TEXAS TECH UNIVERSITY
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2014 Undergraduate Scholar Research Forum

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Development of Transgenic Crops to Enhance Abiotic Stress Response and Crop Yield

Philip C. Jarrett
with Necla Pehlivan, Sun Li, Hong Zhang
Texas Tech University

Crops are commonly grown under unfavorable environmental conditions that inhibit full expression of desirable phenotypes. One of the most prominent stresses that affect crop yield is an excess of salts in plant soil. Due to the constant rise in human population and loss of agricultural land, the need for genetically engineered plants is more prevalent than ever before. A substantial increase (50%) in yield of crops such as maize, wheat, and rice is required to meet the expected global population by the year 2050. An anticipated 69.1% of potential crop yield is lost annually to abiotic stress. As a result, recent plant biotechnology research has focused heavily on the development of crops that more effectively tolerate abiotic stress.

To confer heightened salt tolerance in plants, wild-type *Arabidopsis thaliana* (AT) plants were transformed with a genetic vector encoding two key genes: AtNHX1 and SOS1. AtNHX1 is a proton-sodium antiporter located in plant vacuolar membranes. AtNHX1 permits cellular resistance to salt toxicity by sequestering cytoplasmic sodium ions in the vacuole. SOS1 is a proton-sodium antiporter that shares a similar function with AtNHX1. SOS1 is embedded in the plasma membrane where it pumps sodium ions out of the cell, reducing salt toxicity in the cytoplasm. Both protein antiporters utilize the cell-dependent electrochemical gradient as their energy source.

Prior studies have shown that overexpression of either AtNHX1 or SOS1 may confer salt tolerance in plants. The combined overexpression of AtNHX1 and SOS1 should, as a result, confer more drastic improvements to AT plant salt tolerance. The resulting plants should, therefore, possess an improved genetic potential to yield greater returns in the field. Such hypotheses are testable through standardized protocols in the field of plant biotechnology.

First, wild-type AT plants were transformed with a molecularly engineered construct containing the genes for AtNHX1, SOS1 and a selection marker for screening purposes. Independent, homozygous lines were distinguished through screening. Levels of relative gene expression were assayed via Northern Blotting Analysis to select double- (NSo) and single-gene high-expression lines. Three high-expression NSO lines were discovered. Additionally, AT plants highly expressing either AtNHX1 or SOS1 were also found. Finally, physiological testing was conducted to survey the salt tolerance of double-gene overexpression relative to single-gene and controls.

Physiological tests showed significant improvement in AT plant salt tolerance when compared to wild-type and single-gene overexpression lines. Under incremental salt treatments escalating from 50 mM NaCl to 250 mM NaCl, NSo lines produced greater yield than controls. Additionally, preliminary data suggests that NSo lines exhibit significantly higher rates of photosynthesis than controls. As a consequence, such research may serve as a proof-of-concept for translation into staple crops such as cotton and maize.

Philip Jarrett

I am a senior in cell and molecular biology. I began research in the summer following my high school graduation and have since been involved in research across four separate laboratories. My primary interests lie in medicine and clinical research, but I am currently developing transgenic crops that possess resistance to abiotic stresses such as heat, drought and salt. I am pursuing medical school with a focus in procedural medicine. My other interests include education, fitness and the outdoors!

Understanding Species Limits of *Peromyscus mexicanus* Group Using A Genetic Approach

María Núñez
Mentor: Dr. Robert Bradley

The genus *Peromyscus* deer mice is one of the most widely distributed mammalian taxa in North America. Its range extends from the Atlantic to Pacific coast east to west and from Canada to Panama north to south. Due to their wide distribution this genus consists of more than 50 species and several subspecies. Currently *Peromyscus* is divided into 13 species groups. Before the introduction of molecular analysis most relationships where determined using morphology to resolve which organisms were of the same species or which species were more closely related to one another. Current genetic studies provide another way to determine phylogenetic relationships. Here in, DNA sequence data is used to determine the phylogenetic relationships of the *P. mexicanus* species group. *Peromyscus nudipes* is one of the species of this group, and it is found in southern Costa Rica and northern Panama. *P. nudipes* systematic relationships to the other *P. mexicanus* species has not been studied therefore will be the objective of the study.

For this study, 56 Cytochrome-b (cytb) sequences were used, 28 of these samples were obtained from GenBank and the other 28 were obtained from samples at the Museum of Texas Tech University. Mitochondrial DNA was extracted with standard DNA Extraction methods. Standard Polymerase Chain Reaction (PCR) procedures were followed using primers LH14115 and H15288 for amplification.
The laboratory work will include PCR cleaning, and cycle sequencing using the same primers. Cycle sequencing reactions will be purified using isopropanol clean up protocols, and purified products will be sequenced, with an automated sequencer. Resulting sequences will be aligned and proofed using sequencer 4.0 support; Chromatograms will be examined to verify all base changes. Cytb sequences obtained in this study will be deposited in GenBank. We are going to use different phylogenetic software to generate the phylogenetic tree and the Kimura 2-perameter model of evolution to calculate genetic distances among samples to assess levels of genetic devergency of the species in the *P. mexicanus* group.

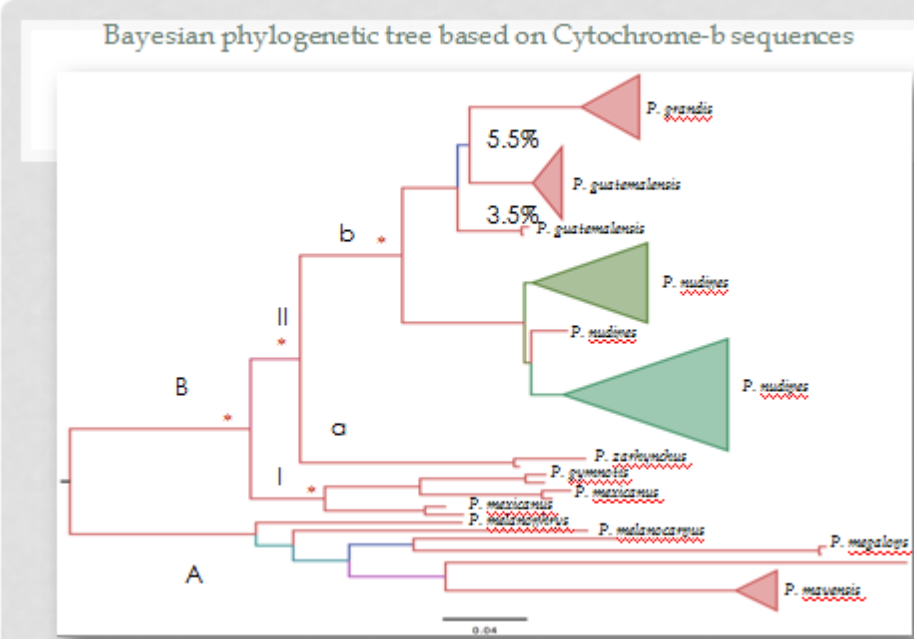
References

Nicté Ordóñez-Garza, John O. Matson, Richard E. Strauss, Robert D. Bradley, and Jorge Salazar-Bravo (2010). Patterns of phenotypic and genetic variation in three species of endemic Mesoamerican Peromyscus (Rodentia: Cricetidae). Journal of Mammalogy: August 2010, Vol. 91, No. 4, pp. 848-859.

Bradley RD, Durish ND, Rogers DS, Miller JR, Engstrom MD, Kilpatrick CW (2007). Toward a molecular phylogeny for peromyscus: Evidence from mitochondrial cytochrome-b sequences. J Mammal, 1;88(5), pp. 1146-1159.

***Peromyscus mexicanus* species group distribution.**

***Peromyscus Grandis P. mexicanus* group**



María Núñez

I am a senior majoring in Zoology and minoring in Spanish and Education. I have been involved in research since I joined the Plains Bridges program while I attended South Plains College, and have just as of this past spring transferred to Dr. Robert Bradley's lab in the department of Biology. My goals are to be able to teach the future generations and inspire them to become involved in science. My research interests are varied, though for the most part I am interested in anything that has to do with understanding how nature works or how we as humans affect the world around us for better or for worse.

Biosynthesis of Granatane Alkaloids in *Punica granatum*

Benjamin Chavez
Mentor: Dr. John D'Auria

Alkaloids are class of organic compounds that are produced in wide variety of organisms such as plants, fungi, and animals. Alkaloids tend to have pharmacological effects on other organisms and for that reason they are used in numerous medical applications such as the tropane alkaloid, cocaine.

The granatane and tropane alkaloids are structurally similar classes of bicyclic alkaloids which have an uneven distribution across flowering plant families. Specifically granatane alkaloids are found in the pomegranate plant (Punica *granatum*) where alkaloids are thought to be biosynthesized in the root and accumulate in the bark. It has been previously shown in the D'Auria lab that tropane alkaloid biosynthesis has evolved independently in different plant families. We are therefore interested in testing the hypothesis that the structurally similar granatane alkaloids also have an independent origin.

A homology based in silico search was performed on a P. *granatum* transcriptome database in order to identify genes thought to be involved in alkaloid biosynthesis. Gene sequences used for this approach originated from known tropane alkaloid biosynthetic enzymes either from E. coca or members of the Solanaceae family. Several putative biosynthetic enzymes were successfully identified during this initial BLAST (Basic Local Alignment Search Tool) search. The genes encoding these enzymes were evaluated to determine whether or not they existed as Open Reading Frames (ORFs) and primers were designed to isolate them from pomegranate tissue. Ongoing studies include the heterologous expression of these enzymes in a host such as E. coli or yeast. Furthermore, these enzymes will then be characterized via enzyme assay for their biochemical activities.

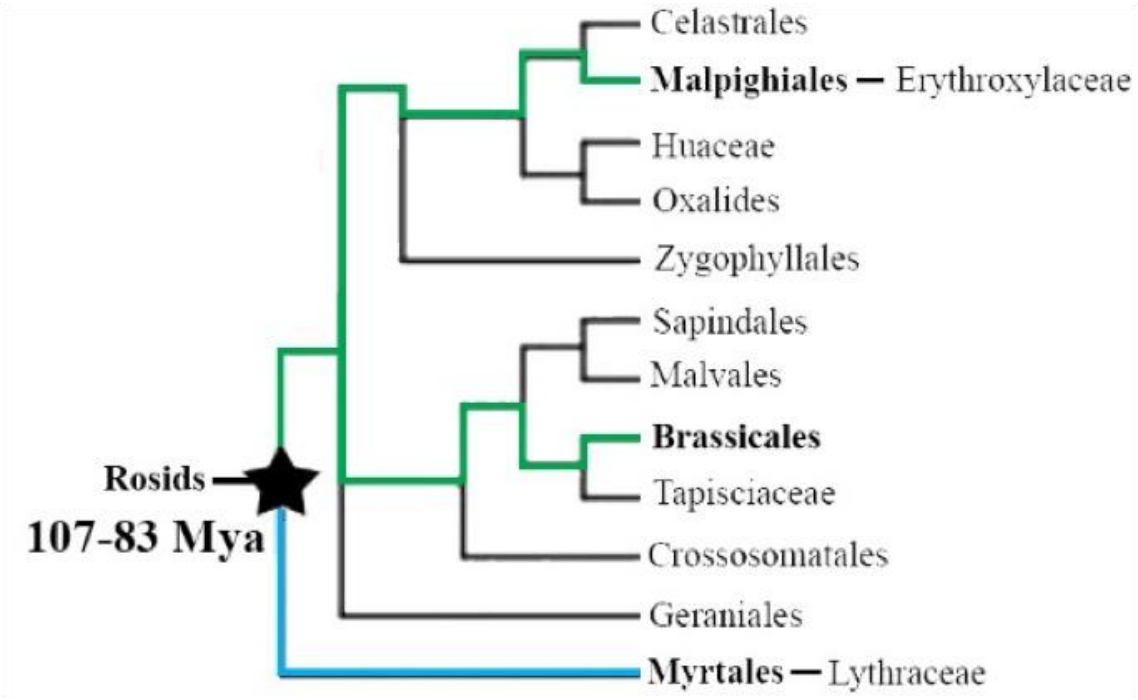


Figure 1: The plant orders Malpighiales and Brassicales contain families of plants capable of making tropane alkaloids. The plant family Lythraceae, in the order Myrtales, has been shown to produce granatane alkaloids. The last shared common ancestor between these orders occurred at the beginning of the formation of the Rosids.

