support for three

key criteria of the plasticity-first hypothesis in the origins of *Spea*'s tadpole cannibalism. First, *S. holbrookii* tadpoles are capable of cannibalism, despite lacking the polyphenism present in *Spea* species. Second, transcriptomic analyses revealed increased heritable variance (*i.e.*, "cryptic genetic variation") in brain gene expression in response to diet and conspecific density cues. Third, a comparison of genes differentially expressed in response to environmental cues between *Scaphiopus* and *Spea* demonstrated that the regulation of brain gene expression plasticity has been modified and refined in *Spea*. These results suggest that, like morphological traits, behavior itself can evolve via genetic accommodation.

GENETIC REGULATORS OF A RESOURCE POLYPHENISM INTERACT TO COUPLE PREDATORY MORPHOLOGY AND BEHAVIOR

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Phenotypic plasticity is the widespread ability of organisms to produce different phenotypes in response to differing environmental cues. Phenotypic plasticity likely requires the coordinated response of multiple traits observed individually as morphological, physiological, or behavioral. The integration, and hence functionality, of this response, may be influenced by whether and how these component traits share a genetic basis. In the case of polyphenism, or discrete plasticity, at least part of the environmental response is categorical, offering a simple readout for determining whether and to what degree individual components of a plastic response can be decoupled. Here, we use the nematode *Pristionchus pacificus*, which has a resource polyphenism, allowing it to be a facultative predator of other nematodes, to understand the genetic integration of polyphenism. The behavioral and morphological consequences of perturbations to the polyphenism's genetic regulatory network show that morphology is the major predictor of both predatory activity and ability, different axes of morphological variation are associated with different aspects of predatory behavior, and rearing environment can decouple predatory morphology from behavior. Further, we found that interactions between some polyphenism-modifying genes have synergistic effects on predatory behavior. Our results show that the component traits of an integrated polyphenic response can be manipulated and, in principle, selected upon individually, and they suggest that multiple routes to functionally comparable phenotypes are possible.

TRAFFIC NOISE EFFECTS ON ANURAN VOCALIZATIONS: METHODOLOGICAL CHALLENGES AND OPPORTUNITIES.

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Noise pollution produced by automobile or airplane traffic is an important threat to animal communication. Over the last two decades, an increasing number of studies have assessed the effects of traffic noise on anuran vocalizations. Drawing general patterns about the impact of traffic noise on this group, however, has been difficult. Here, we systematically examine the methodological approaches used in investigations about how traffic noise affects frog calls.

We found that 39 species have been studied and in most of those species, an effect of traffic noise was reported. Vocal responses, however, vary in which acoustic features are modulated and the direction of the response (increase vs. decrease). Six species have been assessed in two studies, four show consistent results of traffic noise affecting their calling behavior, but two show contradictory results. In the presence of traffic noise, changes in temporal parameters are as frequent as those in spectral features of the calls. We identified three methodological approaches used to assess the effects of traffic noise on calling behavior in frogs and found that which approach was used is not related to whether a study reports a response to traffic noise. Overall, however, our results revealed a lack of standardized methodologies and limited characterization of noise, which among other disregarded methodological steps, constrain our ability to make broad conclusions about the impact of noise pollution on frogs. We discuss common bioacoustics technical omissions and highlight procedures to standardize the methods used to assess frog vocal responses to noise pollution. Ultimately, we outline paths to increase the internal, external, and ecological validity of these investigations.

AMBUSH! THE EVOLUTIONARY AND ECOLOGICAL ORIGINS OF CAT PREDATORY BEHAVIORS IN THE FOSSIL RECORD

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The fossil record offers direct insights into the evolutionary, ecological, and environmental histories of animal groups, with the potential of better understanding the context in which behavioral strategies originated. However, behavior cannot be directly studied from fossils placing severe limitations on this direct line of evidence into the past. Certain kinds of behaviors can be indirectly inferred, however, when they are linked to functional features that are preserved in the skeleton, teeth, or other fossilizable elements. In mammals, specializations in locomotion can often be inferred from fossils, and locomotion is linked in general ways to behavioral strategies like predation. We will look at how locomotion evolved in mammalian carnivorans in North America, how the variety of locomotor types are distributed in ecological communities, how that distribution differs depending on environmental context, and how strategies changed in response to major changes in past climate and vegetation. Felids, which are known for ambush-style predation, appeared on the scene quite late, changing the repertoire of carnivore predation style. We will look at how the immigration of cats into North America affected the range of locomotor behaviors during the spread of grassland habitats, and how the ambush-style predation of cats themselves changed when the Earth entered into the Ice Ages.

