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**CONJUGATED POLYMER NANOPARTICLES FOR FLUORESCENT LABELLING OF LIVE CELLS AND DELIVERY OF BIOLOGICAL MOLECULES INTO PLANT CELLS**

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Conjugated Polymer Nanoparticles are intrinsically fluorescent carbon based structures whose engineered dimensions fall below 100 nm. These nanoparticles possess advantageous photophysical properties such as high fluorescent quantum yield, large excitation coefficient and efficient signal transduction which make them use full in fluorescent Microscopy. Amphipathic structure and flexibility in further chemical modification make them permeable through biological membranes and potential use as a carrier of biological molecules. In this study CPNs were assessed for their ability to fluorescent labeling of Tobacco BY2 cells and protoplasts by co culturing in the culture medium. More than 50% fluorescent positive protoplasts were observed under B2A excitation filter after 2 hours of incubation with 10 mM CPNs. The green fluorescent signal was stable for 2 days without any noticeable reduction in intensity. CPNs uptake of protoplasts was further confirmed by analyzing intracellular fluorescence and side scattering properties of protoplasts using Flowcytometry. Toxicity effects of CPNs were evaluated in terms of cell viability in protoplasts incubated with 5 to 500 mM CPNs. Protoplasts did not show significant reduction in cell viability with CPNs concentrations below 50 mM after 24 hours of incubation. Confocal analysis of BY2 cells treated with similar concentrations of CPNs demonstrated that cell wall provides a barrier for CPNs uptake by BY-2 cells. One of the major goals of this research is to analyze the ability of CPNs to use as a transfection agent to deliver small RNAs in to plant cells. To check the hypothesis protoplasts were incubated with commercially available siGLO small RNAs mixed with CPNs in different ratios of concentrations. CPNs were able to carry those small RNAs into protoplasts after 24 hours of incubation. In order to check whether CPNs can be used as a tool to measure dimension requirements in plasmodesmata transport of molecules in intact leaf cells, CPNs were delivered into *Nicotiana benthamiana* leaf epidermal cells using particle mediated transfer technology. Epifluorescence microscopy was used to monitor their spread immediately upon delivery. This study showed the potential use of CPNs in studying of cell to cell trafficking of molecules in plant cells.

**A COMPARISON OF POPULATION DENSITY, DIFFUSION DISPERSAL AND DEMOGRAPHICS OF THE MEDITERRANEAN GECKO (*HEMIDACTYLUS TURCICUS*) IN TWO CENTRAL OKLAHOMA HABITATS**

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The Mediterranean Gecko, *Hemidactylus turcicus* is a nocturnal species of geckkonid lizard that successfully colonizes urban and suburban areas subsequent to long distance dispersal events facilitated by human beings. We compared population density, dispersal rate, and

demographics of populations of *H. turcicus* at the University of Central Oklahoma (UCO) and at the Oklahoma City Zoo (OCZ). In the United States, *H. turcicus* is most often found on the vertical, exterior surfaces of buildings and is rarely found in areas away from anthropogenic surfaces. We hypothesized that higher building density at UCO would facilitate successful diffusion dispersal there relative to OCZ. Mark and recapture sampling was conducted at UCO during 2005-2008 and at OCZ during 2007-2008 and the sizes of the populations at the two sites were estimated using the Schnabel method. Population densities were calculated as the total population size divided by the total land area occupied and as total population size divided by building area occupied. Age class of all captured animals, sex of adults, and gravid status of adult females was determined at both sites. Dispersal rate was calculated as the total distance dispersed from the point of release per unit time since release or escape of the animals at each site. Population size and density, dispersal distance and dispersal rate were similar at the two sites; however, smaller buildings at OCZ likely indicate denser populations on individual buildings. There was a male sex bias at OCZ that did not exist at UCO. The high density of geckos on the occupied buildings at OCZ suggests that there might be constraints to diffusion dispersal at that site, but more data are needed to elucidate what factors promote or inhibit successful diffusion dispersal of Mediterranean geckos.

