79.6 BIDDULPH, T.A.*; HARRISON, J.F.; Arizona State University; *tbiddulp@asu.edu*

Oxygen Modulates Density Effects on Body Size in Drosophila melanogaster

Larval crowding has been shown to reduce body size in *Drosophila melanogaster* but the mechanisms responsible for this effect remain unclear. While nutritional limitation and larval competition are two factors that likely play an important role in the determination of body size, it may be possible that hypoxia occurs in the media at high densities and this might be a factor in the density effect on body size. To partially test this hypothesis, *Drosophila melanogaster* were reared in 10, 21, or 30 kPa oxygen at three densities from egg to adult. Adults were collected during the first two days after eclosion began in each treatment and were starved for one day with agar gel to prevent desiccation before their masses were recorded. There was a significant interaction between oxygen and density on adult body mass (two−factor ANOVA, N=146, $p > 0.0000001$). At low densities, as seen in prior studies, hypoxia suppressed body size while hyperoxia had no effect, but at high densities hyperoxia increased body size and hypoxia had no effect. These results demonstrate that oxygen needs to be considered as a potential major factor in causing the reduction of body size in *Drosophila melanogaster* at high densities, and suggest that larval crowding causes hypoxia in the media. This study was funded by the SOLUR Program at ASU as well as NSF IOS 1122157 and NSF 0938047.

20.7 BORCHERT, JB*; ANGILLETTA, MJ; Arizona State University, Tempe; *jdborche@asu.edu*

The games that flies play: laying eggs based on temperature and competition

We used game theory to predict how fruit flies, *Drosophila melanogaster*, should compete for oviposition sites. Although flies prefer to lay their eggs within a particular range of temperatures, the potential for competition among offspring should cause females to accept warmer or cooler sites when preferred sites become crowded. To look at this problem, we observed where flies chose to lay eggs under various densities of competing females. In each trial, 4 or 15 flies were placed within a thermal arena containing a choice of two oviposition sites, one at a preferred temperature (25°C) and another at a lower temperature (20°C). In a concurrent trial, 100 eggs were added to the site with the optimal temperature and then 4 flies were added to see if behavior depended on the presence of other females or eggs. After 4 hours, we counted the eggs laid in each portion of the gradient and analyzed how the distribution of eggs was affected by the density of females. Flies at low density laid eggs almost exclusively at 25°C, but those at high density laid a significantly greater proportion of eggs at 20°C than did flies at low density. Surprisingly, flies did not avoid laying at 25°C when eggs were present, suggesting that flies responded to the presence of competing females rather than cues associated with eggs. By drawing on game theory to make quantitative predictions, this research builds on previous empirical

P2.75 BORCHERT, JB*; ANGILLETTA, MJ; Arizona State University, Tempe; *jdborche@asu.edu*

The games that flies play: effects of temperature and density during development on the fitness of Drosophila melanogaster

Both temperature and competition can affect the fitness of an organism. We examined how temperature and competition covary to affect the fitness of *Drosophila melanogaster*. We transferred either 1, 5, 15, or 30 eggs to a petri dish and maintained them in an incubator kept at either 16°, 21°, 25°, or 30°C. We then measured developmental time, survival to adulthood, wing size, and fecundity to approximate the fitness of drosophila under each treatment. These data were used to parameterize a game theoretical model that predicts

where flies should

24.2 COMBES, S.A.*; SALCEDO, M.K.; GAGLIARDI, S.F.; Harvard Univ.; *scombes@oeb.harvard.edu*

Effects of environmental conditions on Drosophila flight performance

Wild insects inhabit a world of radically varying environmental conditions, where temperature, humidity and light levels vary substantially over multiple spatial and temporal scales. While we understand how many of these factors affect development, respiration, reproduction and other physiological traits, our understanding of how environmental conditions affect insect flight performance remains limited. Here, we examined the effects of temperature, light intensity and wind speed on the free flight behavior of fruit flies (*Drosophila melanogaster*), a model species for laboratory−based flight studies, and also an insect that is likely to encounter a range of variable microhabitats in the wild. We allowed lab−reared flies to emerge spontaneously from a vial into a large, open area (>2 m in each dimension) in either the laboratory or outdoors. We quantified flight trajectories within a volume of approximately 1 m^3, while recording temperature, visible and UV light intensity, and wind speed. A variety of locations and weather conditions were sampled, and multiple flies were tracked during each release to account for individual variability. We found that flight velocity was strongly influenced by environmental factors, including temperature and light intensity, and that saccade (turning) behavior also varies with environmental conditions. Past studies have demonstrated a positive relationship between temperature and flight speed, but the strong influence of light intensity (~2.5−fold increase in flight velocity from dim lab lighting to bright sunlight) has not previously been shown. These results have important implications for interpreting flight data collected in various settings, as well as for the behavior and ecological interactions of wild insects, which may vary in their sensitivity to environmental conditions.