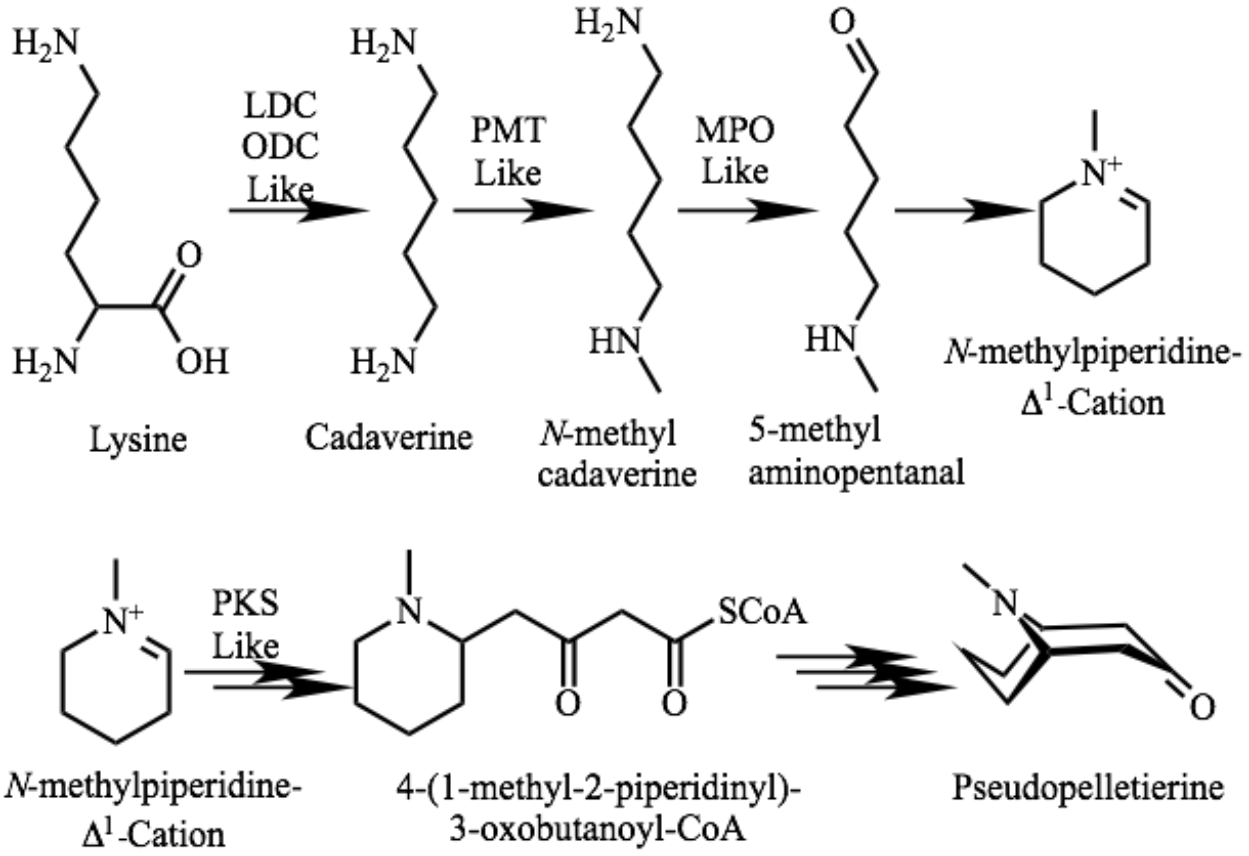


Figure 2: Hypothetical pathway for granatane alkaloid biosynthesis.

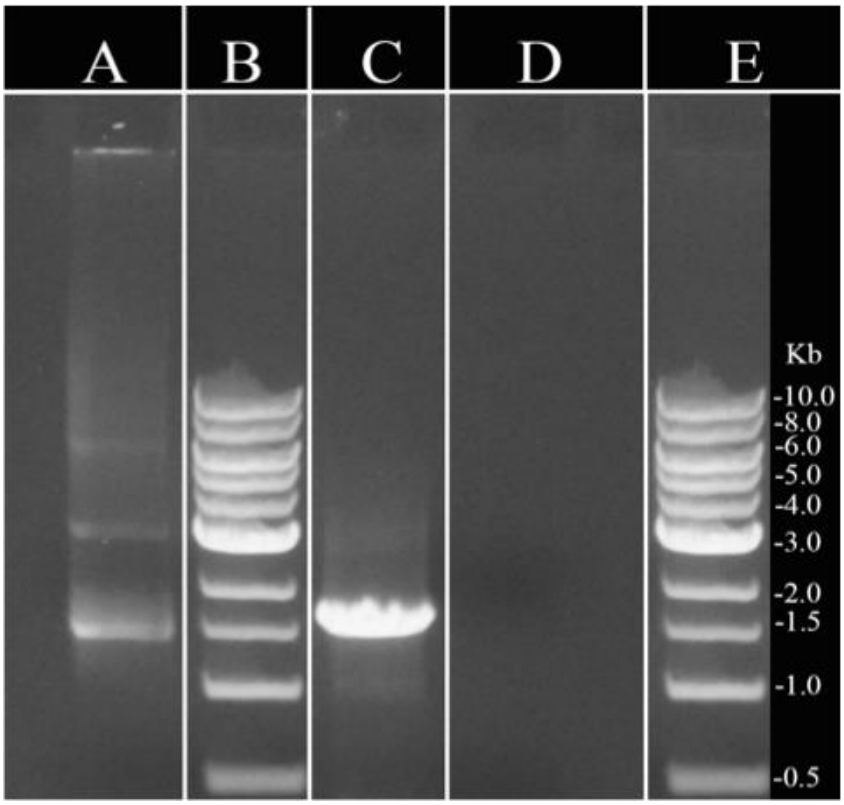


Figure 3: A, Pg_LDC. B, NEB 1 kb DNA ladder. C, Ec_PKS positive control. D, Negative control. E, NEB 1 kb DNA marker (Kb=Kilobase).

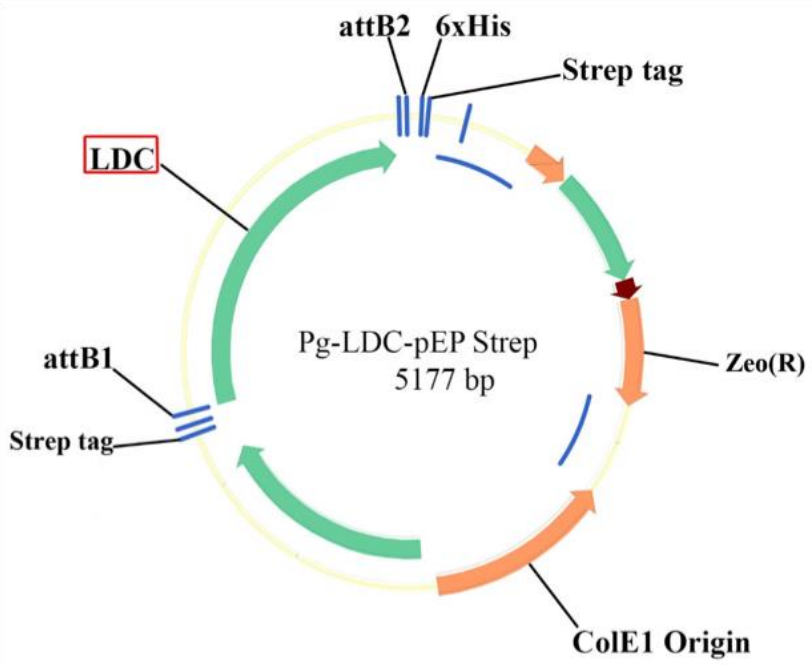


Figure 4: The gene of interest, LDC, from *P. granatum* was inserted into the pEP Step vector. The Zeocin resistance gene (Zeo(R)) codes for antibiotic resistance. The expressed LDC protein will contain an N-terminal Strep tag for one-step affinity purification. A C-terminal 6xHis allows for further protein purification. The attB1 and attB2 site allow for recombination based cloning.

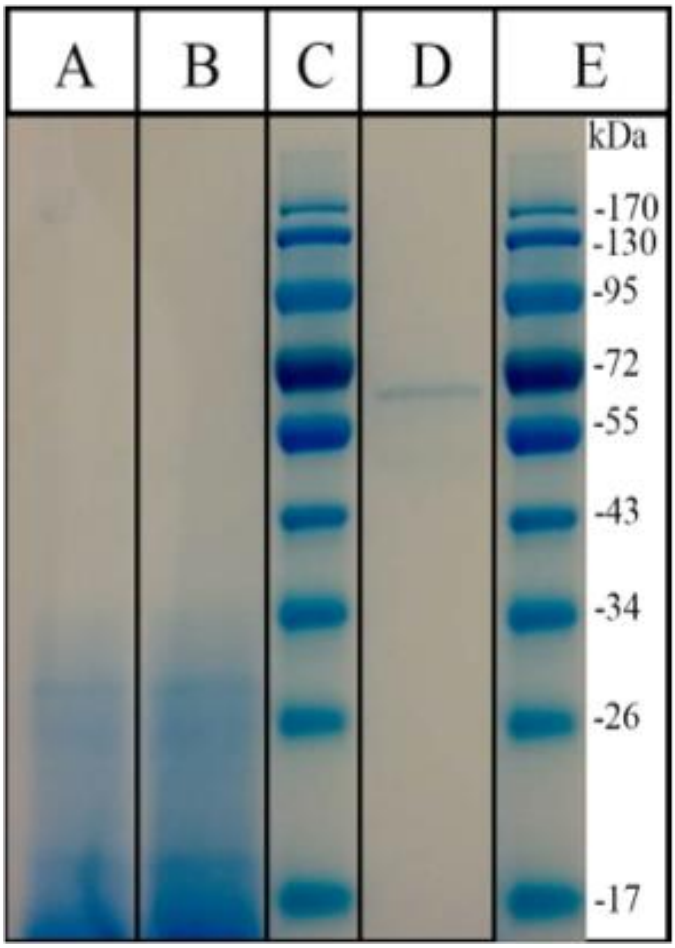


Figure 5: SDS-polyacrylamide gel illustrates the expression and purification of the putative *Punica granatum* LDC. A, crude extract of *Pichia pastoris* KM71 cells transformed with Pg_LDC-pEP Strep. B, flowthrough . C, PageRuler Prestained Protein ladder. D, purified LDC protein. E, PageRuler Prestained Protein ladder (kDa=Kilodaltons).

Benjamin Chavez

I am sophomore chemistry major from Lubbock, Texas. I have been in Dr. D'Auria's lab for almost four months. My interests are in studying the biochemical reactions in both plants and animals. I will likely pursue a career in science and possibly medicine. My favorite hobbies include playing guitar, cooking, and exploring the outdoors.

Synergistic Interactions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an *In Vitro* Wound Model

Stephanie DeLeon
Mentor: Dr. Kendra Rumbaugh

Chronic wounds represent a significant burden to health care professionals. The National Institute of Health (NIH) has estimated that more than 80% of chronic bacterial infections involve biofilms. It has been noted in vitro that bacteria residing in biofilms can be up to 1000 times more resistant to antibiotic agents than free-floating planktonic bacteria. Bacterial biofilms are the causative agents of several chronic wound infections, often isolated from clinical chronic wounds composed of multispecies biofilms. PA and SA are two of the most commonly isolated strains found in wound patients. However, there is very little information describing the interspecies interactions of these two very important wound pathogens. One explanation for the dearth of interspecies studies is that PA quickly kills SA when the two are grown in planktonic co-cultures (Fig.). However, an interesting paradox exists because, although PA can kill SA very efficiently in vitro, co-infections with both species are common clinically. In fact, several types of infections become much more devastating when both species are present. Therefore, understanding the relationship between these two important pathogens is essential. In this study we sought to determine if we could study the interspecies interactions between PA and SA in an in vitro 'wound-like' environment.

Microbes within polymicrobial infections often display synergistic interactions that can enhance their colonization, virulence or persistence. One of the most prevalent types of polymicrobial infection occurs in chronic wounds, where Pseudomonas aeruginosa (PA) and Staphylococcus aureus (SA) are the two most common causes of infection. Although they are the most commonly-associated microbial species in wound infections, very little is known about their interspecies relationship. Evidence suggests that PA and SA coinfections are worse than monoculture infection with either species; however, difficulties in growing these two pathogens together in vitro have hampered attempts to uncover the mechanisms involved. Here we used a simple and clinically relevant in vitro wound model, which supported concomitant growth of PA and SA. We observed that being together increased the ability of PA and SA to survive antibiotic treatment, as did the presence of 'host-derived' matrix components. Our data indicate that PA and SA may mutually benefit by coinfecting wounds.

In preparation of planktonic cultures SA and/or PA were grown, shaking at 250 rpm, in Luria Bertani (LB) broth or wound-like media (WLM) at 37ºC. Samples were taken at the indicated time points, serially diluted and plated on *Pseudomonas* and/or *Staphylococcus* isolation agar to determine the number of colony forming units (CFU). *In vitro* wound-like model: WLM is made up of 45% Bolton's Broth, 50% bovine plasma, and 5% horse-laked red blood cells. After inoculation with SA and grown under static conditions the media coagulates and bacteria are surrounded by a host-derived matrix (HDM). Coagulation typically occurs within 10-12 hours of SA inoculation. *Note:* uninoculated media does not coagulate, nor does WLM media inoculated with PA. Antibiotic tolerance assay: Pellets of planktonic cells or sections of HDM were suspended in 200 µg/mL gentamicin, 32 µg/mL ciprofloxacin, 15 µg/mL tetracycline (in 50% ethanol) or PBS for 5 hours. Antibiotics were neutralized in 1 ml DE neutralizing broth, and then cells were vortexed, serially diluted and plated on *Staphylococcus* and/or *Pseudomonas* isolation agar to quantitate CFU. The percentage of cells viable after antibiotic treatment was determined by dividing the number of cells that survived antibiotic treatment, by the number of cells in the PBS treatment, multiplied by 100.

In conclusion, our in vitro 'wound-like' model supported concomitant PA and SA growth, reflecting what

is seen in vivo. PA/SA cocultures displayed altered antibiotic susceptibilities in comparison to monocultures in wound-like media. Both the host-derived matrix and the bacterial EPS appear to contribute to antibiotic tolerance in vitro and in vivo. Interactions between microbes are clearly dependent on the environment they're in and, in regard to antibiotic susceptibility, the cumulative susceptibility of the entire population should be considered when determining the most effective drug to use.