#### **THE NUMBER OF TUBERCLES AND THEIR DISTRIBUTION ON THE EARS OF MEXICAN FREE-TAIL BATS (*TADARIDA BRASILIENSIS*)**

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The Mexican Free-tail Bat, *Tadarida brasiliensis*, has small tubercles along the dorsal ridges of their ears. We hypothesize these tubercles serve an aerodynamic function similar to the hydrodynamic function of the tubercles along the pectoral flippers of the Humpback Whale, *Megaptera novaeangliae*. To examine this hypothesis, a descriptive morphological analysis of the tubercles of 52 *T. brasiliensis* (23 males and 29 females) was conducted. For all males and females combined, we noted a range of 5 - 12 tubercles on each ear with a median of 9. Males have a median of 9 tubercles on the left and right ears, ranging from 6 - 12 on both ears. Females have a median of 8 tubercles on the left ear and a median of 9 tubercles on the right ear, ranging from 5 - 11 on both ears. We compared male and female tubercle counts on the left and right ears, and there was no significant difference. Descriptive statistics and statistical comparisons of the distances between all the tubercles on the left and right ears of each sex and between the sexes were also made.

#### **USE OF ONLINE PHOTO SHARING ("FLICKR") TO FACILITATE COLLABORATIVE INTERNATIONAL WILDLIFE RESEARCH. E. Terdal, C. Gabbard, and A. Mai, J. Mesh**, Department of Natural Sciences, Northeastern State University, Broken Arrow, Oklahoma 74014.

Keystone predators such as the jaguar (*Panthera onca*) are ecologically important in Mesoamerican forests, but they are difficult to study as they are low in density, nocturnal and elusive. Animal-activated photography, using heat or motion sensors, has played an important role in determining presence/absence from study areas. The challenge has been the travel and labor costs of accessing and transporting the photographs. In the 21st century, researchers switched to digital wildlife cameras that used memory cards as "film." In this study, we

investigated the feasibility of using a free photo sharing website, flickr, to transport digital wildlife photographs over the Internet from a remote study site in the Maya Mountains of Belize to our laboratory in Oklahoma. We placed three Cuddeback G4 cameras in the Eljio Panti National Park on July 1, 2008. In this 10-day pilot study, 29 photographs were uploaded using a satellite internet connection in the nearby village of San Antonio. Twelve photographs depicted staff in the field and 17 depicted data (0.57 data photographs/camera/day). The data photographs were evenly divided between day (8) and night (9). A total of five species (3 mammals, 2 birds) were observed, including a male jaguar. This was the first unambiguous evidence of jaguars in this park, and was obtained just four days after the cameras were placed. There was no cost to transmit the photographs from Belize to the USA using flickr. We conclude that online photo sharing has potential to reduce the costs of wildlife research in developing countries and so may facilitate international collaboration in wildlife research.

#### **LONG TERM EFFECTS OF METAL EXPOSURE: A GENETIC AND TOXICOLOGICAL ASSESSMENT OF *HYALLELA AZTECA* AT THE TAR CREEK SUPERFUND SITE**

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To test the effects of long-term exposure to metal contaminants, amphipods (*Hyallela azteca*) from the Tar Creek Superfund Site and four reference populations (2 lab and 2 field) were subjected to genetic and toxicological testing. Acute toxicity testing revealed that *H. azteca* from the contaminated site were significantly less sensitive to both cadmium and zinc than any reference population using comparisons of 96-hour LC<sub>50</sub> values. Genetic analysis of the populations across multiple nuclear loci using Amplified Fragment Length Polymorphism (AFLP) revealed only minor genetic divergence between the contaminated site and other wild populations (0.093%), while significant genetic divergence was seen between the Tar Creek population and lab populations (0.497%). The reduced genetic differentiation between the clean wild populations and Tar Creek population raises questions of possible biogeographic factors playing a role in genetic divergence of populations. Further work will be conducted using multiple contaminated and reference sites to further evaluate factors affecting genetic diversity within and among populations. Furthermore, additional metals will be used to assess differences in sensitivity of organisms from different sites to a variety of classes of pollutants.

#### **A NOVEL STRATEGY FOR HALOPHILICITY IN THE PHOTOAUTOTROPHIC PROTEOBACTERIUM *HALORHODOSPIRA HALOPHILA***

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*Halorhodospira halophila* is an extremophilic photoautotrophic proteobacterium found in highly saline desert lakes. It is one of the most halophilic organisms known and provides a system to investigate adaptive mechanisms for survival of abiotic stress. Here we report on genome-based studies of halophilic adaptations in *H. halophila*. Two distinct strategies are known to be used by halophilic organisms to cope with high salt conditions, namely: 'Salt-in-cytoplasm' where organisms accumulate potassium ions (up to 5 M), which requires them to have an acidic proteome and 'Organic-osmolyte': where compatible solutes are