DOES PUBERTAL ONSET AFFECT BEHAVIORS MEDIATED BY THE DOPAMINE SYSTEM?

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Adolescence is known to be associated with changes in neuroanatomy and increases in cognitive function, sensation-seeking, and risk-taking behavior. Previous work has also shown that dopamine neuron number changes between the juvenile and young adult period, and dopamine neurons express hormone receptors. However, the developmental trajectory of dopamine-mediated behaviors has yet to be explored during the period of adolescence in relation to the timing of puberty. In the present study, we assess cognitive flexibility, along with motivation and risk-taking across adolescence from P30 (prepubertal), recently pubertal, and P60 (post-pubertal) male and female Long Evans rats. To measure cognitive flexibility, an attentional set-shift task was used, which requires subjects to learn and unlearn different rules for finding a sucrose reward. Changes in risk-taking and motivation for reward were measured using a predator-odor risk-taking task. This task requires the subject to overcome a risk (pass through a chamber with predator-odor-soaked bedding) to obtain a sucrose reward. It was hypothesized that cognitive flexibility would improve following puberty and motivation for reward/risk-taking would increase across adolescence. We found that pre-pubertal rats were unable to perform the attentional set-shift task. In the predator-odor task, post-pubertal males spent less time in the start chamber and consumed the reward earlier than prepubertal males during training for the predator-odor task. These results suggest that adolescents are more likely to participate in risky behavior after puberty, and pubertal effects on risky behavior during adolescence are sex-specific.

TOTAL NUMBER OF NEURONS IS NOT NECESSARILY A BETTER MEASURE OF COGNITIVE ABILITY THAN OVERALL BRAIN SIZE

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What is the best measure of brain anatomy for predicting cognitive and behavioral ability across species? Absolute brain size in Primates is known to be associated with interesting behavioral dimensions, including social group size. Estimates of total number of neurons, from the pioneering work of Herculano-Houzel and colleagues, have been suggested to be a better measure. However, neuron number by itself ignores the degrees and kinds of interconnections between neurons. Abnormal connectivity is specifically thought to underly some cognitive disorders in humans. By contrast, brain volume potentially indexes neuron number plus the complexity of neuronal interconnections and other support cells. Brains with lower neuron densities likely harbor correspondingly greater axonal and dendritic interconnection complexities. Using a small sample (n=10) of Primate species for which estimates of cerebral cortex neuron number (NN), absolute brain size (BR), general cognitive ability (GC), and social group size (GS) have been published, it is shown that BR rather than NN is actually a slightly better predictor (though not significantly) of both GC and GS. For GC the correlation with BR is $r=0.76$ ($p<0.01$), but with NN $r=0.64$ ($p<0.03$). For GS, the correlation with BR is $r=0.75$ ($p<0.01$), but with NN $r=0.72$ ($p<0.01$). Although a small sample, this suggests natural selection has worked to optimize both neuron number and complexity of interconnections, making BR

at least as good an estimate of cognitive/behavioral abilities as NN. Implications for interpreting the evolution of human cognition from the fossil record will be addressed.

HOT OR NOT? THE EFFECTS OF TEMPERATURE ON AMPHIBIAN GLUCOCORTICOID REGULATION

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Climate change is emerging as the biggest peril to biodiversity loss, with amphibians as the most threatened vertebrate class. Therefore, a critical question is: how do amphibians respond to changing temperatures? Specifically, because many frogs choose mates in the same ponds where their offspring will develop, it is imperative to examine how adults respond to warming water. We hypothesized that adult frogs may respond to a rapidly changing environment, specifically the ponds in which they mate and reproduce, through an elevated glucocorticoid response (i.e., corticosterone or CORT). To evaluate the impact of warming water on the adult frog stress axis, we used a non-invasive assay that measures stress-induced corticosterone (CORT). Using the spadefoot toads (genus *Spea*), we evaluated whether warmer water temperatures modify levels of stress-induced CORT. Our results indicate that CORT levels are higher in adults that experience relatively warm water, including temperatures within the range of temperatures found in *Spea*'s natural habitat. Because elevated CORT is associated with changes in behavior, our study suggests that warmer water might modify adult frog behavior, especially in the context of reproductive habitats. These results inspire future projects examining how differences in adult reproductive behaviors influence offspring quality in a changing climate.