P1.189 DAVIS, SM*; ZACHARY, ED; JAMES, TA; LATHAM, KL; BALTZLEY, MJ; Western Oregon University; *sdavis12@mail.wou.edu*

Using a sequential y−maze and selective breeding to create Drosophila melanogaster strains with magnetic orientation preferences

There is some experimental evidence that the fruit fly, *Drosophila melanogaster* uses the Earth's magnetic field as an orientation cue. The ability to detect and orient using the Earth's magnetic field has been shown in diverse species, including sea turtles, birds, lobsters, newts, and sea slugs, yet the cellular basis for magnetosensory behaviors has remained elusive. If *D. melanogaster* orients to magnetic fields, the species offers an unprecedented opportunity to study the genetic basis of magnetic orientation and navigation because of their status as a model organism. We are going to attempt to confirm that *D. melanogaster* can orient using the magnetic field and will do this by creating populations with strong magnetic orientation preferences using artificial selection. We developed a maze that allowed us to isolate different individuals with north and south orientation preferences. Two populations of *D. melanogaster* were then created through selective breeding: a north−seeking population and a south−seeking population. We will present the preliminary results of this orientation and selection research. The experiment will continue through 15 generations of selection. The experiment was modeled after a study by Hadler (1964) which was used to ascertain the genetic basis of phototaxis in *D. melanogaster*. We are using this

52.1 DREYER, AP*; SHINGLETON, AW; Michigan State University; *dreyerau@gmail.com*

Does Size Really Matter? The Effect of Genital Size on Reproductive Success

From flies to spiders and crabs to beetles, genital size remains near constant among individuals in a population despite considerable phenotypic plasticity in body size and the size of other organs. Several competing hypotheses have been proposed to explain the selective pressures underlying this commonly observed phenomenon, however, a lack of experimental data has made it difficult to distinguish between them. Any comprehensive test of these hypotheses requires high levels of variation in genital size alone, which does not exist in natural populations. We have designed a method to circumvent this problem and test the hypotheses experimentally. Our design uses targeted gene expression to up− or down−regulate insulin−signaling in the developing genitalia of *Drosophila melanogaster* and produce male flies with extreme genital morphologies. Male flies with proportionally small, wild−type or large genitalia are then paired with female flies in up to three contexts; (1) no male competition, (2) direct male−male competition, (3) indirect male−male competition. Specific aspects of male reproductive success are measured to compare across the three genital sizes: courtship and copulation latency, and duration; post−mating egg production, proportion of fertilized eggs and egg paternity. Our results suggest that females prefer males with wild−type size genitalia even though they are physically able to mate with, and fertilize eggs using sperm from, males that have proportionally small or large genitalia.

11.6 EGGE, AR*; ELLER, OC; MORGAN, TJ; Kansas State University; *aegge@ksu.edu*

Genotype−by−environment interactions of demographic values in fluctuating thermal environments using Drosophila melanogaster

Organisms often experience a wide range of temperatures in nature, exhibited by daily and seasonal fluctuations. Ectotherms are particularly susceptible to these fluctuations and must alter their physiology in order to survive and reproduce in potentially stressful conditions. Assessment of egg laying and survivorship at different temperature regimes provides significant information on how different genotypes are affected by thermal fluctuations. *Drosophila melanogaster* have adapted to a range of thermal regimes and inhabit much of the world. They also provide us with a large base of genetic resources including the *Drosophila melanogaster* Genetic Reference Panel (DGRP). We chose forty genotypes from the DGRP to assess absolute lifetime fitness measures at two different fluctuating environments: 18° ± 6° C and 25°± 6° C (average 18.3° C and 25.3° C, respectively). Preliminary results indicate significant variation in survival and egg laying rates among these forty genotypes, which may lead to significant differences in demographic parameters such as lambda, net reproductive rate, and generation time. These parameters are important in assessing long−term population−wide, genotype−specific survival under natural conditions. Association mapping of these 40 genotypes will provide candidate genes involved in these thermally sensitive reproductive values.