accumulated in the cytoplasm. The salt in cytoplasm strategy is mainly used by extreme halophiles, which gives them ability to grow in high salt environments (up to saturation levels) while the organic osmolyte strategy is used by moderate halophiles, which gives them adaptability to grow over wide range of salt concentrations. We found that *H. halophila* has an acidic predicted proteome. In line with this, based on flame photometry and X-ray micro probe analysis we found that *H. halophila* accumulates up to 3 M K<sup>+</sup> in its cytoplasm. However it can also grow over a broad range of NaCl concentrations (3.5 - 35% NaCl). When grown in 5% NaCl, it has K<sup>+</sup> concentration similar to *E. coli* despite its acidic proteome. We have also obtained evidence that *H. halophila* switches to accumulation of compatible solutes when grown in media containing a reduced potassium concentration. These data indicate that *H. halophila* is the first extreme halophile which uses both halophilic strategies, which not only enables it to grow in high salt environments but also over wide range. The potassium concentration at which it switches its halophilic strategy is similar to that of its natural habitat (Wadi Nantrun lakes, Egypt), and therefore is likely to be ecologically relevant.

### **ANATOMICAL AND CLINICAL PERSPECTIVES ON AN UNREPORTED VARIATION OF THE RARE TENSOR FASCIA SURALIS MUSCLE.**

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In the late 1800s', a rare muscular variant (tensor fascia suralis) of the posterior thigh was reported. This muscle is described as having a single belly arising from the "hamstrings" and inserting into the sural fascia and/or the calcaneal tendon. Herein, we describe a previously unreported variation of this muscle and provide insights on the developmental and the clinical aspects associated with this muscular variation. The unusual configuration of this muscle was discovered during cadaveric dissections at Oklahoma State University Center for Health Sciences (OSU-CHS). The muscle variant is bicipital with a distal intertendinous junction that separates proximal and distal bellies. The proximomedial head arises from the lateral aspect of semitendinosus muscle, whereas the proximolateral head arises from the medial aspect of the long head of biceps femoris muscle. A prominent, proximal muscular belly is fusiform shaped and extends into the superior part of the popliteal fossa. A smaller, distal muscular belly is fusiform shaped and inserts into both the calcaneal tendon and the deep investing fascia of the leg. This variation likely occurred during the migration of myogenic cells from somites to the developing lower limb buds or from the disruption of myogenic formation factors, which play a role in myogenic gene activation and the subsequent formation of individual muscles. The tensor fascia suralis muscle was likely benign for this individual. When evaluating masses in the popliteal fossa, however, this muscular variant could be included in the differential diagnosis.

### **SUPPLEMENTAL FERTILIZERS FOR ORGANIC GREENHOUSE SOIL MEDIA**

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The consumer's perceptions that organic food tastes better and is healthier are two major factors driving the increasing demand for organically produced crops in the U.S. It was necessary to develop organic certification to provide consistent standards across the U.S. for the benefit of producers, processors, wholesalers, retailers, and consumers. All compo-

nents entering into the organic crop production system must be approved for organic use, including the seed, soil media, and fertilizer used in transplant production.

Research was conducted to determine whether the addition of supplemental fertilizer to an organic soil media enhance seedling growth. The factorial experiment included 4 fertilizer rates (5-4-4, 6-2-2, 7-3-7, and 0-0-0 N-P-K) added at 1% by weight prior to planting tomato (*Lycopersicon esculentum* Mill.) seeds ('Florida 47') and 2 liquid fertilizer rates (0.4% and 0%) added at 3 weeks after planting (WAP) with 6 replications with 6 plants per replication. Adding fertilizer prior to planting produced significantly greater plant heights and fresh weights at 3 and 5 weeks (WAP) compared to the control (0-0-0). Although there were few height differences among 3 fertilizer treatments at 3 WAP, the addition of 0.4% solution of a fish and seaweed fertilizer at 3 WAP resulted in a significant height and dry weight increase at 5 WAP for the 6-2-2 fertilizer treatment. To produce suitable tomato transplants supplement fertilizer was required at a level of 1% by weight of a 6-2-2 N-P-K organic fertilizer, which was further enhanced by applying a 0.4% solution of a fish and seaweed fertilizer at 3 WAP. Further research should investigate additional organic soil media, fertilizer sources and rates, and crops.