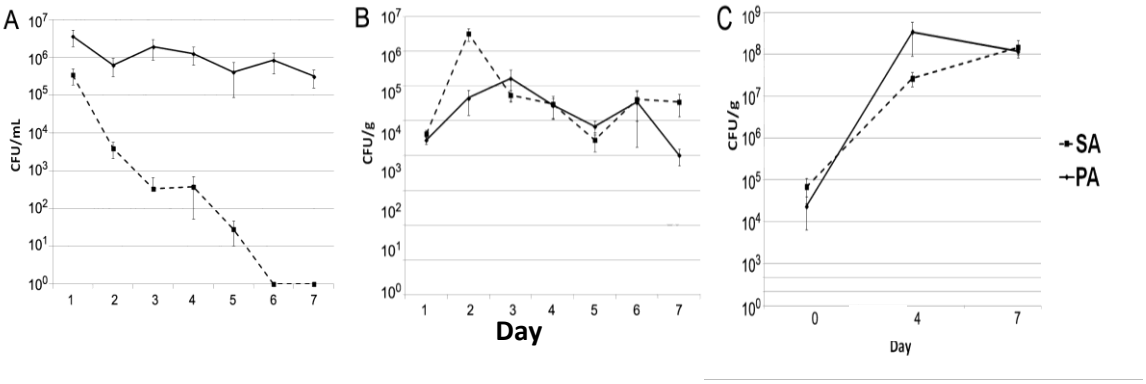


Figure 1. SA/PA cocultures were initiated in LB (A) or WLM (B) with approximately 10^4 CFU of each species and were grown in glass culture tubes under static, aerobic conditions at 37ºC for 7 days. Cultures were sampled at the indicated time points and the number of bacteria was estimated by CFU enumeration on PA and SA isolation media. Experiments were performed in triplicate. (C) PA and SA maintained equal numbers over 7 days when grown in mouse surgical excision wounds (n=6 mice/time point).

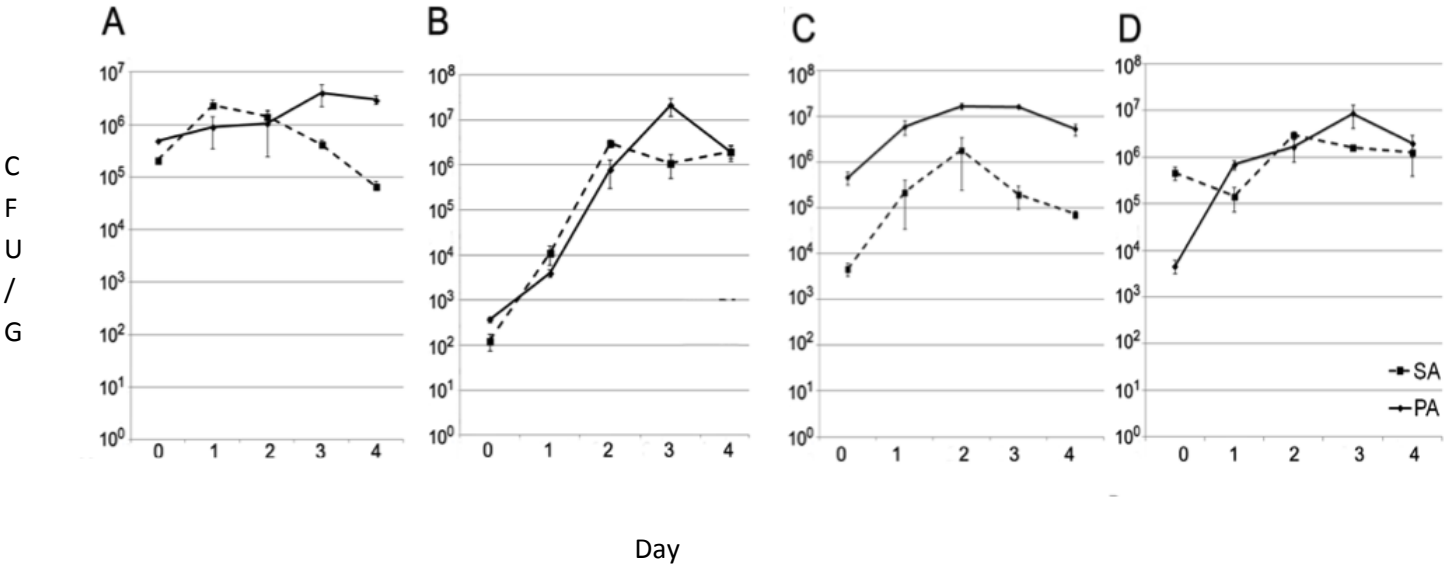


Figure 2. Cocultures were initiated in WLM with a high inoculum (10^5 CFU/species, A) or low inoculum (10^2 CFU/species, B), and at a PA:SA starting ratio of 1:1 (B), 100:1 (C) or 1:100 (D) and cultures were grown in glass culture tubes under static, aerobic conditions at 37ºC for 4 days. Cultures were sampled at the indicated time points and the number of bacteria was estimated by CFU enumeration on PA and SA isolation media. Experiments were performed in triplicate.

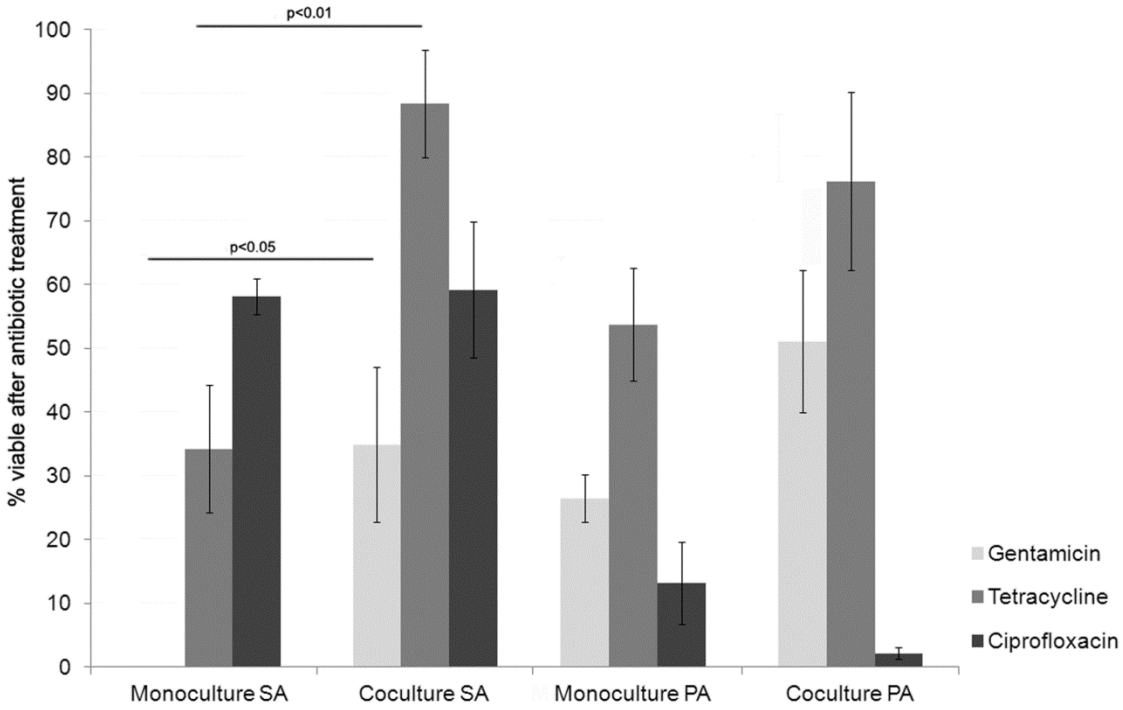


Figure 3. Planktonic mono or cocultures of PA and SA were grown overnight in WLM and tolerance to gentamicin, ciprofloxacin, and tetracycline was determined. Cultures were initiated with an approximate 1:1 starting ratio of PA to SA and were grown in flasks at 37ºC with vigorous shaking, which prevented the coagulation of the media. One-way Analysis of Variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test were used to test for differences in tolerance between SA and PA mono and cocultures. Each group included at least 6 individual cultures and bars represent the standard error of the mean.

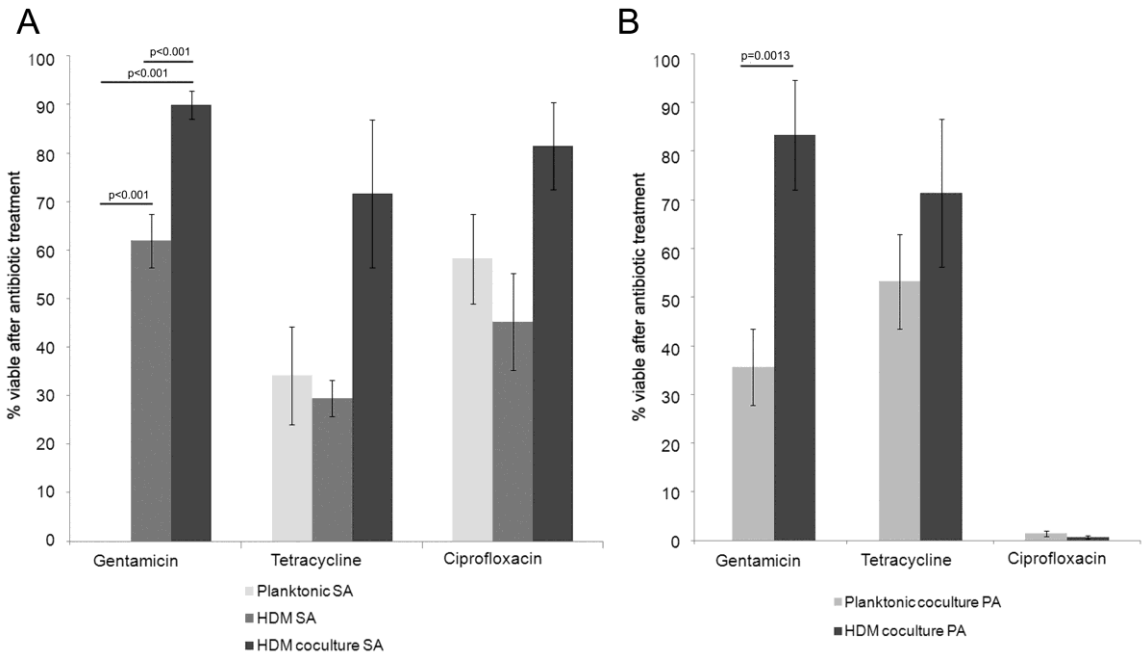


Figure 4. SA monocultures and SA/PA cocultures were grown aerobically overnight at 37ºC either in culture tubes under static conditions, which allows for the coagulation of the WLM, or in flasks with vigorous shaking, which inhibits coagulation and results in a planktonic population. Gentamicin, tetracycline and ciprofloxacin tolerance was then measured in samples from these cultures. One-way Analysis of Variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test were used to test for differences in tolerance between SA cultures in each antibiotic group. Two-tailed, unpaired t-tests were used to test for differences between planktonic and HDM-associated PA within each antibiotic group. Each group included at least 6 individual cultures and bars represent the standard error of the mean.

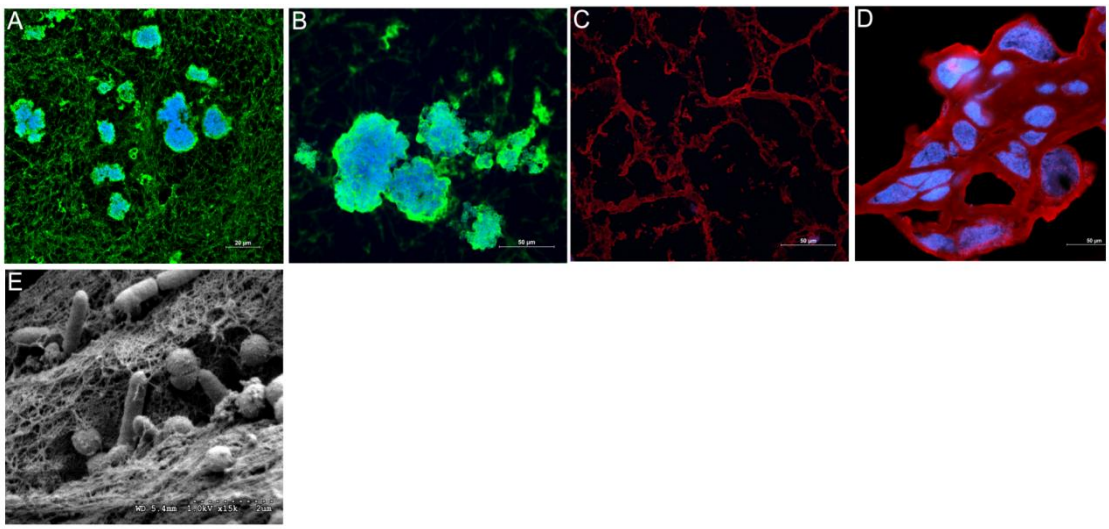


Figure 5. Imaging PA and SA in the HDM. 1-day-old PA/SA cocultures in coagulated WLM were frozen in Cryomatrix and sectioned. Sections were stained with either FITC-conjugated (A and B) or Texas-Red-conjuagted (C, D and F) ConA and DAPI and visualized by fluorescence microscopy. These images revealed discrete clusters of bacteria interspersed between fibrous host matrix, which stained intensely for ConA. SEM was performed on glutaraldehyde/ paraformaldehyde-fixed sections from 1-day-old PA/SA cocultures in coagulated WLM and revealed cocci and rods in close proximity enmeshed in a web of fibrous material (E).

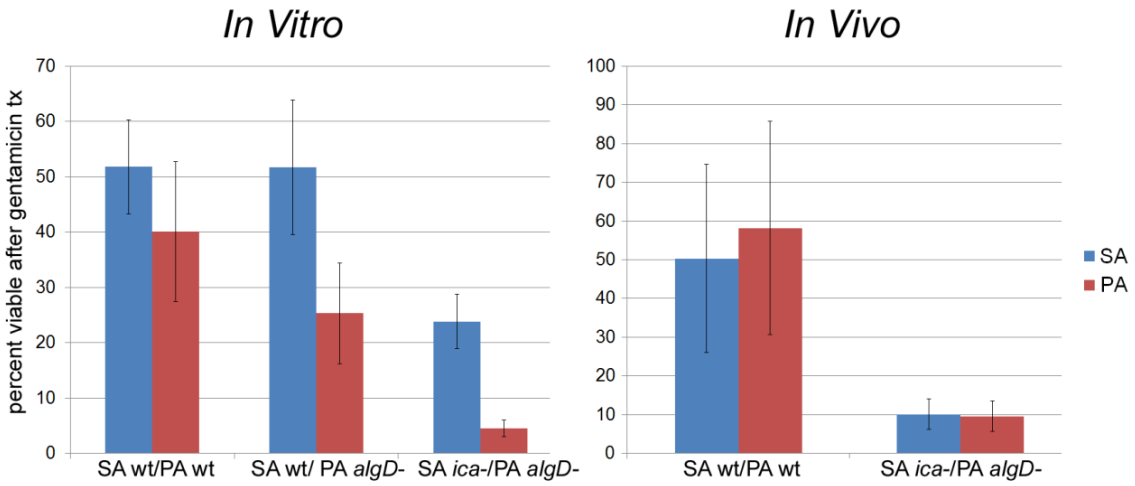


Figure 6. Mutations in SA and PA genes involved in biofilm matrix formation affect gentamicin *tolerance in vitro* and *in vivo*. Cocultures were grown in the *in vitro* wound model for one day or in the chronic wounds of mice for 4 days and then the tolerance to gentamicin was determined. Cocultures made up of an SA strain with a deletion in the intercellular adhesion (*ica*) gene and a PA strain with a deletion in the alginate (*algD*) gene displayed reduced gentamicin tolerance in comparison to cocultures made up of wild-type (wt) Sa and PA.