P3.90 ELLER, OC*; EGGE, AR; MORGAN, TJ; Kansas State University; *oceller@ksu.edu*

Effect of Cooling Rate on the Rapid Cold Hardening Response in Drosophila melanogaster

Climate is a significant environmental factor that influences the distribution and abundance of most organisms on Earth. One constantly changing component of climate is temperature and we are interested in how organisms respond to stress brought on by

temperature fluctuations coupled with cold stress. *Drosophila melanogaster* is a cosmopolitan species that inhabits many different environments throughout the world and exhibits a wide−range of physiological thermal adaptations. Rapid cold hardening (RCH) is a short−term acclimation response in which an organism is exposed to a non−lethal cold temperature before being exposed to an extreme cold temperature. This non−lethal pretreatment improves cold survival for individuals that have the ability to acclimate over a short period of time. Previous experiments investigating the RCH response have directly transferred flies from rearing temperature to pretreatment to extreme cold exposure. These direct shifts do not translate to natural conditions where organisms are gradually exposed to new temperatures. Our goal was to compare experimental environments of a more natural context to see if or how the RCH response changed in relation to direct transfer experiments. We used thermal ramping to cool the experimental environment at two ecologically natural but different rates and then compared survivorship after an extreme cold shock between flies that received a ramping pretreatment and flies that received a direct transfer pretreatment. Our results indicate that neither the speed of cooling nor direct transfer pretreatment have a significant effect on an individual's ability to acclimate and survive extreme cold temperatures. These results are significant as they demonstrate that direct and ramping RCH pretreatments are both ecologically relevant measures of thermal performance.

P3.89 EVERMAN, ER*; MORGAN, TJ; Kansas State University; *evermane@ksu.edu*

Age−related change in cold stress tolerance in Drosophila melanogaster

Organisms occur in environments that vary spatially and temporally throughout their lifespans. Resistance to cold stress is one important fitness trait that is expected to decline through ontogeny; however this general expectation is based on the response of a small number of genotypes of *Drosophila melanogaster*. To further characterize this change in cold stress resistance, we performed Rapid−Cold Hardening (RCH) screens on 49 genetically distinct lines of the *Drosophila melanogaster* Genetic Reference Panel (DGRP) at early (5−7 days) and late (20−22 days) age. Consistent with previous investigations of RCH and the DGRP lines, we observed a wide range of variation between lines at each age point. In addition, cold stress resistance differs significantly ($p < 0.05$) between early and late aged flies. However, we observed that the direction of the change in expression of cold stress resistance varies among lines as well, with several lines increasing in cold stress resistance as they age. The difference in cold resistance between ages combined with the difference in direction of the change between lines suggests that cold stress resistance as characterized by RCH screens is influenced by a number of complex genetic interactions. To fully characterize these interactions, we will continue screening the remaining 143 lines in the DGRP and use association mapping to isolate regions of the genome that are likely tied to the age−related change in cold stress tolerance.

120.1 FRANKINO, W. A.*; STILLWELL, R. C.; DWORKIN, I. M.; SHINGLETON, A. W.; University of Houston, Michigan State University, Michigan State University; *frankino@uh.edu*

Tipping the scales: Evolution of the allometric slope independently of average trait size

The scaling of body parts is central to the expression of morphology across body sizes and to the generation of morphological diversity within and among species. Although patterns of scaling relationship evolution have been documented for over one hundred years, little is

known regarding how selection acts to alter these patterns. In part, this is because the degree to which the elements of scaling relationships, mean trait size and the slope, can evolve independently is not known. Here, using the wing:body size scaling relationship in Drosophila as an empirical model, we demonstrate that the slope of a morphological scaling relationship can evolve independently of mean trait size. Our success is likely due to in part to our employment of a developmentally−timed diet manipulation to isolate the nutritional static allometry from the genetic static allometry and to our application of selection over many (17) generations. We discuss our findings in the context of how selection likely operates on scaling in nature, the developmental basis of the integration of mean trait size and the scaling relationship slope, and the general approach of using individual−based selection experiments to study the expression and evolution of morphological scaling.