MAPPING MOUSE AUDITORY BEHAVIOR IN THE BRAIN

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Anthropogenic noise is known to impact auditory behaviors like social communication, yet we have limited understanding of it impacts on brain organization. Specifically, noise has been shown to influence migration routes, and ability to perceive auditory signals that are dependent upon auditory cortex (AC). Furthermore, functional methods have shown that individual variation in the location of auditory cortical subfields obscures our understanding of auditory cortical organization, making it difficult to understand the effects of hearing impairment on the brain. However, histological measures of the differential distribution of microstructure, like cells and myelin, across the various auditory subfields in primates and cats suggest microstructure is a true biological marker of mammalian AC. Therefore, we propose to validate the microstructural organization of AC by integrating multiscale measurements of the brain in pursuit of understanding the effect of hearing impairment. I hypothesize that microstructural distribution of mouse AC can be a biomarker to distinguish AC from adjacent regions that is measurable across multiple scales using histology and MRI. Specifically, I will show qualitative histological results demonstrating the differential myelination (10+%) and cellular density across AC in mice and rats. I also present our current work using a supervised

deep learning (80% object detection accuracy) to quantify cell and myelin microstructure, as well as various MRI modalities designed to reveal neural microstructure. Ultimately, our demonstration of the validity of microstructure to localize AC provides insight to the mechanism(s) of learned auditory behavior and enables the noninvasive assessment of brain health to improve the conservation of mammals exposed to anthropogenic noise pollution.

UNVEILING THE NEURAL CORRELATES OF MOSQUITO BEHAVIOR: USE OF ACOUSTIC SIGNALS ACROSS DIFFERENT ECOLOGICAL CONTEXTS

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Mosquitoes exhibit a diverse array of behaviors facilitated by acoustic signals including foraging for blood meals and mating interactions. They hear using flagellar ears, Johnston's organ (JO), located within the antennae's pedicel. Our understanding of mosquito audition predominantly stems from species of medical importance, which use sound primarily during mating. Yet, the complexities of sound-mediated interactions extend beyond close-range mating encounters to long-distance host-seeking behaviors. Here, we investigate how the JO auditory innervation varies across species using sound in different ecological contexts such as mating and foraging. To do so, we performed immunohistochemistry and confocal imaging on vibratome sections of male and female mosquito heads from different species. We examined the JO of five species of mosquitoes that use sound in different contexts: only for mating (*Aedes aegypti* and *Toxorhynchites rutilus*), for both mating and foraging (*Culex territans*), only for foraging (*Uranotaenia lowii*), and neither for mating or foraging purposes (*Sabethes cyaneus*). Given that sound use across those contexts varies between the sexes with males heavily relying on wingbeats in mating and females depending on host-emitted calls to find a blood meal, we also examined sexual differences in the auditory innervation of the JO. Through comparative analysis of auditory innervation in these diverse mosquito species, we shed light on the neural mechanisms underlying different sound-mediated behaviors. By identifying sex- and species-specific differences in the JO auditory innervation, we identify potential auditory adaptations supporting feeding and mating strategies in mosquitoes.

DINING ON THE CROAK TUNE: ACOUSTIC HOST-SEEKING BEHAVIOR IN FROG-BITING MOSQUITOES, *URANOTAENIA LOWII*

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Mosquitoes use multiple sensory cues to locate their hosts and obtain a blood meal for egg production. While most mosquitoes feeding on endothermic hosts use various cues such as chemical gradients, heat, and visual markers, some mosquitoes use auditory cues to feed on ectothermic hosts like frogs. These frog-biting mosquitoes find their hosts by detecting species-specific mating calls. In this study, we investigate the host-seeking behavior of *Uranotaenia lowii*, a mosquito species that feeds on frogs by exploiting their communication system. We hypothesized that the acoustic preferences of these mosquitoes are shaped by selection to maximize offspring production. We first confirmed phonotaxis behavior towards