Stephanie DeLeon

I am a fifth-year student majoring in Biology. I have been in the lab since the summer of 2011. My research in microbiology consists of interactions of dual-species in-vitro biofilms. I am likely to pursue a career in science and medicine, and eventually hope conduct research while practicing medicine. Other things that I am passionate about include traveling, medical missions, and volunteering.

PEDF Induces the Migration, Differentiation and Phagocytic Activity of Macrophages

Dalia Martinez-Marin
Mentor: Dr. Stephanie Filleur

Macrophages have been described as one of the main inflammatory components in tumor growth, including in prostate cancer. Pigment Epithelium-Derived Factor (PEDF) is a secreted glycoprotein, which blocks angiogenesis, promotes neuronal survival and differentiation, and was recently suggested as an immune-modulating factor. PEDF also acts as an inflammation-modulating factor in prostate cancer. Prior studies have shown that PEDF expression increased the recruitment of tumor-cytotoxic M1-type macrophages into orthotopic MatLyLu rat prostate tumors suggesting a new way through which PEDF curbs prostate cancer growth. The totality of these data reinforces the anti-tumor properties of the PEDF gene in human castration-refractory prostate cancer (CRPC). It also emphasizes the critical need to develop a specific and efficient delivery system for PEDF gene. The objective of the present study is to investigate PEDF gene therapy using bone marrow-derived macrophages (BMDMs) as a novel therapeutic modality for advanced CRPC, using immunohistochemistry and in-vitro migration assay. Our central hypothesis is that the expression of PEDF will induce the migration and differentiation of BMDMs into a tumor-cytotoxic phenotype and, as a corollary, will block tumor growth and metastases formation, and prolong survival. We have formulated this hypothesis on the basis of our preliminary data that showed that PEDF stimulates the migration of monocytes/macrophages in-vitro and our observation that PEDF expression levels correlate with macrophage density in human prostate specimens.

In addition, we are looking at the mechanisms at which PEDF induces the migration and differentiation of macrophages. To test our hypothesis the techniques we used two established cell lines, RAW 264.7 monocytes and THP-1 monocytes. We studied different markers for two (M1 and M2) macrophage phenotypes. M1 macrophages are tumor-cytotoxic, and tumor progression promotes a phenotype switch to M2 macrophages, which promote tumor growth, survival and metastasis. As explained earlier, PEDF expression has been shown to be associated with an increase in density of M1 macrophages. We evaluated the effect of PEDF expression on monocytes and macrophages by using different concentrations of PEDF (0nM; 0.5nM; 1nM; 5nM; 10nM). We studied the different concentrations by using Enzyme-linked immunosorbent assay (ELISA), western blotting, and real-time PCR (RT-PCR). In these techniques, we studied M1 specific markers iNOS, and M2 specific markers Arginase 1. We found that an increase in PEDF expression up-regulates the expression of iNOS and down-regulates the expression of Arginase 1.Which shows us that PEDF is associated with an increase of macrophages of an M1 phenotype. Our data of PEDF expression correlates to our previous findings that PEDF induces the migration of monocytes and macrophages in-vitro. As a result of their differentiation, we have found that PEDF stimulates the phagocytosis of tumor cells, which suggest another mechanism by which PCa growth is halted.

We are looking at PEDF mechanisms by investigating the expression levels of PEDF receptors (ATP5B, PNPLA2, and LRP6) in RAW 264.7 macrophages and BMDMs. So far we have found that the ATP5B and PNPLA2 are the two main receptors expressed in both cell types. While all three receptors in RAW 264.7 increased, only ATP5B and PNPLA2 receptors increased in BMDMs. This is being studied using the same techniques as the macrophage markers such as western blotting and real-time PCR. Using immunocytochemistry we were able to see an increase in expression of both ATP5B and PNPLA2 in cells treated with PEDF than those without PEDF.
We are also investigating the PEDF-derivative synthetic 18-mer peptide and its mechanism of action on macrophages. P18 has been shown to block endothelial cell chemotaxis and induces apoptosis in vitro,

and has been shown to be more effective than its parental 34-mer peptide in blocking growth and angiogenesis in prostate cancer. We were able to demonstrate using western blotting, RT-qPCR, as well as immunocytochemistry that macrophages treated with P18 in comparison to PEDF (parental 34-mer peptide), shows a higher efficacy of PEDF expression. Using confocal microscopy we were also able to demonstrate a much larger rate of phagocytosis of prostate cancer cells by macrophages than compared to PEDF.

Finally, we will assess PEDF gene therapy using bone marrow-derived macrophages (BMDMs) as a novel therapeutic modality for advanced PCa. This project may lead to development of improved therapeutic approaches to treat PCa. Our central hypothesis is that PEDF expression will induce the migration and differentiation of BMDMs into a tumor-cytotoxic phenotype and, as a corollary, will block tumor growth and metastases formation, and prolong survival. The results of our study are of importance as they suggest that macrophages may play a key role in PEDF anti-tumor effects. A better understanding of PEDF may help lead to the further development of PEDF based anticancer therapy or improvement of alternatives to chemotherapy for prostate cancer.

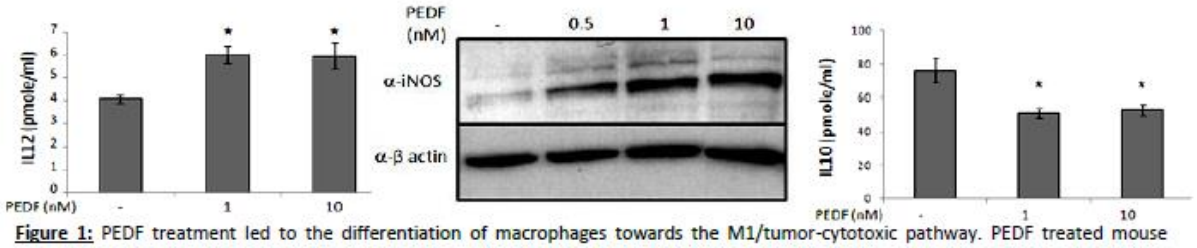


Figure 1: PEDF treatment led to the differentiation of macrophages towards the M1/tumor-cytotoxic pathway. PEDF treated mouse macrophages led to an increase in M1-type specific markers in RAW 264.7 IL12 (left) and iNOS (center), and led to a decrease in M2-type specific marker IL10 (right).

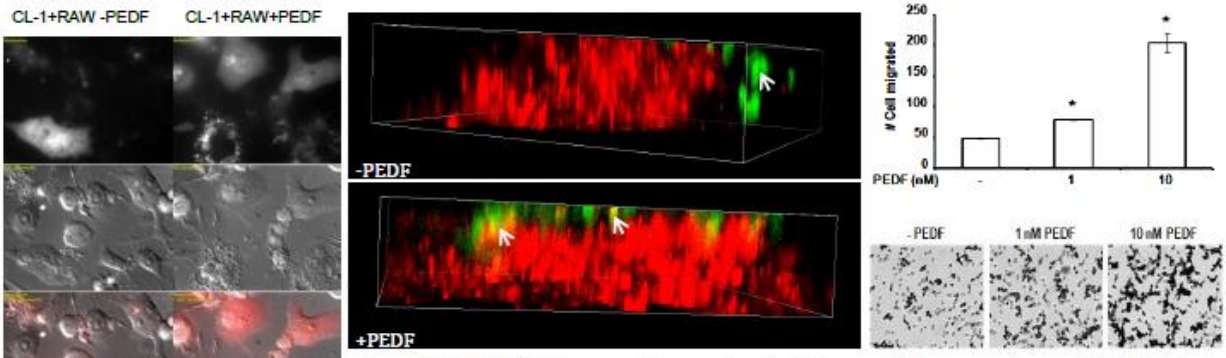
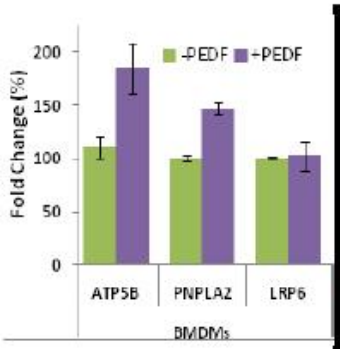
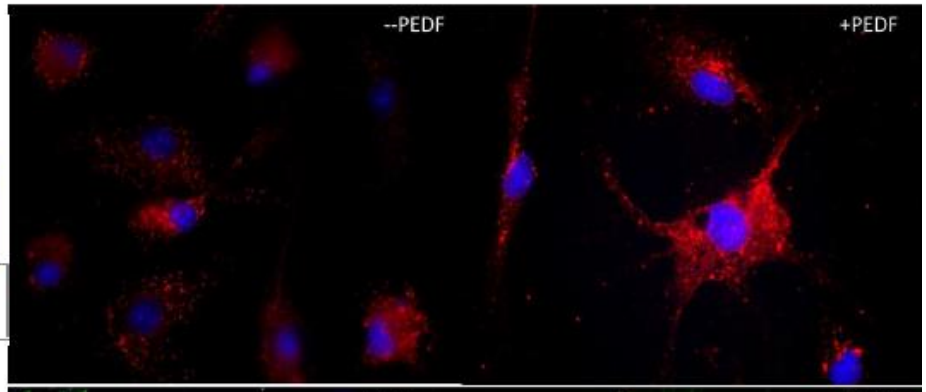


Figure 2: **(Left)** PEDF increases the engulfment of tumor cells by macrophages. **(Center)** Using confocal imaging we were able to determine that PEDF leads to migration of macrophages towards the CL-1 spheroid. **(Right)** PEDF induces the migration of macrophages in vitro.





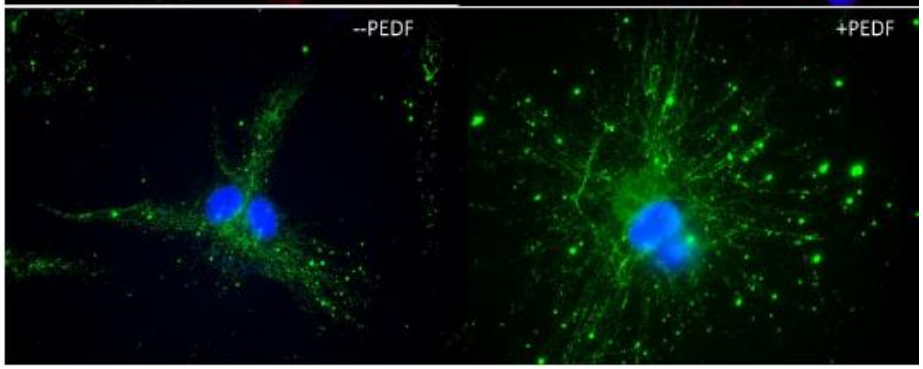


Figure 3: PEDF biological activities are thought to depend on its interaction with cell surface receptors. Expression of PEDF receptors in macrophages in the absence or presence of PEDF treatment was demonstrated via Immunocytochemistry and RT-qPCR.

Dalia Martinez-Marin

I am a senior getting a B.S in Cell and Molecular Biology. I started doing research the summer of my junior year in high school and have done research in four labs here at TTU-TTUHSC. I am very interested in medical research specifically in cancer research. After graduation I plan on pursuing a PhD and continue doing research in cancer. My other hobbies is playing in the TTU symphony orchestra and playing video games

Pseudomonas aeruginosa quorum sensing in diabetic and non-diabetic chronic wound infections

Angel R Cueva
Mentor: Dr. Kendra Rumbaugh

Pseudomonas aeruginosa is a gram negative bacterial species which is ubiquitous and a common environmental contaminant, and can be a transient colonizer of human skin. P. aeruginosa is associated with a number of acute and chronic infections, such as burn wounds, cystic fibrosis, pneumonia, and diabetic foot ulcers which contribute greatly to high rates of morbidity and mortality globally. The production of some virulence factors that contribute to the pathogenesis of *P. aeruginosa* is regulated by cell to cell signaling, or quorum sensing (QS). *P. aeruginosa* QS involves three distinct regulatory systems which alter gene expression in response to the amount of specific chemical signals, or auto inducers, within an environment. We have previously demonstrated that QS is essential to the pathogenesis of *P. aeruginosa* in burn wound infections; however, little has been elucidated about the role of QS in the chronic wound environment. Likewise, the efficacy of QS inhibiting compounds as effective treatments for chronic wound infections has not been investigated.

Our role is to investigate QS in the chronic wound environment. We utilize a chronic wound mouse model for in vivo experiments. To create the chronic wound, the mice are first anesthetized with an intraperitoneal injection of Nembutal, their backs are shaven, and the remaining hair is removed with Nair. The mice are then given a localized injection of Lidocaine, then a full-thickness surgical exiction of less than 2cm is given. A surgical op-site bandage is applied to the wound through which either *P. aeruginosa* wild-type strain PA01 or PA quorum sensing mutant JM2 is injected. The progression of the chronic wound is monitored as is the healing of the wound itself. Healing progression is determined by percent wound closure over time. At some time points mice were euthanized and the bacterial load in the wounds was determined by CFU determination.