129.6 HATEM, N.E.*; SUZUKI, Y.; Wellesley College; *nhatem@wellesley.edu*

Regulation of critical weight in the tobacco hornworm, Manduca sexta.

The regulation of the timing metamorphosis is a critical event in many organisms. In insects, a size assessment point called the critical weight marks the time at which metamorphosis is no longer delayed even when animals are starved. The timing of metamorphosis in the tobacco hornworm, *Manduca sexta*, is primarily regulated by juvenile hormone (JH). During the final instar, metamorphosis is inhibited by JH until the larva reaches the critical weight when JH titers drop. In contrast, studies have shown that in the fruit fly *Drosophila melanogaster*, the critical weight is regulated by nutrition−dependent insulin signaling in the prothoracic gland, the major site of ecdysteroid biosynthesis. Alterations of prothoracic gland size through the manipulation of insulin signaling lead to shifts in the critical weight. In this study, we examined the role of insulin/TOR signaling in the determination of the critical weight in *Manduca*. By feeding *Manduca* rapamycin treated diets, we inhibited TOR signaling in final instar larvae, and the effect on the critical weight was examined. In wildtype larvae, the peak size was unaffected when fed rapamycin although the growth rate was reduced. Critical weight was also unaffected when fed rapamycin. However, the size of the prothoracic gland at the critical weight was disproportionately smaller in larvae fed rapamycin. These results indicate that insulin/TOR signaling does not play a major role in the determination of critical weight in wildtype *Manduca*. Thus, JH overrides the nutritionally dependent critical weight regulation in this species. Our study shows that the critical weight is regulated by two distinct mechanisms, one that senses body size (mediated by JH) and another that senses nutritional input (mediated by insulin/TOR signaling). The relative contribution of these two mechanisms therefore determines whether body size or nutritional availability determines the timing of metamorphosis.

108.5 LANE, S.J.*; FRANKINO, W.A.; ELEKONICH, M.M.; ROBERTS, S.P.; Central Michigan Univ., Mount Pleasant, Univ. of Houston, Texas, National Science Foundation & Univ. of Nevada, Las Vegas; *steven.lane@umontana.edu*

The Effects of Age and Lifetime Flight Behavior on Flight Capacity in Drosophila melanogaster

The effects of flight behavior on physiology and senescence may be profound in insects due to the extremely high metabolic costs of flight. Previous studies have shown that flight capacity decreases with age, and that flies prohibited from flying had longer lives and slower age−related loss of antioxidant capacity and accumulation of oxidative damage in flight muscles. Using *Drosophila melanogaster*,

we tested the effects of age and lifetime flight behavior on wingbeat frequency, metabolic rate, and the ability to fly in a hypo−dense gas mixture. Specifically, 5−day old adult flies were separated into three life−long treatments: (A) those not allowed to fly (no flight), (B) those allowed − but not forced − to fly (voluntary flight), and (C) those mechanically stimulated to fly (induced flight). Flight capacity senesced earliest in flies from the no−flight treatment, followed by the induced−flight group and then the voluntary flight group. Wingbeat frequency senesced with age in all treatment groups but was greatest in the voluntary and induced flight groups. Metabolic rate during agitated flight senesced earliest and most rapidly in the induced flight group, and was low and uniform across ages in the no−flight group. Early senescence in the induced flight group was likely due to the acceleration of effects such as the rapid accumulation of damage at the cellular level, while the early loss of flight capacity and low metabolic rates in the no−flight group demonstrate that disuse effects can also significantly alter senescence patterns of whole−insect performance.