frog calls testing them in a controlled environment. To do so, we characterized their behavior in response to the calls of barking treefrogs. We then investigated i) relative attractiveness to the calls of six frog species that vary in their evolutionary history with these mosquitoes, and ii) clutch size and larvae development when feeding from such frogs. By evaluating the acoustic preferences and offspring production when attacking native sympatric and allopatric frogs, as well as their invasive counterparts, we examine the prediction that more attractive calls result in increased fitness. This study contributes valuable insights to deepen our understanding of host-seeking strategies and the sensory strategies used to attack ectothermic hosts.

THE INTERACTIVE EFFECTS OF *WOLBACHIA* AND *NOP60B* ON SINDBIS VIRUS REPLICATION

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Arboviruses pose a threat to more than half of the world's population. The intracellular bacterium, Wolbachia, is found in 40-60% of insect species and can share a mosquito host with human pathogens. Indeed, Wolbachia colonization protects invertebrate hosts against infection from single-stranded, positive-sense RNA viruses, a phenomenon known as pathogen blocking. While vector control strategies leveraging Wolbachia have been successful to date, the details regarding the mechanism are unknown. Here we performed a screen to identify host factors involved in Wolbachia-mediated pathogen blocking of Sindbis virus (SINV) in *Drosophila melanogaster*. We identified a host pseudouridine synthase gene, Nop60B, which was previously shown to display significant differential isoform usage in flies colonized with Wolbachia compared to Wolbachia-free counterparts. Thus, we hypothesized that Wolbachia-induced changes to Nop60B may affect SINV replication. We induced variation in Nop60B expression in transgenic RNAi flies with and without Wolbachia to measure SINV replication. There is a significant positive correlation between SINV RNA and Nop60B expression in Wolbachia-free flies. In contrast, Wolbachia-colonized flies show a weak negative correlation between SINV RNA and Nop60B expression. We identified Nop60B isoform differences in these samples, which may explain the opposite trends. To narrow down on a mechanism, we used a *D. melanogaster* cell line cleared of Wolbachia to overexpress Nop60B protein. Overexpression leads to an increase in SINV infectivity and intracellular RNA levels. Overall, these experiments suggest that the pseudouridine synthase, Nop60B, is proviral and Wolbachia-colonization dismantles the proviral effect. Results of these experiments will contribute to our understanding of Wolbachia-virus-host interactions and antiviral mechanisms in general.

TADPOLE FIGHT CLUB: THE ONTOGENY OF AGGRESSION IN POISON FROG TADPOLES

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Juvenile aggression is an ecologically and evolutionarily important phenomenon that spans taxa and ranges from social play to lethal combat. As organisms develop and grow, the costs and benefits of fighting may change. As a result, I hypothesize that juvenile aggression changes across development. To test this hypothesis, I studied tadpoles of the Dyeing Poison Frog

(*Dendrobates tinctorius*), as they are aggressive and change morphologically and behaviorally across ontogeny. I first categorized tadpoles into three developmental categories: early stage (no external limb development), middle stage (subtle external leg development), and late stage (prominent external leg development). I then conducted aggression trials between pairs of tadpoles and recorded avoidance and aggressive behaviors. I found that while avoidance behavior does not significantly differ across development, aggressive behavior does. Late-stage tadpoles bit significantly less than early or middle stage tadpoles. I speculate that this may be due to the physiological demand of metamorphosis that the late-stage tadpoles are currently facing or to their legs hindering their physical fighting capability. The next step of this research will be to discover the mechanisms behind the behavioral differences observed via RNA sequencing of the brain tissue. The results of this project inform about developmental stage vulnerabilities, behavioral plasticity, and ecological and social dynamics.