Diabetes is induced in mice by using streptozotocin (STZ), a compound that has a preferential toxicity toward pancreatic β cells. The mice are injected intraperitoneally with a single dose corresponding to its body weight. After injection with STZ the mice are allowed five to seven days before being tested for diabetes. Blood is obtained from the tail and diabetic state is confirmed by glucose readings. Once diabetes is confirmed the mouse begins insulin treatment and allowed a few days to adjust before a chronic wound is performed.

Our most recent results show that the quorum sensing negative mutant JM2 has similar percent wound closure to the wild type PA01 as do non diabetic and diabetic experiments. This data could imply that quorum sensing may not play a significant role in chronic infections, unexpected based on our results of QS in burn wounds. There is unpublished data in our lab that shows blood proteins, present in chronic wounds, can alter and possibly suppress QS. In addition, there is evidence in the literature that QS inhibitors in vivo are not effective, despite high efficacy in vitro. This gives more evidence to the possible decreased role of QS in the chronic wound environment.

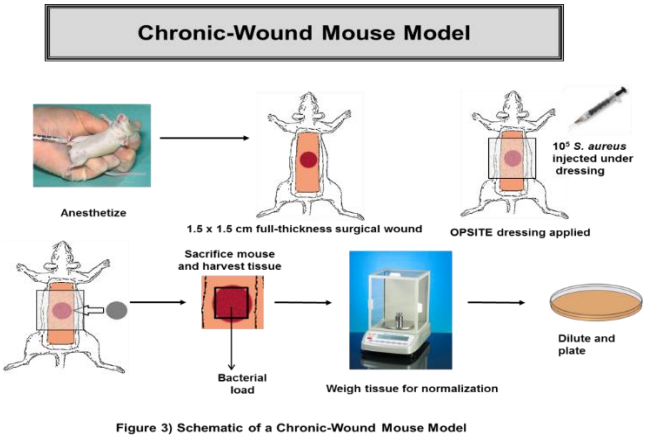
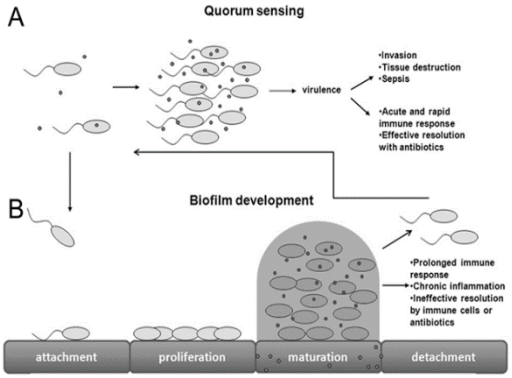
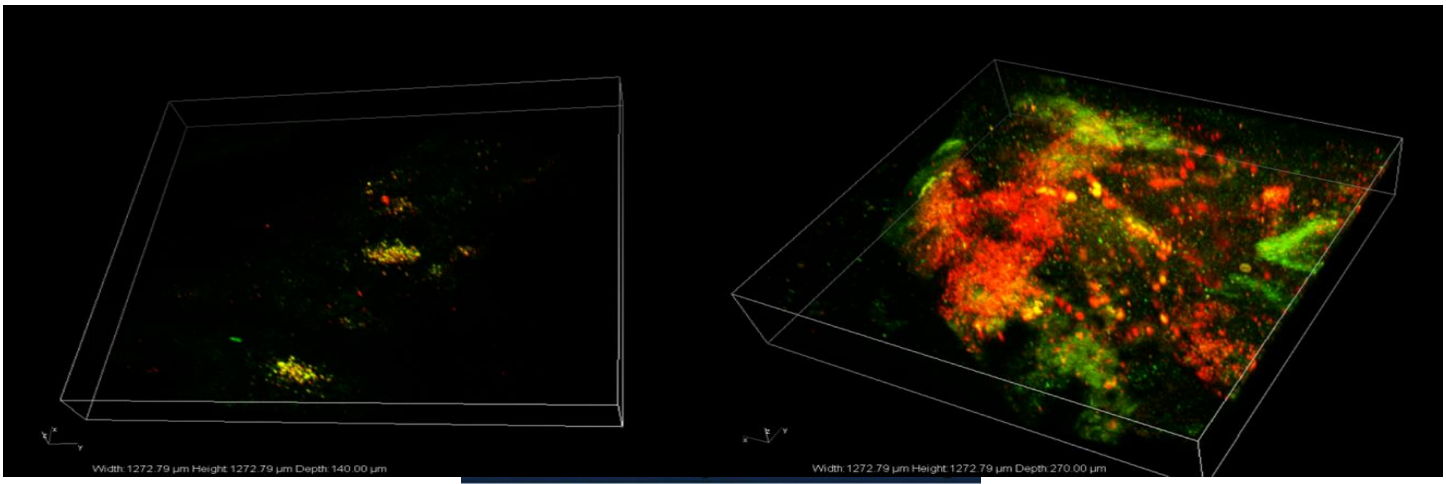
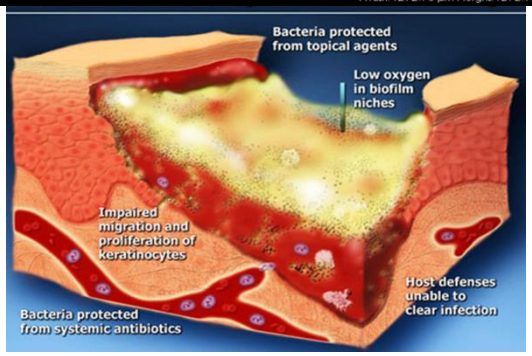
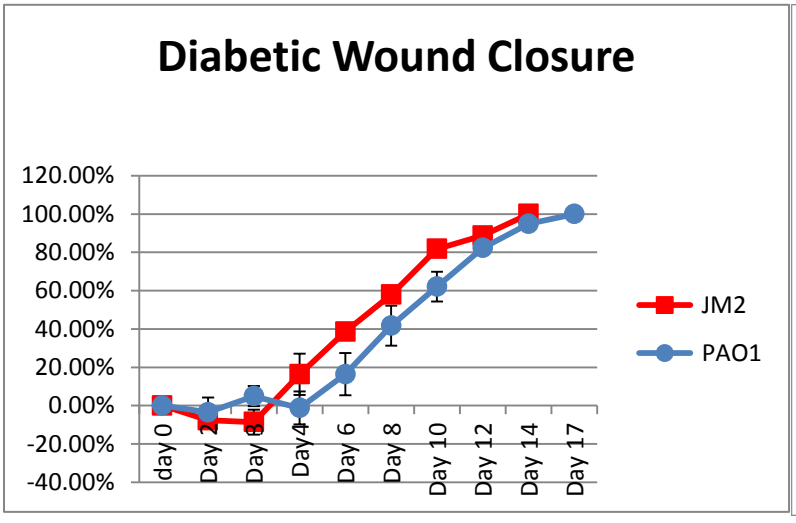


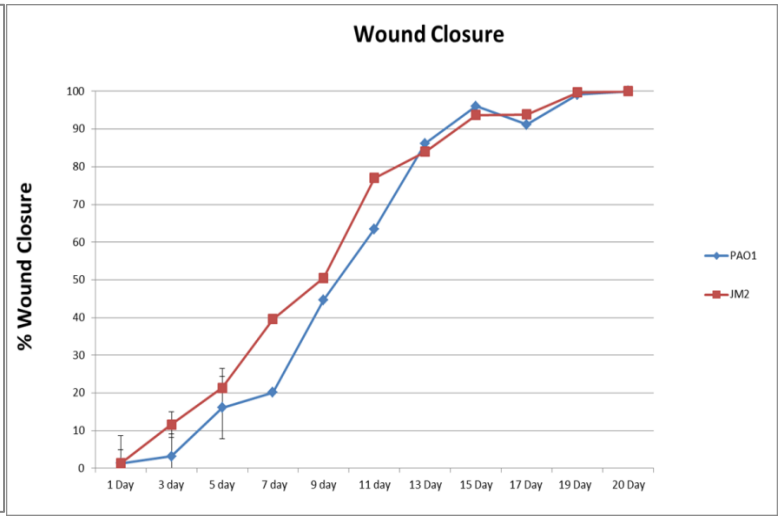
Figure 3) Schematic of a Chronic-Wound Mouse Model











Angel R. Cueva

I am a fourth-year student in microbiology. I've previously worked in Dr. Kai Zhang's lab and for the past year have been doing research in Dr. Kendra Rumbaugh's lab. I am pre-dental and my general interests are in pathology. I am likely to pursue a career in science, as a Doctor of Dental Surgery or a research scientist. My other interests are the outdoors and dancing.

Sertoli Cell Reinitiate Proliferation After Loss of Cell-Cell Contact

Rachel Dziuk
Mentor: Jannette Dufour

Sertoli cells (SCs), located in the seminiferous tubules of the testis, are immune privileged cells that survive and protect co-transplanted cells after allo- and xeno- transplantation. Recent studies have proposed utilizing genetically engineered SCs to express various therapeutic proteins for cell based gene therapy. Specifically, our lab is aiming to engineer SCs to secrete functional insulin to ameliorate diabetes, a disease that affects approximately 9.3% of the U.S. population.[1]

In a preliminary study, immature SCs were transduced with an adenoviral vector expressing human insulin and transplanted into diabetic animals. While long term lowering of blood glucose levels (BGL) was expected, the lowering of BGL was transient. The epichromosomal nature of the adenoviral vector or the proliferating nature of the immature SCs could have caused the loss of insulin expression in transplanted SCs without evidence of SC death. The current dogma is mature SCs are terminally differentiated and do not proliferate, so transplantations were repeated using mature transduced SCs. Surprisingly, transplantation of mature SCs also resulted in transient lowering of BGL and loss of insulin expression. We then tested whether mature SCs could resume proliferation after transplantation. SCs from mature Lewis rats were transplanted under the kidney capsule of NSG mice or syngeneic rats. Animals were injected daily with 5-bromodeoxyuridine (BrdU), and their graft bearing kidneys were removed at day 10, paraffin embedded, and immunostained for SC marker, Wilms' Tumor (WT) 1, and cell proliferation marker, BrdU. The mature SCs reinitiated proliferation after transplantation.[2] Quantification of BrdU labeled SCs revealed lower proliferation rates in SCs arranged in tubule-like structures compared to the SCs arranged randomly throughout the graft. We questioned if transplanted SCs in tubule structures proliferated less by forming cell-cell contact through tight junction interactions. We hypothesized SCs expressing tight junction proteins after transplantation have lower proliferation rates compared to SCs that do not express tight junction proteins.

Using the same animal model as before, the graft bearing kidneys were immunostained for SC marker, WT1, and tight junction protein marker, Claudin-11. Analysis of both mice and rat grafts indicated the grafts with defined tubule structures had high SCs expression of Claudin-11, with similar localization to the normal testis. This correlated with overall lower SC proliferation rates. Grafts with undefined tubule structures showed lower SCs expression of Claudin-11, which similarly correlated with overall higher SC proliferation rates. Statistical analysis showed this significant difference of Claudin-11 expression by SCs within and outside of tubules in both mice and rat grafts. Our results suggest adjacent SCs arranged in tubule structures express Claudin-11 tight junction proteins, similar to their arrangement that forms the blood testis barrier (BTB) in the normal testis environment. Our results also suggest the loss of cell-cell contact between mature SCs reinitiates proliferation until tight junctions form, indicating that SCs can be used safely in gene therapy. From these data, future studies are planned to test the phenotypic state of the proliferating SCs and determine if the observed tight junctions are functional.

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[1] According to statistics from 2012. *Statistics About Diabetes.* Retrieved from www.diabetes.org.