### ORGANIC WATERMELON PRODUCTION SYSTEMS

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The increasing perception by consumers that organic food tastes better and is healthier continues to expand the demand for organically produced crops. Research investigating certified organic production requires a systems approach to determine the optimum combination of individual components to maximize crop yields. Research was conducted at two locations on organically certified land to determine the impact of weed control and variety selection on watermelon (*Citrullus lanatus* var. *lanatus* L.) yields. Six watermelon varieties were transplanted at two locations (Lane, Ok and Center Point, OK), into randomized complete block designed experiments with four replications. The six varieties included three seeded varieties ('Early Moonbeam', 'Sugar Baby', and 'Allsweet') and three seedless varieties ('Triple Crown', 'Triple Prize', and 'Triple Star'). The weed control system at Lane utilized black plastic mulch on the crop row, while the area between rows was cultivated to control weeds. The no-till organic system at Center Point used a mowed rye and vetch cover crop, hand weeding, and vinegar (5% acetic acid) for weed control. When averaged across watermelon varieties, the organic production system at Lane produced significantly more fruit per plant (4.2 vs. 2.3 fruit/plant), greater marketable yields (35.2 vs. 18.5 lb/plants), and higher average marketable weight per fruit (13.4 vs. 8.9 lb) than at Center Point. Plants at Center Point produced a greater percentage of marketable fruit, 92%, compared to plants at Lane, 63%. When comparison was made between locations, four of six varieties had significantly greater numbers of fruit per plant and higher marketable yields at Lane than at Center Point. Except for 'Early Moonbeam', all other varieties produced significantly heavier fruit at Lane than at Center Point. In contrast, the Center Point location produced a greater percentage of marketable fruit for all varieties except 'Allsweet'. The plastic mulch and cultivation between crop rows was a successful method of weed control at the Lane location and provided a stronger weed barrier to prevent than the cover crop mulch at Center Point. Further research will specifically investigate the impact of weed competition on watermelon fruit quality.

**DNA EXTRACTION AND SEQUENCING IN THE TURTLE GENUS *TERRAPENE***  
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The evolutionary relationship between species within the genus *Terrapene* is unclear (Milstead, 1969). There are two different species of the genus *Terrapene* found in Oklahoma; *Terrapene carolina trigunguis* and *Terrapene ornata ornata*. There have been reports of the two species interbreeding. DNA was extracted from both species as well as putative hybrids for analysis. DNA was extracted from liver tissue and processed using the DNeasy kit. This DNA was examined with two different methods. One method was the performance of gel electrophoresis. This produced the bands that were compared to a standard scale. This allowed for differentiation of the species although a clear understanding of putative hybrids could not be discerned. DNA was also sequenced for further analysis. This process provided a much better understanding of the genetic variability within this genus. It is hoped that the continuation of these two processes with an increased number of specimens will allow for a clearer understanding of the possible interbreeding of these species.

**UNEXPLORED PROKARYOTIC BACTERIAL PHOTOBIOLOGY: DEMONSTRATING LIGHT SENSING IN A DEEP SEA BACTERIUM USING A PHARMACOLOGICAL STRATEGY**

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The genomes of many chemotrophic bacteria unexpectedly contain putative light sensing proteins, implying that they exhibit unexplored photobiological responses. A major barrier in studying the function of these photosensory processes is the lack of genetic tools. We are studying photoactive yellow proteins (PYP), first found in photosynthetic purple bacteria. PYP is a blue-light receptor triggered by photoisomerization of its *p*-coumaric acid (*p*CA) chromophore. We recently identified six novel PYPs in chemotrophic bacteria, and are studying their properties after heterologous overproduction. The photocycle rates of PYPs vary considerably, from less than 1 second in the  $\alpha$ -Proteobacterium *Idiomarina loihiensis* to ~1 hour in the Bacterioidetes *Salinibacter ruber*. *I. loihiensis* is a deep sea bacterium with no known photobiology. We found that the *I. loihiensis* genome encodes a functional PYP. Bioinformatics analysis of its genome revealed that its *pyp* gene is located in the immediate vicinity of a gene encoding a diguanylate cyclase, which suggests that *Il* PYP might be involved in regulating biofilm formation. Indeed, the presence of light suppresses surface attachment of *I. loihiensis* cells as monitored by crystal violet staining. We demonstrated that PYP is the photoreceptor for this response using a novel pharmacological approach, based on the exogenous addition of *trans*-locked and *cis*-locked *p*CA analogs which constitutively inactivate and activate PYP, respectively. This light regulation of cell physiology is unexpected for a chemotrophic deep sea bacterium, and suggests that planktonic *I. loihiensis* is present near the ocean surface. The results also demonstrate that locked *p*CA analogs provide a general approach to study *in vivo* function of PYP that can be used in the absence of genetic tools.

