

ited knowledge of the interaction of avian behavior and external temperature during nesting, especially from multiple temporal and thermal perspectives. We predicted that natural thermal variation and mean thermal conditions would significantly alter time parents spent away from nestlings, and the effects of increased temperature and altered behavior would negatively influence nestling growth. In nest-boxes, we examined how natural thermal variation and mean temperature impacts nesting behavior in Eastern Bluebirds and Tree Swallows. We quantified adult breeding behavior using NestIQ, a machine learning software which recognizes off-bouts by comparing multiple nesting temperatures. As thermal variation increased, individual and daily measurements of off-bout duration decreased while off-bouts lengthened as mean temperatures increased. Also, nestling growth rates are moderately influenced by adult behavior, but not environmental temperature. As off-bout duration increased, growth rates decreased regardless of the thermal environment, suggesting that behavioral adjustments have more direct control than natural thermal conditions. We conclude that thermal variation and average temperatures have opposing, equally important effects on breeding behavior. Parents may exhibit altered behavior in response to the thermal environment, which can influence nestling physiological development. To define thermal drivers of behavior, we must assess relationships between thermal conditions and behavior at multiple timescales to explore responses to long-term trends in thermal conditions and acute exposure to suboptimal thermal conditions. There is limited knowledge of the interaction of avian behavior and external temperature during nesting, especially from multiple temporal and thermal perspectives. We predicted that natural thermal variation and mean thermal conditions would significantly alter time parents spent away from nestlings, and the effects of increased temperature and altered behavior would negatively influence nestling growth. In nest-boxes, we examined how natural thermal variation and mean temperature impacts nesting behavior in Eastern Bluebirds and Tree Swallows. We quantified adult breeding behavior using NestIQ, a machine learning software which recognizes off-bouts by comparing multiple nesting temperatures. As thermal variation increased, individual and daily measurements of off-bout duration decreased while off-bouts lengthened as mean temperatures increased. Also, nestling growth rates are moderately influenced by adult behavior, but not environmental temperature. As off-bout duration increased, growth rates decreased regardless of the thermal environment, suggesting that behavioral adjustments have more direct control than natural thermal conditions. We conclude that thermal variation and average temperatures have opposing, equally

important effects on breeding behavior. Parents may exhibit altered behavior in response to the thermal environment, which can influence nestling physiological development.

### **Inhibition of sphingosine kinase disrupts symbiosis of two cnidarian model systems**

*Sheila Kitchen, Angela Poole, Alexa Bilsky, Mary Rowland, Samuel Piorkowski, Kira Turnham, Mónica Medina, Aki Ohdera*

Bioactive lipids play a pivotal role in determining cell fate and mediating host-microbe interactions. Modulation of intracellular sphingolipids by the enzyme sphingosine kinase (SPHK) creates a pro-survival environment for the host, microbe or both partners. In the symbiosis between cnidarians and dinoflagellates of the family Symbiodiniaceae, sphingolipid metabolism mediates partner interactions at various stages, from the onset to the establishment of long-term associations. In this study, we examined the role of SPHK in the early stages of symbiosis by disrupting its function through pharmacological inhibition and RNA interference in two symbiotic cnidarians, the jellyfish *Cassiopea* and sea anemone *Exaiptasia*. In both species, the inhibitor-treated animals significantly reduced uptake of symbionts in a dose-dependent manner. At the highest concentrations, symbiont numbers were comparable to those found when either animal was provided heat-killed symbionts, suggesting a failed symbiosis. SPHK inhibitor also prompted nearly complete symbiont loss in fully colonized *Cassiopea* but not *Exaiptasia* after three days of exposure. To identify the conservation between *Cassiopea* and *Exaiptasia* SPHK-mediated lipid signaling pathways, we compared orthologous gene expression of treated and non-treated animals during the onset of symbiosis within and between species. Overall, these findings support the conserved regulatory role of SPHK during symbiont uptake in distantly related symbiotic cnidarians.