[2] Mital, P., Kaur, G., Bowlin, B., Paniagua, N.J., Korbutt, G.S., and Dufour, J.M. (2014) Nondividing, Postpubertal Rat Sertoli Cells Resumed Proliferation after Transplantation. *Biology of Reproduction*, 90(1):13, 1-10. Doi:10.1095/biolreprod.113.110197

Lewis rats

NSG mice

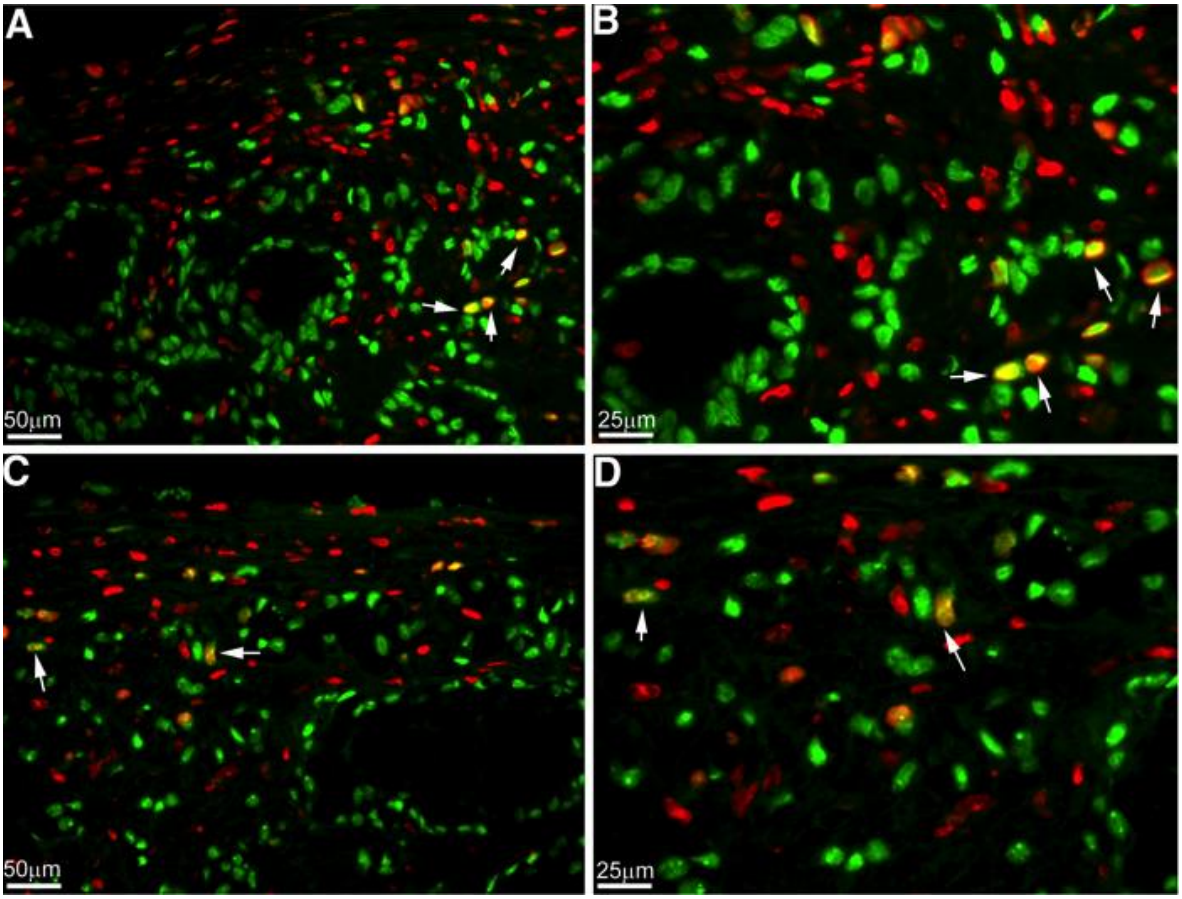

Figure 1: Preliminary data: mature SCs Proliferate. Cells are fluorescently labeled through immunofluorescence staining. Positive SCs are labeled green by Wilms' Tumor (WT)1, positive proliferating cells are labeled red by Bromodeoxyuridine (BrdU), and proliferating SCs are double-positive and are observed as yellow.[2]

Table 1: Preliminary data of proliferating SCs outside of tubules compared to proliferating SCs inside of tubules. *p≤0.05 compared to outside of tubules.[2]

Recipients	% of BrdU+ SCs outside of tubules	% of BrdU+ SCs within tubules	Total % of BrdU+ SCs
Mice (n=4)	9 (4-21)	2* (0.8-6)	11 (5-27)
Rats (n=4)	11 (4-34)	6 (2-15)	17 (6-49)

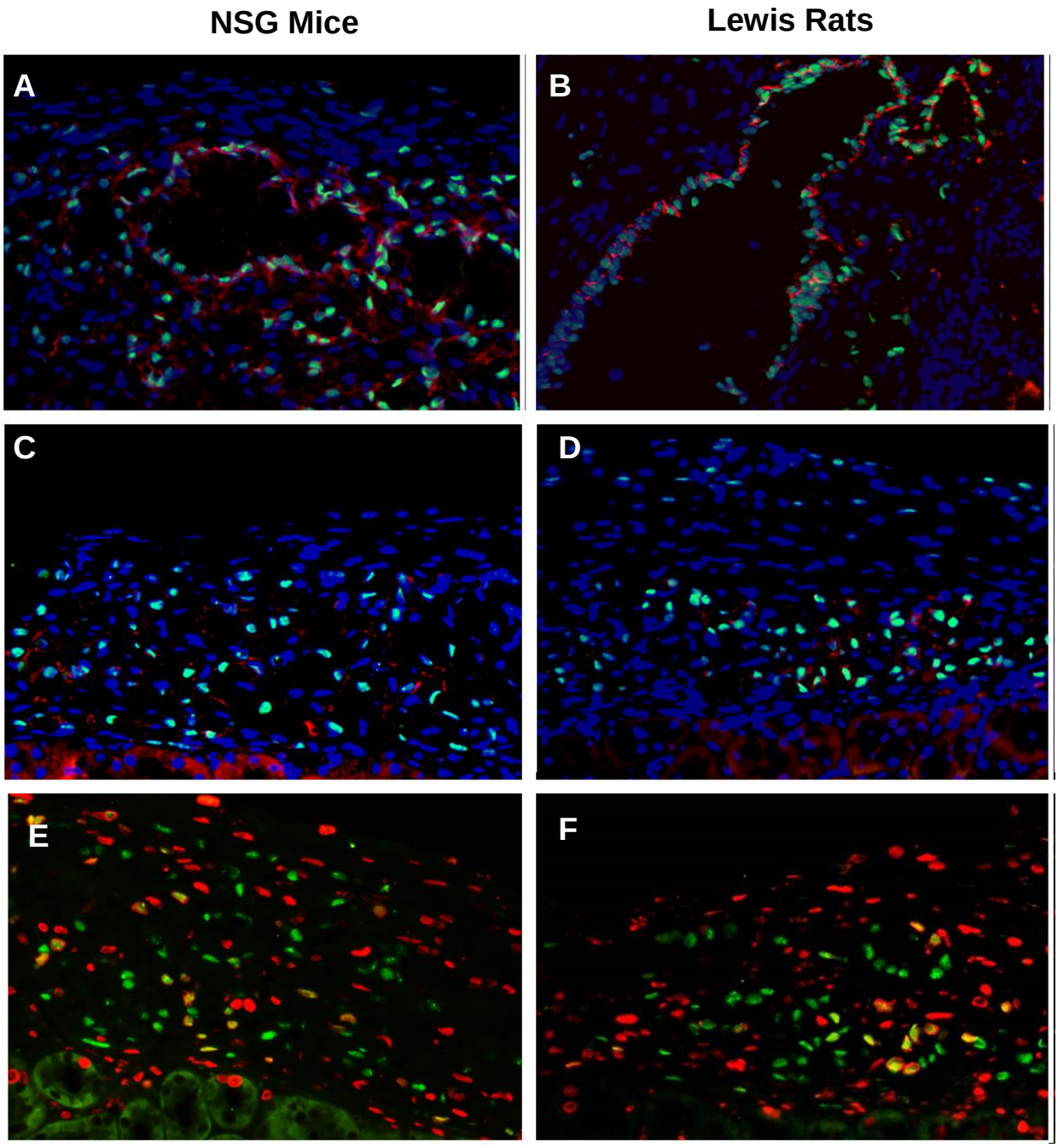

Figure 2: Immunofluorescence staining results of mature SC in syngeneic Lewis rats and NSG mice grafts at day 10. Colored labeling is as followed: Grafts 1-D: Coral/pink: CLaudin-11 tight junction protein positive, Aqua blue: WT1 positive SCs, deep blue: DAPI cell staining as control. Grafts E and F: Red: BrdU proliferation marker, Green: WT1 positive SCs, yellow: double positive proliferating SCs. Grafts A and B showed high Clauidn-11 expression in the tubule formations. Grafts C and D paired with their respective proliferation grafts E and F showed very little tubule formation, decreased Claudin-11 expression by SCs, and a higher percentage of proliferating SCs.

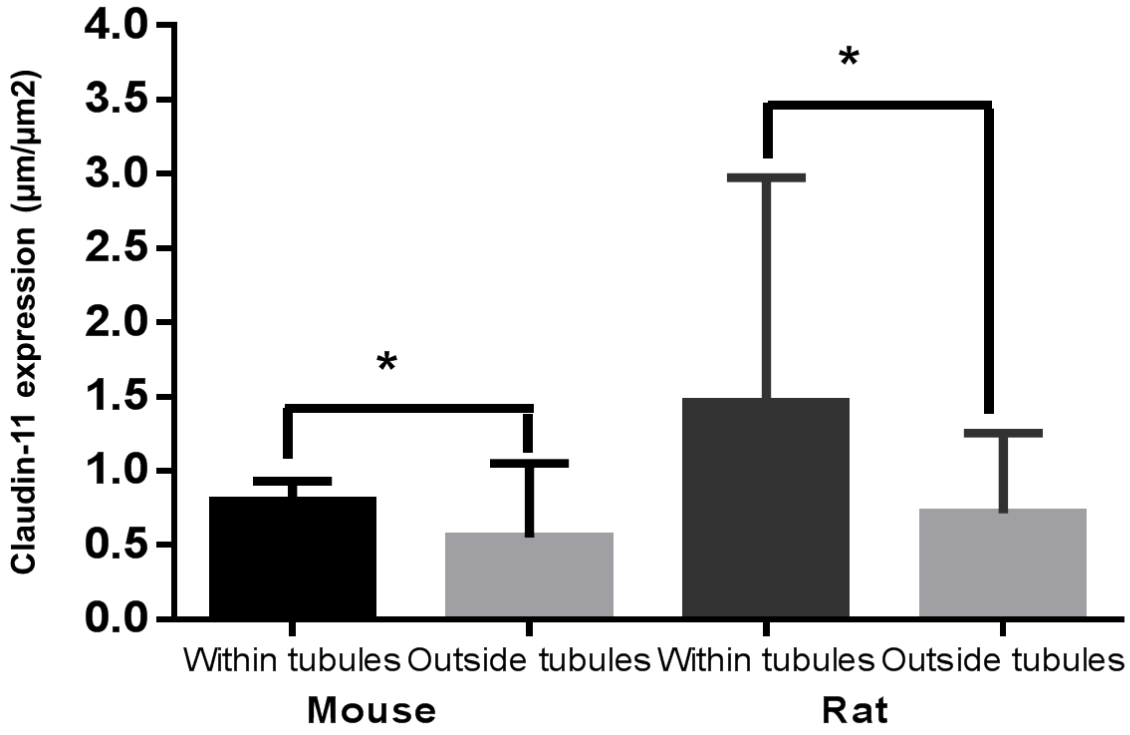

Table 2: SCs arranged in Tubules Express High Amounts of Claudin-11 tight junction protein. Statistical analysis using student's unpaired t-test, *p≤0.05.

Rachel Dziuk

I am a third-year cell and molecular biology student. After completing my first year at Texas Tech, I entered the Dufour lab and discovered my love for research! My research projects investigate the mechanisms behind Sertoli cell immune-privilege, and their potential uses in transplantation and gene therapy. I find joy and excitement in learning, and hope to obtain my Ph.D. in immunology or cell and molecular biology. Outside of the lab, I enjoy the outdoors, weight-training, cooking, and piano playing.

Listening to the Blink of an Eye: Testing that Sonification of EEGs can Lead to the Recognition and Classification of Types of Brain Activity

Kate Ehnis and Stacy Philip
with Fredrick Ramirez, Renato Gonik, andCarl Seaquist

Electroencephalograms (EEGs) monitor brain activity, comparing differences in surface voltages. The analysis and display of EEGs for visual analysis is ubiquitous. When one can understand a normal EEG, then identifying abnormalities and better understanding how the brain works follows.

Recent work indicates that auditory analysis may also be useful in detecting irregularities in EEGs. Because brain waves consist of frequencies inaudible to the human ear, it is necessary to use digital signal processing techniques to convert the signals to sound without losing valuable information. The analysis of EEG signals will help in the detection of abnormal EEG signals to better understand the human brain.

Ten patient Electroencephalogram (EEG) recordings were selected from a study conducted at a Lubbock hospital. These recordings include 2 normal and 8 abnormal EEGs, which were stripped of personal identifying information. Recent publications indicate that sonification (converting data to sound) allows the human ear to analyze series data and detect irregularities that might otherwise go unnoticed. Since brain rhythms are typically lower than the human hearing range, signal-processing techniques, including but not limited to modulation, Fourier transforms, wavelet analysis, and digital filtering, will be applied to convert EEGs to sound. Our objective is to demonstrate that in addition to traditional visual analysis, auditory acuity may be useful in the analysis of EEGs and aid in the early detection of abnormal EEG activity. The project will be a success if an algorithmic approach to sonification leads to the identification of important features of the EEGs by listening to the transformed signals.

An EEG from patient one, a normal patient, was analyzed in order to find a blink artifact, which occurs at about 80 seconds. The window observed was seventeen seconds and includes the blink artifact followed by alpha rhythms.

Using EDF browser electrodes from the frontal, occipital and parietal regions were selected. These raw signals were read into MATLAB, combined using the Double Banana montage, and then a Butterworth filter was applied to remove noise.

The signals were then resampled and used to modulate others in the auditory range; namely, A3, E6, and C6 creating a chord. A sound file was created that was read into a digital audio workstation for further analysis and playback.

Although the blinking of an eye is visually an easily detectable artifact in an EEG, it is now possible to detect blinks and to determine whether the eye is open or closed simply by listening to the appropriate EEG channels.

As this research progresses, this study hopes to go beyond listening to and assessing blinking towards the analysis of other situations; for example, the detection of various types of seizures. Hopefully, this insight about brain signals will be helpful in the early detection of abnormalities. By sonifying EEG signals it appears that new insight into EEG analysis is possible.