**AN *ab initio* COMPUTATIONAL STUDY OF VOLATILE COBALT HYDROXIDES.**

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Formation of volatile hydroxides at elevated temperatures is an important mechanism for corrosion of metal alloys or oxides in combustion environments. Thermodynamic data for these species is obtained by studying the reaction of water vapor with a metal oxide in a flowing gas stream. We are currently studying the reactions of cobalt oxides with water vapor. In addition to experimental methods, computational chemistry methods are useful because they allow one to evaluate the geometry and expected vibrational modes exhibited by the metal hydroxides. Such methods are necessary because most of the volatile metal hydroxides cannot be produced in sufficient concentrations to perform spectroscopic measurements. We have examined the equilibrium geometry and vibrational modes for  $\text{CoOOH}(\text{g})$ ,  $\text{CoO}(\text{OH})_2(\text{g})$ , and  $\text{Co}(\text{OH})_3(\text{g})$ , using the *ab initio* computational chemistry program GAMESS (M.W.Schmidt, K.K.Baldrige, J.A.Boatz, S.T.Elbert, M.S.Gordon, J.H.Jensen, S.Koseki, N.Matsunaga, K.A.Nguyen, S.J.Su, T.L.Windus, M.Dupuis, and J.A.Montgomery, *J. Comput. Chem.* **14**, 1347-1363(1993). Partition functions and energies for each molecule will be presented. The ultimate goal is to obtain a G2 level energy calculation for comparison to experimental data. To date frequencies and enthalpies have been obtained for the three molecules using a 6-311G level of computation, resulting in  $H(\text{tot}) = 31.073 \text{ kcal/mol}$  and  $S(\text{tot}) = 75.030 \text{ cal/mol K}$  for  $\text{Co}(\text{OH})_3(\text{g})$ , and for  $\text{CoO}(\text{OH})_2(\text{g})$   $H(\text{tot}) = 14.741 \text{ kcal/mol}$  and  $S(\text{tot}) = 64.262 \text{ cal/mol K}$ . Preliminary results and the current status of this project will be presented.

**ESTIMATING THE VOLUMES OF THE EAR TUBERCLES OF THE MEXICAN FREE-TAILED BAT**

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The Mexican Free-tail bat, *Tadarida brasiliensis*, has small tubercles along the dorsal ridges of their ears. We hypothesize these tubercles serve an aerodynamic function similar to the hydrodynamic function of the tubercles on the pectoral flippers of the Humpback whale, *Megaptera novaeangliae*. As an initial step in examining this hypothesis, we conducted a morphological analysis of the tubercles. The volumes of the tubercles of the 52 *T. brasiliensis* (23 males, 29 females) were estimated. They ranged from  $5.29 \times 10^6 \mu\text{m}^3$  to  $1.20 \times 10^7 \mu\text{m}^3$ . Male tubercles ranged from  $5.29 \times 10^6 \mu\text{m}^3$  to  $1.20 \times 10^7 \mu\text{m}^3$ , and female tubercles ranged from  $5.45 \times 10^6 \mu\text{m}^3$  to  $1.97 \times 10^7 \mu\text{m}^3$ . The volumes were greatest for the most medially located tubercles and decreased towards the distal edge of the ear. This pattern of the greatest volume in the most medial tubercle with decreasing volume towards the distal tubercle was consistent for most specimens. Statistical comparisons of the tubercle volumes between the left and right ears of males and females as well as between sexes were made.

**A SUMMER SCIENCE PROGRAM: EARLY CHILDHOOD THROUGH ELEMENTARY SCHOOL**

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Science is a journey of learning while traveling explore, discover, and experiment trails. Summer science camps for Pre-K through fifth grade students afford the student an oppor-

tunity to succeed in the sciences and to encourage a continued interest in science, whether astronomy earth science, weather studies, life sciences or squishy-squashy sponges. Pre-K to 5<sup>th</sup> grade students has the opportunity to complete the camp and enjoy the benefits of a summer science program. Fifteen – 20 students enroll in each grade level class and complete a two-week on-campus event. Each grade level is mentored by science content major and an elementary education or a secondary science education major. The elementary education major provides pedagogy and the science major provides content enhancement. The intent is to combat science conceptual struggles because there are no associations, no past experiences from which the students can draw to be able to imagine or conceptualize ideas later in their education. “Ideas stated but not imagined”.