75.3 LOCKWOOD, B.L.*; MONTOOTH, K.L.; Indiana University; *bllockwo@indiana.edu*

The consequences of thermal stress on early embryonic development: from cells to the whole−organism

For ectothermic organisms like *Drosophila*, changes in environmental temperature alter cellular processes. During early development, a rapid series of mitotic divisions bring an embryo from having a single nucleus to having thousands of nuclei, before gastrulation occurs and cell differentiation begins to take place. These early mitotic divisions are driven by the cytoskeleton, and previous work in cell culture has shown the cytoskeleton to be sensitive to thermal stress. Does thermal variability disrupt developmental progression by adversely affecting the cytoskeleton? Here we use confocal fluorescence microscopy to investigate the effects of thermal stress on the cytoskeleton in vivo in early stage embryos of *Drosophila melanogaster*. We find that exposure of eggs to heat stress causes gross defects in cytoskeletal arrangement and leads to the disruption of mitosis and cellularization of the blastoderm, both key developmental events. Moreover, brief heat stress events experienced in early embryogenesis lead to a decrease in survival to adulthood. These findings suggest that the thermal sensitivity of the cytoskeleton plays a key role in determining thermal tolerance at both the cellular and whole−organism levels.

P2.149 MCKEE, A*; NEWTON, S; CARTER, A; California State University, Long Beach; *biologymajor8@gmail.com*

Influence of Inbreeding on Female Mate Choice in Two Species of Drosophila

Many organisms have been reported to choose their mates in order to increase the heterozygosity of their offspring by avoiding mating with relatives or homozygous individuals. Most previous studies using *Drosophila melanogaster* have used artificial chromosomes or extreme inbreeding treatments, situations unlikely to be matched in nature. Additionally, few studies have examined the interaction between female inbreeding status and her choice of mate. Using females and males from populations that had experienced either random mating or one generation of sib−sib inbreeding, we measured the preferences of females for males. Our results indicate that outbred males were chosen more often than inbred males and that this preference may be more pronounced in outbred females than in inbred ones.

16.1 RUSSEY, WA*; FRANKINO, WA; Univ. of Houston; *warussey@gmail.com*

Do clines in Drosophila flight morphology result from adaptive environment−phenotype matching?

Drosophila exhibit convergent genetically−based clines and phenotypic plasticity in absolute and relative wing size; relative to warm environments, cooler environments produce large flies with disproportionately large wings. Although this pattern has been documented for many insect species on several continents, the reasons for the pattern remains unknown. The leading hypothesis is that selection on flight performance across thermal environments favors the observed patterns in morphology, although data addressing this hypothesis are scant and the importance of phenotypic plasticity in producing and maintaining these patterns remains unknown. Discussed here is a two−part study to (I) impose natural selection on flight performance in warm and cool environments to create experimentally derived populations of flies possessing morphology suited to flight at each temperature, and (II) assess the adaptive nature of the derived morphologies in ecologically relevant flight assays. Two experimental evolution studies were performed. In the first, warm and cool−selected populations were both reared at an intermediate temperature, removing effects of thermally−induced phenotypic plasticity. In the second experiment, warm and cool−flight selected lines were reared such that temperature of rearing was the same as the temperature of adult flight, providing a reliable cue during development. The experiments discussed here rigorously test the hypothesis that selection on ecological performance has produced a biogeographic morphological pattern frequently cited as one of the most compelling cases of adaptation known. In addition, the proposed work will offer general understanding into how developmentally and functionally integrated suites of traits might change rapidly under different thermal

57.3 SALEEM, S*; CARNEY, G.E.; Texas A&M University; *ssaleem@bio.tamu.edu*

Sexual experience enhances mating behavior and success of male Drosophila melanogaster

Competition for mates is a wide−spread phenomenon affecting individual reproductive success. The ability of animals to adjust their behaviors in response to changing social environment is important and well documented. *Drosophila melanogaster* males compete with one another for matings with females and modify their reproductive behaviors based on prior social interactions. However, it remains to be determined how male social experience that culminates in mating with a female impacts subsequent male reproductive behaviors and mating success. Here we show that sexual experience enhances future mating success. Previously mated *D. melanogaster* males adjust their component courtship behaviors and are able to out−compete sexually inexperienced males for copulations, when placed in the same mating arena. Interestingly, courtship experience alone was not sufficient in providing this competitive advantage, indicating that copulation plays a role in reinforcing this social learning. We also show that females use their sense of hearing to preferentially mate with experienced males when given a choice. Our results demonstrate the ability of previously mated males to learn from their positive sexual experiences and adjust their behaviors to gain a mating advantage. These experienced−based changes in behavior reveal strategies that animals likely use to increase their fecundity in natural competitive environments.