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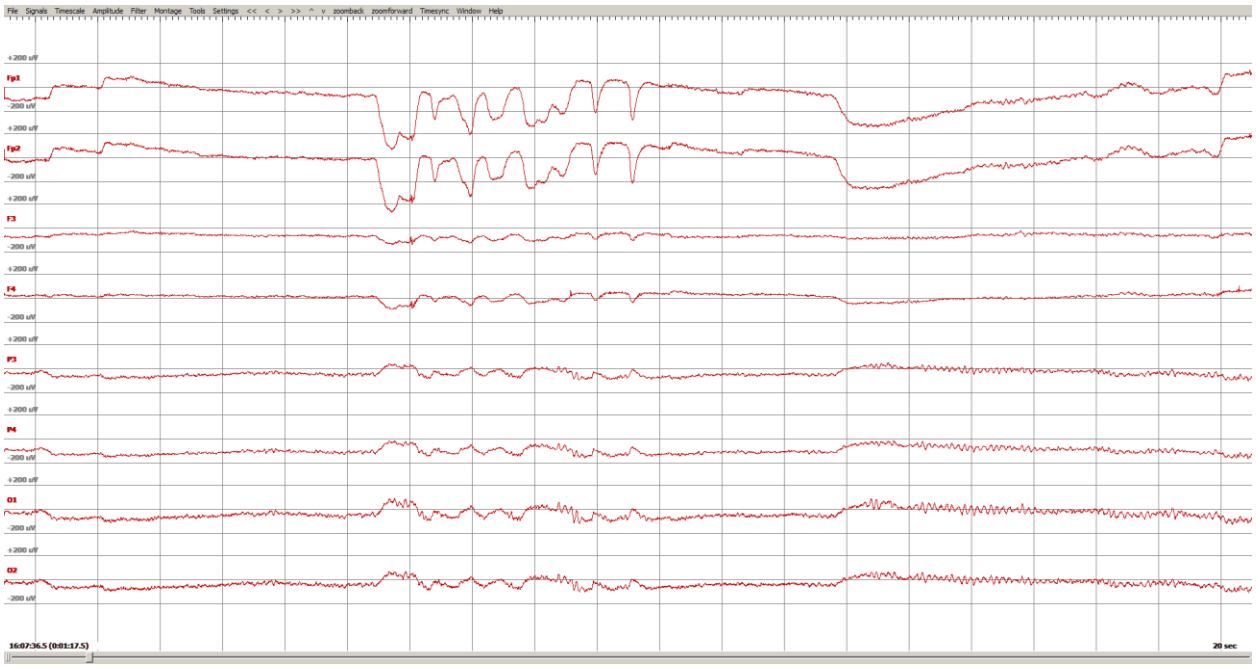
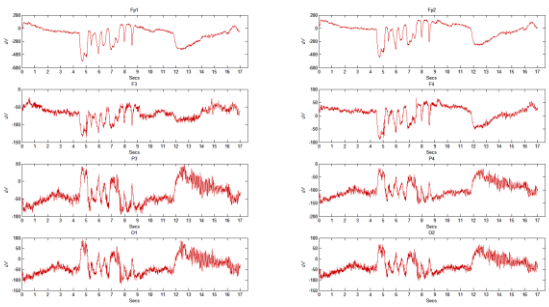
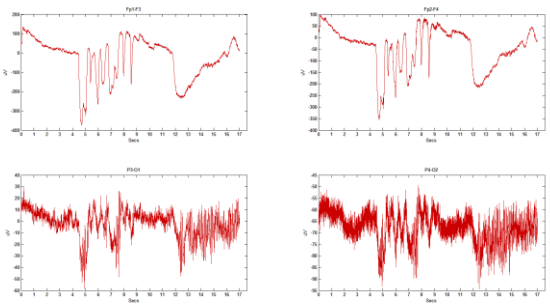


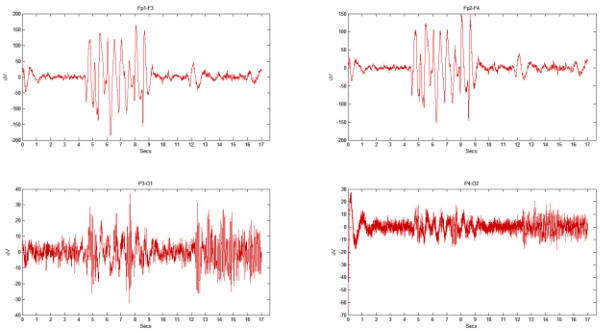
Raw Data Using a Referential Montage in EDFbrowser



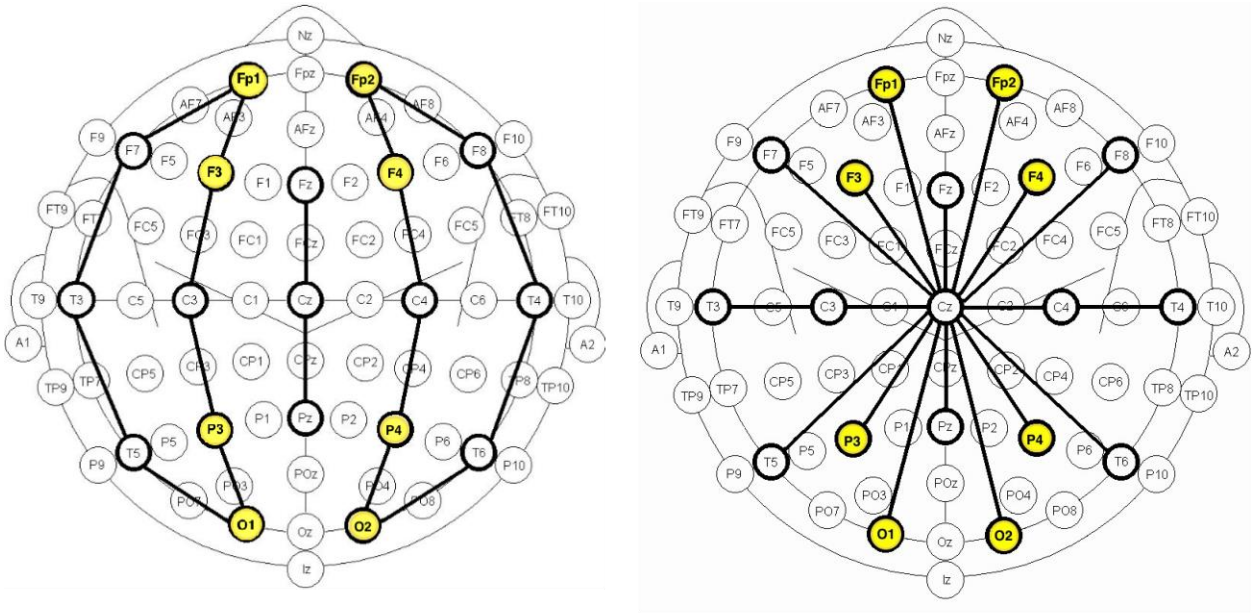
Raw Data Using a Referential Montage in MATLAB



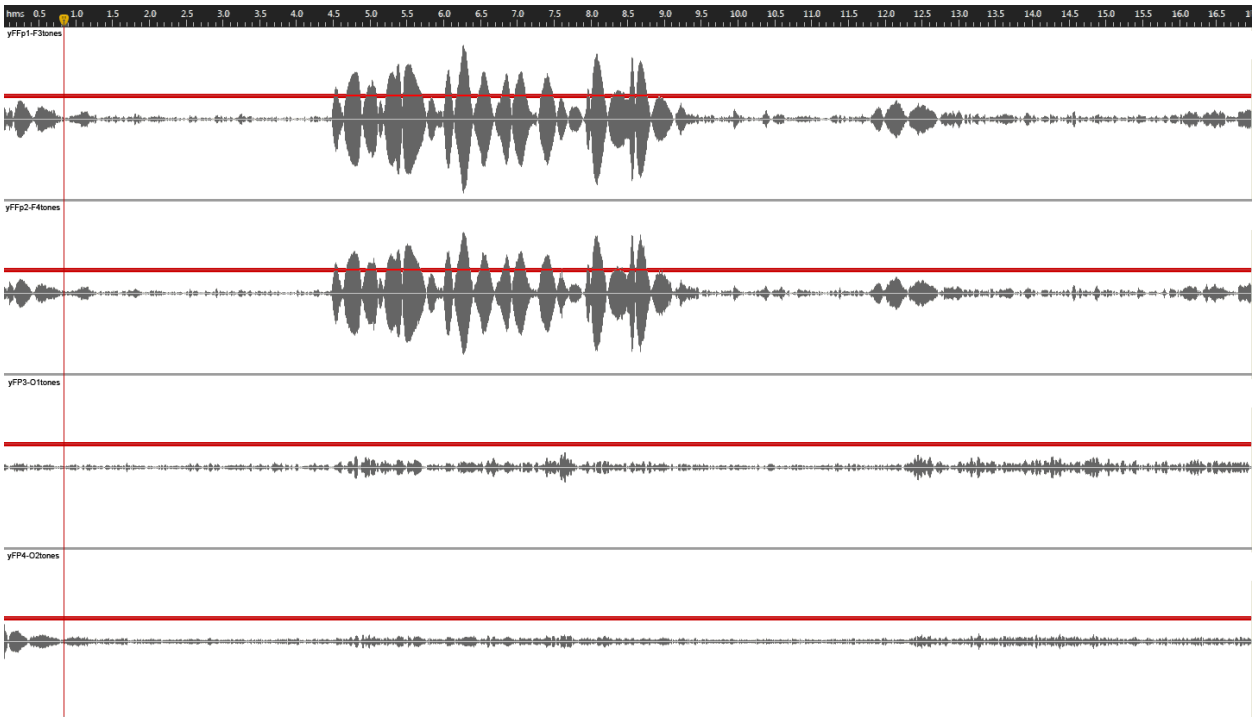
Raw Data Using a Bipolar Montage in MATLAB



Butterworth Filter Applied in MATLAB



Bipolar montage (left) and Referential montage (right) with primary electrodes highlighted that were used to create sound file



Blink of an Eye Converted to A-E-C Chord Using Adobe Audition Recording Software

Kate Ehnis

I am a third year mathematics major at Texas Tech. I have been working on this research with Dr. Seaquist and Stacy Philip for a little over a year and we enjoy presenting it at conferences around the United States. I am currently finishing my BS and beginning my MA in mathematics. When not studying, I enjoy spending time with family and friends, traveling, and shopping.

Stacy Philip

I am a 3rd year graduating senior Biology student. I have been working on a research project with my research partner, Kate Ehnis, and faculty mentor, Dr. Carl Seaquist, in the Mathematics and Statistics Department since the spring of 2013. After I graduate this year, I hope to earn my MBA and then attend medical school the year after, in order to fulfill my aspiration to be a Missionary Surgeon. I also enjoy volunteering, singing, and playing the violin.

The Effect of Human Leptin on food intake in the African clawed frog Xenopus Laevis

Carlos Jesus García
Mentor: Dr. James A. Carr

Currently, in the United States, 63.1% of Americans are considered obese and/or diabetic. A main contributor to obesity and diabetes is over eating. Leptin, a hormone produced in fat cells, serves as a regulator in body weight. Leptin is a product of the obese (ob) gene which is synthesized by adipocytes. Adipocytes being signals to the brain to represent that there is still energy reserves which in case effect development, growth, metabolism and reproduction. Leptin in most mammals functions as an adiposity signal. Adiposity signal meaning the circulation of leptin throughout the body, and fluctuates in equal to fat mass, and act on the hypothalamus to suppress food intake. The human appetite is suppressed by brain signals caused by leptin, in turn burn more calories. Leptin was originally discovered by Friedman and collaborators in 1994.

At present little is known about the evolution of leptin signaling in vertebrates. While leptin genes have been cloned in fish and amphibians, it is unclear whether heterologous leptin peptides are functional across species. Our current study is to determine whether human recombinant leptin (hrLeptin) will have an effect in food intake in the amphibian species, the South African clawed frog *Xenopus laevis*. Despite having very little similarity based upon primary amino acid sequences, vertebrate leptins are surprisingly conserved based upon tertiary structure. Thus, we hypothesized that hrLeptin will be effective in suppressing food intake in an amphibian species.

Reverse Transcriptase PCR was an instrument that we utilized in this experiment. We were trying to examine the expression of the Leptin gene in different regions of the brain including the optic tectum, telencephalon, and hypothalamus of the *Xenopus laevis*. Normally, in humans the production of Leptin is common in these regions of the brain. Using this method we were able to show that there was significant expression of leptin production in the brain of the *Xenopus laevis* demonstrating that this species is an exemplary model for our experiments.

In our initial experiments the juvenile frogs were injected via the dorsal lymph sac with one of five doses (0, 0.02, 0.2, 2, 20 ug) of leptin and food intake was assessed. The frogs were given peripheral injections of hrLeptin, and after 90 min they were given 300 mg or 1.2 mg of beef liver. After 30 min, any uneaten liver was removed and weighed. Food intake was measured by the remaining mass of food subtracted from the original food mass, divided by the body weight of frog. We concluded that hrLeptin had no effect on food intake in *Xenopus laevis* after peripheral administration in the two independent trials. As a supplementary experiment, hrLeptin was directly injected to the roof of the large midbrain structure in order to determine if injection of hrLeptin will yield different results when compared to the peripheral injections. We administered 1µg of hrLeptin via intracerbroventricular (i.c.v) injection, and repeated the same food intake assessment used in the previous experiment. The i.c.v injection had no effect on food intake in the juvenile frogs.

Through the food assays we concluded that Human recombinant leptin had no effect on food intake in an amphibian after peripheral or i.c.v injection. According to the findings we suggest that there are differences in the interaction between human leptin and leptin receptors in other tetra pod species. Future work will examine other satiety neuropeptides to investigate their effects on subconscious sensory pathways detecting food in amphibians, as well as synthesize leptin extracted from *Xenopus laevis* and re-inject the frogs with different concentrations of the protein, to determine its effects compared to human leptin.

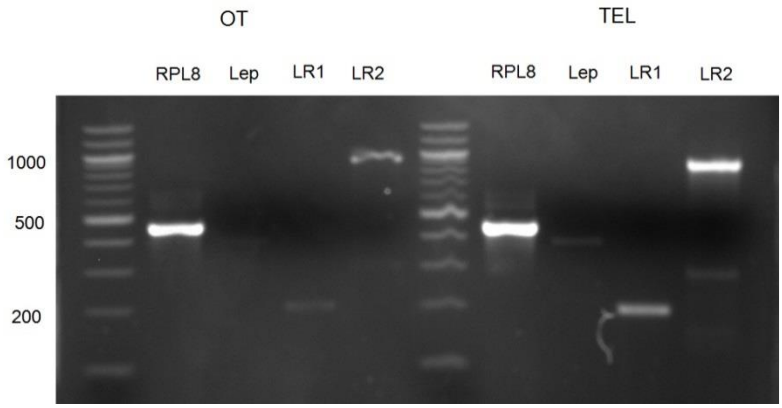


Figure 1: Using Reverse Transcriptase PCR expression of Leptin was found in the RPL8, Lep, LR1 and LR2 in the Optic tectum and Telencephalon.