### **BROAD-SPECTRUM ANTIMICROBIAL ACTIVITY OF THE REACTIVE COMPOUNDS GENERATED IN VITRO BY *Manuca sexta* PHENOLOXIDASE**

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Although quinone production and melanin formation are widely recognized as an integral part of the insect defense system, experimental evidence is lacking that the proteolytic activation of prophenoloxidase (proPO) participates in the direct killing of invading microbes – active phenoloxidase (PO) generates quinones that polymerize to form melanin. Here, we report the antimicrobial effect of reactive intermediates produced in PO-catalyzed reactions. After being treated with *M. sexta* PO and dopamine, *E. coli* and *B. subtilis* ceased to grow whereas the growth of *P. pastoris* was slightly affected. Microscopic analysis showed melanin deposition on cell surface, aggregation of bacteria, and loss of cell mobility. Viability tests revealed major decreases in the bacterial colony counts and, since the decrease remained significant after dispersion of the cell clumps, the reactive compounds were surmised to have aggregated and killed *E. coli* and *B. subtilis* cells. Under the experimental conditions, 60-94% of the Gram- bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. typhi-murium*) and 52-99% of the Gram+ bacteria (*B. cereus*, *B. sub-tilis*, *M. luteus*, and *S. aureus*) were killed. As one of the oxidation products of dopamine, 5,6-dihydroxyin-dole (DHI) killed bacteria (*E. coli* and *B. subtilis*), fungi (*P. pastoris*, *S. cerevisi-ae*, *C. albicans* and *B. bassiana*), and viruses (baculovirus and lambda bacteriophage). These results established that proPO activation is an important component of the insect immune system, which immobilizes and kills invading microorganisms.

### **MLST, PFGE, AND RIBOTYPING FOR MOLECULAR TYPING OF ADHERENT PHENOTYPES AMONG ISOLATES OF *Listeria monocytogenes***

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*Listeria monocytogenes* is an intracellular Gram-positive bacterium causing foodborne illness (listeriosis). Isolates from raw and ready-to-eat (RTE) meats and environmental samples from RTE meat processing facilities were divided into weak or strong surface-adherent phenotypes based on a microplate fluorescence assay we recently developed. Further testing using tissue culture virulence assays with the Caco-2 cell line showed higher cellular adherence and invasiveness with low incubation time (15 min) and low multiplicity of infection (1:10) with strong adherent strains. We further subtyped several strong (CW50, 99-38, CW62 and CW77) and weak (CW34, CW35, CW52 and SM3) adherent strains of *L. monocytogenes* to see if



molecular typing methods could segregate the adherent phenotypes into different groups. Molecular typing was performed using multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) and ribotyping (RT). For MLST, sections of different genes of *L. monocytogenes* (*hlyA*, *inlA*, *prfA*, *actA1* and *actA2*) were amplified and sequenced. The strains were also subtyped by PFGE using *AscI* and *Apal* and by automated RT using *EcoRI*. Clustal analysis of data from all three methods grouped strain 99-38 (raw beef) and CW50 (RTE meats) in one group though these strains were isolated from different sources. Typing of additional strains by both PFGE and MLST demonstrated several instances whereby strains from raw sources co-typed with strains from RTE sources, suggesting that outbreak strains that are only compared to RTE-derived isolates (i.e., USDA-FSIS regulatory samplings) may not identify raw sources for *L. monocytogenes* should they occur because their database contains only RTE-sourced isolates. The adherence and virulence analyses suggest that strong adherence not only promote retention of such strains in food processing facilities, but enhanced virulence as well. We are currently examining the virulence of these strains *in vivo* by orally infecting A/J mice to examine *in vivo* tissue invasiveness to confirm our observations. Keywords: *Listeria monocytogenes*, MLST, PFGE, Ribotyping, virulence assay.

**GENE SILENCING OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) AND ITS EFFECT ON BLOOD MEAL ACQUISITION IN AMERICAN DOG TICK, *Dermacentor variabilis***

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Macrophage Migration Inhibitory Factor (MIF) is a pro-inflammatory cytokine and has been identified in salivary glands and midgut tissues of *Amblyomma americanum*, lone star tick (Jaworski et al., 2001). MIF protein is 12.6 kDa in size and is expressed during early blood feeding stages. Our hypothesis is that MIF functions in tick blood meal acquisition and impacts pathogen transmission to hosts. We have identified expression of a MIF homolog in *D. variabilis* during early blood feeding. Here we report the effect of gene specific gene silencing of MIF on blood feeding in *D. variabilis* using specific double stranded RNA (dsRNA) for *D. variabilis* MIF. RNA interference was carried out by injecting unfed female and male *D. variabilis* adults with double stranded RNA specific for *D. variabilis* MIF. Positive and negative controls were made by injecting ticks with subolesin dsRNA (de la Fuente et al, 2006) and elution buffer respectively. Following injections, ticks were fed on a calf until females reached repletion. Gene silencing was confirmed by performing RT-PCR on midgut (MG), salivary gland (SG) and remaining carcass (CA) tissues. Effect of gene silencing on blood meal acquisition was evaluated by measuring the female tick weights. Our results show that MIF dsRNA injection did not silence the expression of MIF in *D. variabilis*, but rather the expression was enhanced. Additionally, MIF expression was suppressed in subolesin dsRNA injected ticks. Replete female tick weights were consistent with the results observed in RT-PCR experiments. That is, no significant difference in female body weights were observed for MIF injected ticks versus buffer injected controls. Currently we are continuing to clone & sequence full length cDNA from *D. variabilis* MIF. Our results also suggest the importance of further research on subolesin's effect on regulating MIF expression in ticks.