85.4 SAXENA, N.*; NATESAN, D.; SANE, S.P.; National Centre for Biological Sciences, Bangalore, India; *niteshs@ncbs.res.in*

Odor tracking behavior of fruit flies in the presence of landmark cues

Flying insects rely heavily on visual cues for flight. For example, during pheromone tracking, moths are known to utilize surrounding visual patterns to maintain upwind heading. Similarly, fruit flies also

require wide−field visual cues to reach the vicinity of the odor source location. Whereas odor cues in combination with far−field visual cues may serve to attract a fly towards the general area of odor source, landmark cues may be important to pinpoint the location of an odor source. Thus, we hypothesized that the presence of multiple landmarks or landmarks that are dissociated from odor sources will elicit a search behavior that is dependent on the distribution of visual objects. We studied the behavior of the fruit flies, *Drosophila melanogaster* as they honed in on the odor source within a wind tunnel. Our results show that in presence of multiple closely−spaced but identical visual cues, flies often make errors in identifying the exact location of the odor source. Moreover, in experiments where the odor cues are dissociated from visual cues, flies are often likely to be attracted to high−contrast visual cues in the vicinity of the odor cues. Thus, these experiments highlight the importance of landmark cues in the odor tracking behavior.

P3.48 SCHULTZHAUS, JN*; MOEHLMAN, AT; CARNEY, GE; Texas A&M University; *jschultzhaus@bio.tamu.edu*

The role of fit in Drosophila melanogaster starvation response, eating behavior, and lipid storage

female specific independent of transformer (*fit*) expression increases in *D. melanogaster* courting males and in both sexes after mating. In contrast, *fit* expression decreases during starvation in males and females. *fit* expression is enriched in the fat body, an insect tissue that is analogous to vertebrate fat. The fat body stores excess nutrients that are mobilized during starvation. This information led us to investigate how *fit* mutants respond to starvation. We found that both *fit* mutant males and females are starvation resistant, a phenotype that is reversed in males by *fit* expression in the fat body. *fit* mutants may be starvation resistant because they consume more food than controls prior to starvation. To test this, we monitored the total amount of food consumed from eclosion until starvation using the CAFE assay. Another possible explanation for starvation resistance is either increased storage before or slower use of lipids in the fat body during starvation. We used Nile Red and Oil Red O to stain lipids in the fat bodies of fed *fit* mutants and controls to examine whether the absence of *fit* leads to altered lipid storage. We also examined the fat bodies of *fit* mutants and controls throughout starvation to determine when lipid stores are depleted.

S9.1−5 WILLIAMS, C.M.*; SUNNY, N.; EDISON, A.S. ; MORGAN, T.J.; HAHN, D.A.; U. Florida, Gainesville, Kansas State U., Manhattan; *carolinewilliams@ufl.edu*

Nutrient flux through glycolysis and gluconeogenesis and the evolution of cold−stress tolerance in Drosophila melanogaster.

Ectotherms must maintain energy homeostasis in rapidly changing thermal conditions; a considerable challenge given that metabolism comprises a complex suite of processes that differ in their thermal sensitivities. Cold−adaptation on a broad phylogenetic scale involves changes to enzymes and pathways that allow them to function more effectively at low temperatures, but we lack an understanding of the microevolutionary variation in energy metabolism segregating within populations that may contribute to cold−stress tolerance. Without an integrative understanding of this naturally segregating variation, from the genomic through the physiological to the organismal levels, we cannot predict the evolvability of cold−stress tolerance, an important component of predicting the impacts of global climate change. We hypothesize that susceptibility to cold stress is set by an imbalance between energy supply and demand incurred at low temperatures, and that resistance may be conferred by reorganizing metabolic networks to maintain energy balance more effectively at low temperatures. Using complementary resources of the *Drosophila melanogaster* Genetic Reference Panel (192 fully−genotyped isogenic lines), and *Drosophila* lines selected in the laboratory for

fast or slow recovery from a cold−induced coma, we show that tolerant flies have higher metabolic rates, maintain metabolic homeostasis more effectively during cold exposure, and show considerable restructuring of metabolic networks. Using stable isotopes, we demonstrate a reallocation of nutrients among core energetic pathways. These alterations to nutrient flux may rebalance energy supply and demand in the cold, and assist in maintaining energy balance in the face of changing temperatures.

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