EVALUATING REWARD RELATIVITY IN ALCOHOL PREFERRING RATS USING AN INCENTIVE CONTRAST PARADIGM

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Individuals with Alcohol Use Disorders (AUDs), who are having symptoms of alcohol withdrawal, report having strong cravings for sweet products. Research has indicated that high sugar diets can induce similar behavioral changes that are produced by drugs of abuse (such as cocaine, heroin) and AUD; these behavioral characteristics include bingeing, withdrawal, and cravings. Research has revealed that individuals with high cravings for sweeter products can take ten times longer to attain abstinence and have higher relapse rates than those who do not have high sugar cravings. This coincides with research indicating that a high sugar diet could be leading to an increased disposition to AUD and other drug abuse disorders. Animal studies have observed the functional changes which occur in the reward pathways of the brain when sugar is consumed. These studies have further indicated that sugar can have a similar effect on the reward pathways as alcohol intake and other drugs of abuse. Despite the amount of research that has been conducted, the understanding of how sugar and ethanol specifically affect shifts in motivation in a typical Alcohol Preferring (P) rat model still remain vague. Thus, the current project observes motivational behavior in P rats using an incentive contrast (IC) paradigm (demonstrating how varying reward values can alter behavior based on previous outcomes) by exploring if P rats behaviorally discriminate and respond to contrast changes similarly to both sugar and ethanol concentrations. Female rats followed a 16-week timeline receiving sucrose and ethanol within an operant box apparatus. Rats were given a total of 240 seconds of access to the concentrations over a time span provided through 5 seconds, 10 seconds, or 20 seconds of access per nose poke. The measures obtained were: consumption (g/kg) rates for both sucrose and ethanol, nose poke latencies, and lick rates.

CAUSES AND CONSEQUENCES OF BEHAVIORAL VARIATION: INSIGHTS THROUGH THE STUDY OF HONEY BEES AND DUNG BEETLES

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Among-individual variation is common for many animal behaviors, yet its causes and consequences are often poorly understood. In this presentation, I outline recent work studying factors that influence behavioral variation and the possible effects of this variation on development and evolution. I examine these factors through the lens of two non-traditional insect models: the European honey bee (*Apis mellifera*) and the bull-headed dung beetle (*Onthophagus taurus*). I begin my presentation building on previous work which documented the existence of behavioral plasticity in aggression due to social conditions in honey bees. To explore the underlying causation, I performed two experiments to assess if variation in larval food quality or parental care given to larvae is associated with variation in colony-level aggression. I found high variation in honey bee larval food, yet it was not associated with colony-level aggression. In contrast, I detected significant variation in the parental care behaviors of nurse bees as a function of colony level aggression. Nurses from high aggression colonies decreased larval care behaviors in the presence of non-task-specific pheromones, while nurses from low-aggression colonies did not. This finding supports the hypothesis that larvae from colonies of different aggression levels receive different care regimes during this critical phase of growth and development. I end my presentation by describing currently ongoing work in the bull-headed dung beetle. This work extends my investigation of variation in adult behavioral repertoires by allowing me to investigate the consequences of such variation in more diverse ecological and evolutionary contexts. I am once again exploring the contributions of early-life nutrition but also sex and maternally inherited microbiota. In addition, I am exploring the consequences of behavioral variation in the context of range expansions and the colonization of novel, challenging habitats.

PREDICTING THE FUTURE: MECHANISMS OF ANTICIPATORY REPRODUCTION IN NORTH AMERICAN RED SQUIRRELS

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Microevolutionary changes and phenotypic plasticity both (or in combination) drive organismal responses to changing environments, however, plasticity has been responsible for most of the documented responses to global climate change in mammals. Global climate change inherently threatens the persistence of reliable environmental cues and whether organismal responses due to plasticity are adaptive, especially in environments in northern latitudes. In order to understand how animals integrate environmental information, we investigated the coevolutionary relationship between North American red squirrels (*Tamiasciurus hudsonicus*)

and white spruce trees (*Picea glauca*) in the Yukon, Canada, as part of the Kluane Red Squirrel Project. Red squirrels are specialized seed predators subject to an episodic resource pulse system in which white spruce trees produce a synchronous superabundance of seeds in “mast years” followed by multiple “non-mast years” of little to no seed production. Red squirrels anticipate future seed crops and increase reproductive effort prior to the availability of additional resources in mast years. Red squirrels exhibit adaptive reproductive plasticity in mast years, yet the mechanism inducing phenotypic plasticity in this system is unknown. We are testing the hypothesis that a hormonally active phytochemical in white spruce buds adaptively modifies red squirrel reproductive physiology and behavior in mast years. We will present our initial results testing this hypothesis through analyses of life-history, behavioral, and physiological data.