**THE EFFECT OF M<sub>n</sub>II RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN THE MELATONIN RECEPTOR 1a GENE ON REPRODUCTIVE SEASONALITY IN EWES.**

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Melatonin is important in regulating circadian rhythms of many biological processes. To evaluate the relationship between melatonin receptor 1a gene (MTNR1A) and reproductive performance, reproductive records were compared between Polled Dorset and Polled Dorset x East Friesian ewes expressing different genotypes for MTNR1A at the M<sub>n</sub>II restriction fragment length polymorphism (RFLP) site. One hundred sixteen ewes with first lambing records and 104 ewes with second lambing records were genotyped for the M<sub>n</sub>II RFLP in the MTNR1A gene. Genotypes were determined by PCR amplification of an 824-bp fragment of ovine MTNR1A followed by digestion with M<sub>n</sub>II restriction enzyme. The M allele has a restriction site for M<sub>n</sub>II while the m allele does not; genotypes are presented as MM, Mm, or mm for each ewe. Ewes were managed according to the STAR system, which divides the year into five, 73-d periods that begin on Jan 1, Mar 15, May 27, Aug 8, and Oct 20. Ewes were exposed to rams for the first 30 d of each period. Lambs were weaned on d 70 of each period, ranging from 40 to 70 days of age. Three days following weaning, ewes were again exposed to rams. Season of conception was defined as early favorable (EFV; conception in Jul, Aug, Sep, Oct), late favorable (LFV; conception in Nov, Dec, Jan, Feb), or unfavorable (UFV; conception in Mar, Apr, May, Jun). The effect of breed, season of conception for first lambing, and each polymorphism on days to first lambing and days between first and second lambings were evaluated. Breed of ewe did not affect days to first lambing or days between first and second lambings. Ewes with first conception in EFV or LFV seasons had 127 fewer ( $P = 0.09$ ) days to first lambing and 100 more ( $P = 0.07$ ) days between first and second lambings than ewes with first conception in UFV seasons. Ewes conceiving for the first time in UFV seasons may be the result of failure to conceive in EFV or LFV seasons, making them older at time of first conception and more likely to conceive on time for second conception. Ewes conceiving in LFV seasons tended to have 68 fewer days between first and second lambings than ewes conceiving in EFV seasons. This is explained by the sequence of breeding in the STAR system in which the next breeding exposure for ewes conceiving in EFV is UFV season and in LFV is EFV season. Days to first lambing and days between first and second lambings were decreased by 207 ( $P = 0.02$ ) and 140 ( $P = 0.03$ ) days for ewes expressing MM or Mm genotypes relative to ewes expressing mm genotype. There was no difference in reproductive performance between ewes expressing MM or Mm genotypes. These results show ewes that express at least one M allele are able to conceive at a younger age, better able to breed in an UFV season, and have a shorter interval between first and second lambings than ewes expressing only the m allele.

**STRUCTURAL BASIS FOR ANTAGONISM OF HUMAN INTERLEUKIN-18 BY POXVIRUS INTERLEUKIN-18 BINDING PROTEIN**

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Human interleukin-18 (hIL-18) is a cytokine that plays an important role in inflammation as well as host defence against microbes. Its activity is regulated *in vivo* by a naturally occurring antagonist, the human IL-18 Binding Protein (IL-18BP). Functional homologs of human IL-18BP are encoded by all orthopoxviruses, including variola virus which is the causative agent of smallpox. These orthopoxvirus homologs contribute to virulence by suppressing IL-18-mediated immune responses. Here we describe the 2.0Å resolution crystal structure of