### **Differential gene expression between growth plate and non-growth plate forming ends of bones**

*Kelsey Kjosness, Sungdae Park, Sherrie Wallace, Sarah Doelp, Maria Biancaniello, Douglas Menke, Philip Reno*

Several bones within the developing mammalian autopod possess a growth plate on only one end. This includes the metatarsals (MT), the pisiform in the wrist, and calcaneus in the ankle. While regulation of growth plate cartilage is well understood, little is known about

which genes specify where growth plates do and do not form in the skeleton. We used RNA-seq to identify differentially expressed genes (DEGs) between four tissues with a growth plate forming and non-forming region in postnatal day 4 (P4) and P9 mice: 1) proximal versus distal MT1, 2) distal versus proximal MT3, 3) pisiform versus all other carpal, and 4) proximal versus distal calcaneus. We compared DEGs that had a positive fold change and a significance value of  $p < 0.05$  for all four datasets within each age group to identify common genes with higher expression in the growth plate forming region. We identified 4 genes at P4 and 8 genes at P9 that met these criteria. *Stra6*, a member of the retinoic acid signaling pathway, was the only gene to be shared between both age groups. *In situ* hybridization shows *Stra6* to be strongly expressed in the perichondrium adjacent to proliferative chondrocytes in growth plate and minimally expressed in the non-growth plate forming cartilage. This analysis demonstrates the utility of using variation in growth plate location to identify growth plate specific genes.

### **Examining cuttlefish suckers using FEM and biomimetics: An open-source workflow for studying suction**

Brett Klaassen-van-Oorschot, Timo van-Leeuwen, Lara-Marie Jess, Guillermo Amador

Cuttlefish suction cups contain a hardened proteinaceous ring. The function of this ring is unknown. We hypothesized that in common cuttlefish (*Sepia officinalis*) this ring provides structural support to resist collapse under the low pressures exerted during suction. Alternatively, the ring may provide a sealing surface in concert with papillae that grow from the ring's edge. We tested the ring's function using a finite element model in FEBio and validated this model empirically using a biomimetic suction cup. Our findings demonstrate that sucker rings resist buckling of the suction cup rim and dramatically improve attachment. Moreover, we present 1) a novel biomimetic suction cup that is reversible, strong, and capable of adhering to a variety of surfaces; and 2) an open-source workflow for exploring suction cup biomechanics across morphologies.

### **Mistaken synapomorphy: The evolutionary developmental origins of the arachnid patella**

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The patella is the leg segment that confers a “double-bend” architecture to the pedipalps and walking legs of Euchelicerata (arachnids and horseshoe crabs). It was postulated that the patella was a synapomorphy of Arachnida and resulted from neofunctionalization of a new copy of the gene *dachshund* (*dachshund-2*). Two aspects of this reconstruction are difficult to reconcile across the literature. First, some arachnid orders are thought to lack patellae (e.g., Solifugae, Pseudoscorpiones, some acariform mites). Second, *dachshund-2* is restricted to a subset of six arachnid orders (Arachnopulmonata); various arachnid groups outside of the arachnopulmonates possess a true patella, but not *dachshund-2* (e.g., Opiliones), suggesting that *dachshund-2* evolved after the patella. Thus, neither the developmental genetic basis for patellar formation, nor when the patella evolved, are clearly understood. Here, we show that a novel expression domain of the gene *extradenticle* is associated with the patellar segment in embryos of the harvestman *Phalangium opilio*. Gene silencing of *extradenticle* results in the loss of the patella, suggesting that this transcription factor underlies the origin of the patellar segment. We tested whether this novel *extradenticle* expression domain was regulated by Notch-Delta signaling, which is responsible for leg segmentation. Knockdown of Notch resulted in unsegmented appendages, in addition to diminution of the median *extradenticle* domain. With this developmental genetic definition of the patella, we surveyed chelicerate orders to pinpoint patellar origin.

### **Foraging Behavior of Fruit Flies (*Drosophila melanogaster*) in a 3D Arena**

Michael Knabe, Jimena Aracena

Fruit flies (*Drosophila melanogaster*) exhibit negative geotactic behavior, which has many advantages including the ability to escape from predators. We tested the effect of negative geotactic behaviors on foraging. We presented the flies with a feeding cube (2.5 cm side length) to test their feeding preference in a 3D arena (10 x 10 x 10 cm) to determine their preference to feed from the top, sides, or bottom of a cube with 9 wells on each side filled with colored sucrose solution. The top, bottom, or sides had either a red, yellow, or blue solution. The flies were deprived of food for 24 hours and tested in the dark for one hour. They were then frozen, and the contents of their abdomens were evaluated for color. There was a preference for feeding on the top over the sides and/or the bottom of the cube. The flies also preferred the yellow solution, while avoiding the red solu-