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an orthopoxvirus IL-18BP, ectromelia virus IL-18BP (ectvIL-18BP), in complex with hIL-18. The complexed hIL-18 structure shows significant conformational changes at the binding interface when compared to the structure of ligand free hIL-18, indicating that the binding is mediated via an induced-fit mechanism. The complex structure reveals a canonical immunoglobulin-fold in ectvIL-18BP, which interacts via one edge of its  $\beta$ -sandwich with three induced cavities on the hIL-18 surface through extensive hydrophobic and hydrogen bonding interactions. The ectvIL-18BP residues that participate in these interactions are mostly conserved in both human and viral homologs, explaining their functional equivalence despite limited sequence homology. EctvIL-18BP blocks a putative receptor-binding site on IL-18 thus preventing IL-18 from engaging its receptor. Our structure provides insights into how IL-18BPs modulate hIL-18 activity. The unveiled unique binding interface provides basis for rational design of inhibitors against orthopoxvirus IL-18BP (for treating orthopoxvirus infection) or hIL-18 (for treating certain inflammatory and autoimmune diseases).

### **DIETARY MANIPULATION OF n-ALKANES OF CUTICULAR HYDRO- CARBON PROFILES IN TERMITES**

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Previous studies have shown that analysis of cuticular hydrocarbons in termites can be an effective tool in identifying termite species. However, little is known about how environment and diet may affect termite cuticular hydrocarbon profiles. This study was conducted to determine if adding dietary hydrocarbons can affect their cuticular hydrocarbon profiles. Termites collected from the summer burn site from the tallgrass prairie preserve in Osage county, Oklahoma were used. Hydrocarbons including nC23, nC25, nC27, nC29, and nC31 were added to filter paper that the termites fed on for a period of two weeks with a chase period where termites fed on hydrocarbon-free paper of 4 days, 1 week or 2 weeks. The length of the chase period did not significantly affect incorporation of hydrocarbons. Preliminary results indicated that termites that were fed nC23 showed a sharp increase in nC23, nC25, and nC27. However when fed with longer chain hydrocarbons such as nC31, the same effect was not observed. Similar results were observed when mixtures of hydrocarbons were fed to the termites. Feeding short chain hydrocarbons resulted in similar changes in the internal hydrocarbon profiles of termites. Based on the preliminary data, it seems that hydrocarbons in the diet may affect the cuticular hydrocarbons of termites. It also indicates termites may metabolize nC23 differently than the other hydrocarbon but further studies need to be conducted to confirm these findings.

### **IN UTERO PROCESSING OF LUMINAL INTERLEUKIN 18 (IL-18) BY ENDOMETRIAL CASPASE-1 IN THE PREGNANT AND CYCLIC GUT**

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Interleukin (IL)-18 is a pleiotropic cytokine expressed in mouse and human reproductive tissues. IL-18 expression involved with reproductive processes is poorly understood. IL-18 is secreted as a 24-kDa inactive product which yields an 18-kDa product following Caspase-1 cleavage. In the current study, IL-18 and Caspase-1 expression patterns are characterized in the endometrial tissues of cyclic and pregnant gilts. Objectives for the present study were to establish genetic and translational expression patterns of IL-18 and Caspase-1 in cyclic and pregnant gilts. Crossbred cycling gilts of similar age (8-10 mo) and weight (100-130 Kg) were

checked twice daily for estrus behavior with intact males. Gilts assigned to be bred were naturally mated with fertile crossbred boars at first detection of estrus, and bred subsequently at 12h and 24 hr post detection. Gilts were hysterectomized on either d 0, 5, 10, 12, 15 and 18 of the estrous cycle (n =24) or Days 10, 12, 15 and 18 of pregnancy. Endometrium and uterine flushings were harvested from cyclic and pregnant gilts. IL-18 and Caspase-1 gene expression was evaluated using quantitative Real-Time PCR. IL-18 protein was quantified using a commercially available porcine specific ELISA kit. IL-18 endometrial gene expression increased more than 5 fold from days 10 to 18 ( $P<0.003$ ) regardless of reproductive status. IL-18 quantification revealed total luminal protein expression significantly increased in the pregnant gilts on days 15 and 18 of pregnancy. Caspase-1 endometrial gene expression remained nadir and unchanged in the cyclic gilts however, in the pregnant gilts, endometrial gene expression for Caspase-1 was almost 6 and 10 fold higher than cyclic contemporaries on days 15 and 18 respectively. The current study provides some evidence for a possible biological relationship between IL-18 endometrial gene and protein expression and its processing counterpart Caspase-1. This study also infers that processing of IL-18 product is a pregnancy associated event.