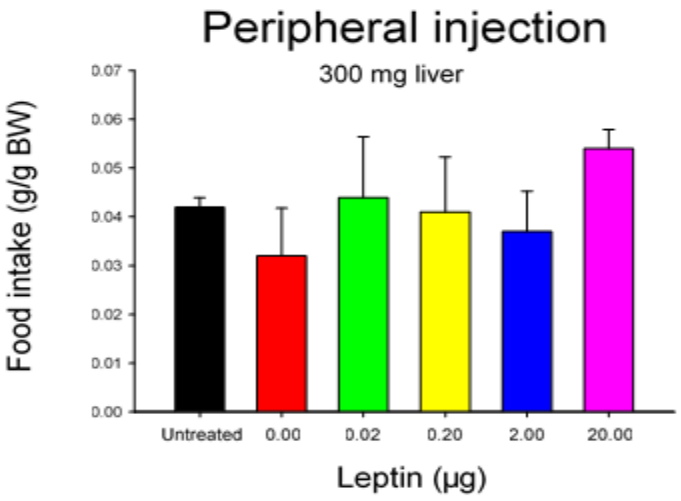


Figure 2: Effect of leptin peripheral injections on Food intake of juvenile Xenopus laevis. Various concentrations of leptin were used from 0 µg up to 20 µg, and where fed 300 mg of liver

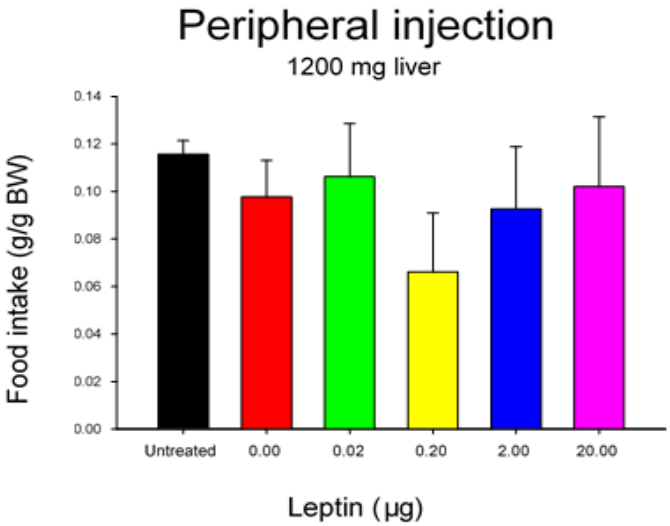


Figure 3: Effect of leptin peripheral injections on Food intake of juvenile Xenopus laevis. Various concentrations of leptin were used from 0 µg up to 20 µg, and where fed 1200 mg of liver

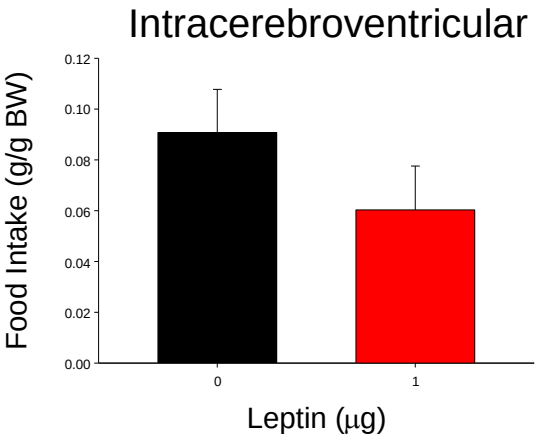


Figure 4: Effects of i.c.v injection of hrLeptin on meal size in juvenile *Xenopus laevis*. Using 1µg of leptin and were fed 1200 mg of liver.

Carlos Jesus García

I am a senior wildlife biology major and will be graduating in the spring 2014. I am from Oceanside, California. I have been in undergraduate research since I was at South Plains College. I have had the pleasure to work with Dr. James Carr, and now I am transitioning into a different lab in the Natural Resource Management department with Dr. Richard Stevens. My plans after graduation are to apply to grad schools and obtain my PhD. My ideal job would be working overseas in either India or Thailand to work at one of their conservations for tigers. In my free time, I enjoy longboarding, reading, and learning how to play the violin.

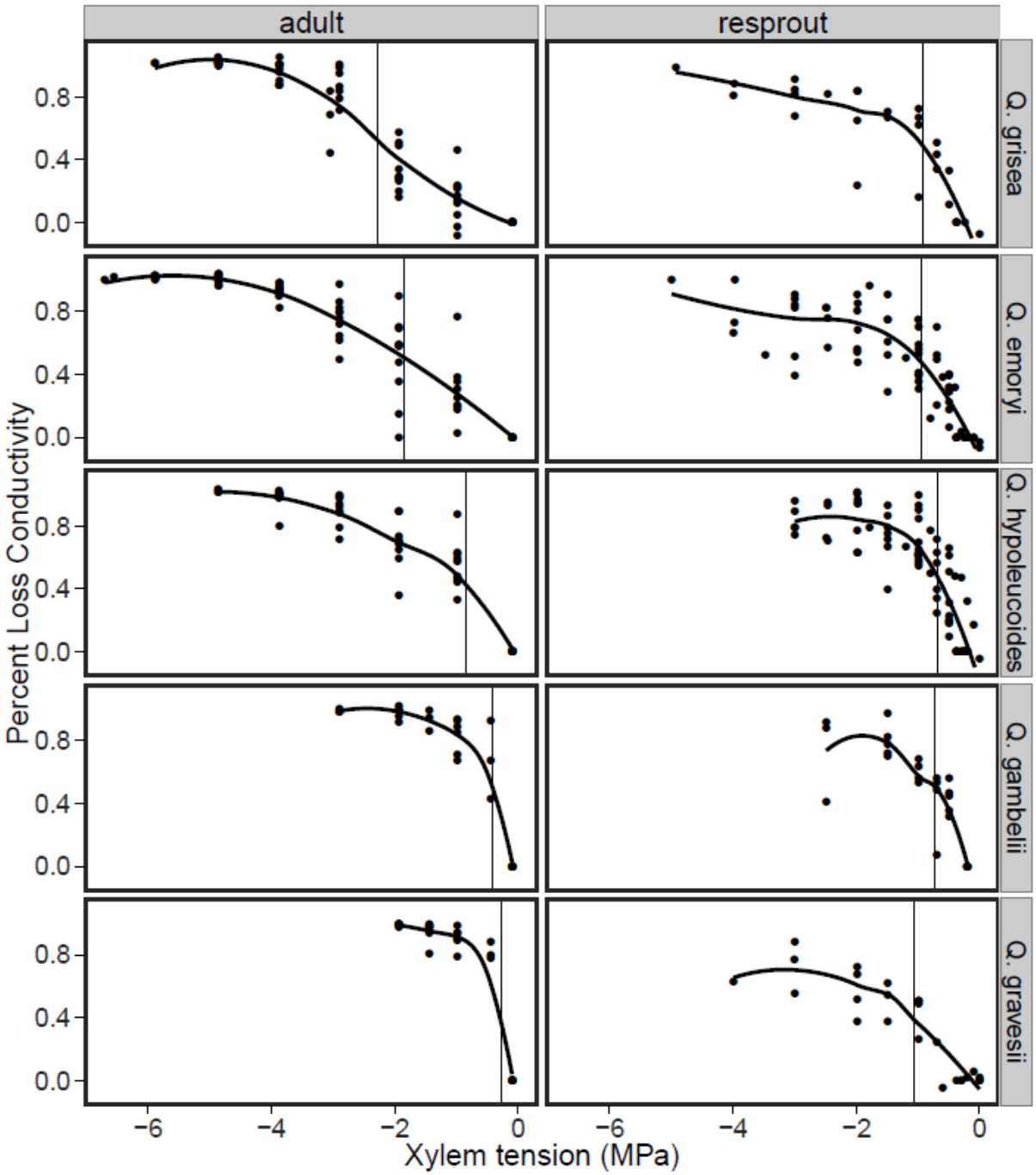
Drought Tolerance in Adult versus Post-Fire Resprout Oaks in a Chihuahuan Desert Sky Island

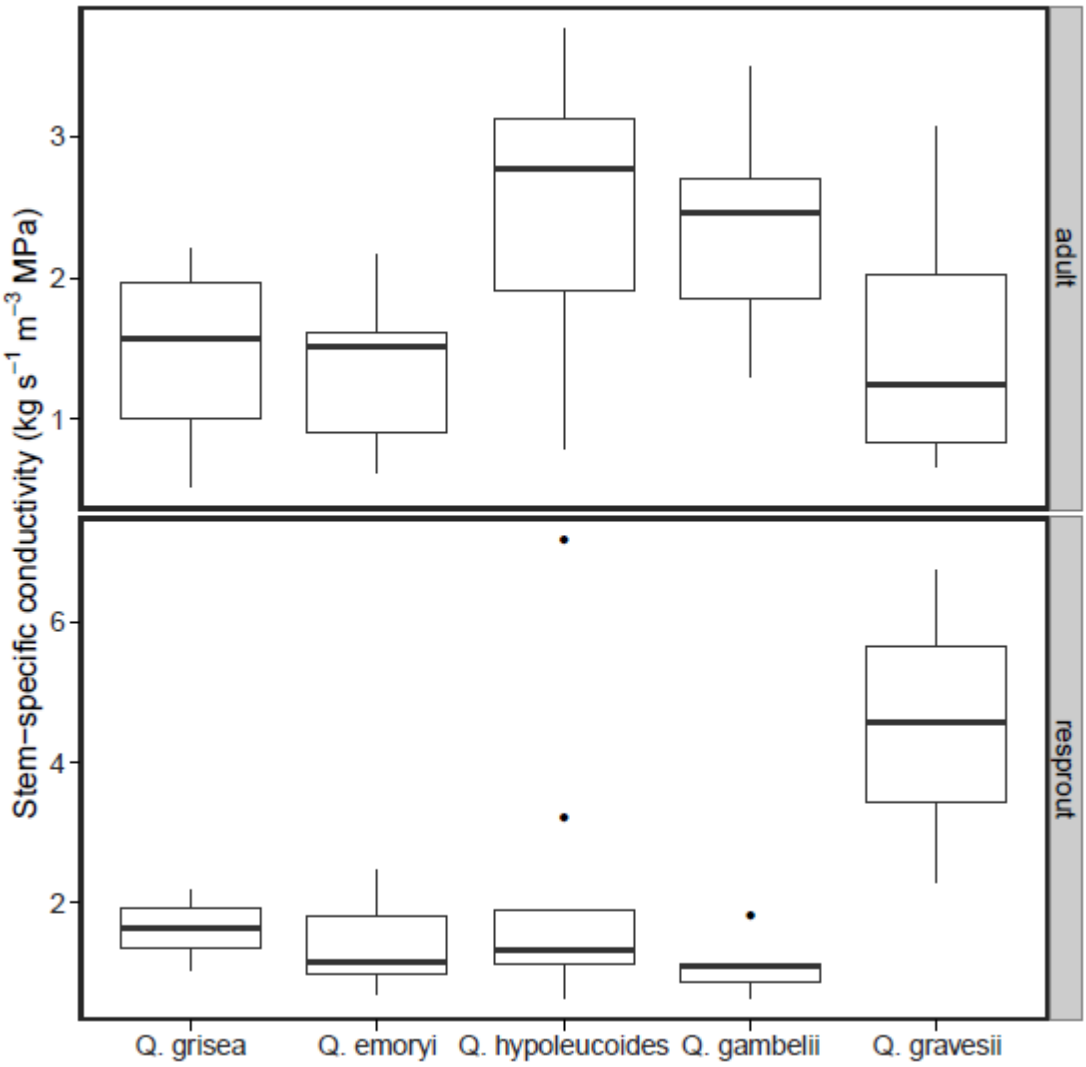
Tailor Brown
Ecology
Mentor: Dr. Dylan Schwilk

Water availability is a deciding factor of tree distribution. Past work has demonstrated that oaks inhabiting "sky island" forests of the northern Sierra Madre Oriental have differing susceptibilities to drought-induced xylem failure (cavitation). Embolism occurs in xylem due to increasing tension from inadequate water availability. This results in death of the stem, and ultimately the plant. These oak species, are all post-fire resprouters: they can basally resprout from underground storage organs when fire kills above ground tissue. Post-fire resprouts should have increased root:shoot ratios relative to adults and therefore have access to increased water relative to leaf demand. We expected that if resprouts exhibit plasticity, they should favor water transport efficiency over safety, show higher maximum xylem conductances, but greater susceptibility to drought-induced cavitation indicated by higher PLC50 values (the water potential at which conductance is reduced to 50 of maximum). We examined five species of oaks common in the Davis Mountains in west Texas. We measured the xylem hydraulic conductivity of adult trees and first-year post-fire resprouts before and after spinning stems in a centrifuge to generate negative xylem pressure, mimicking drought. PLC curves, stem specific conductivity, and leaf specific conductivity were calculated for adults and resprouts.

We found support for our prediction that resprouts would be more vulnerable to drought-induced cavitation: The two species with the lowest P_{50} values for adults had resprouts with significantly more vulnerable xylem. The species for which adults were more vulnerable, however showed little difference by life stage.

Contrary to our prediction, we found no evidence for an efficiency-vulnerability trade-off. Stem specific conductivity was not greater in the more vulnerable species nor was it greater in resprouts than in adults. We have not yet investigated true sapwood-specific conductivity values and pith may make up a proportionally greater area of the stem for resprouts than for adults. Leaf area for adults may be lower than normal values because 2011 was a drought year, and many species dropped leaves.


adult
resprout
Q. grisea
Q. emoryi
Q. hypoleucoides
Q. gambelii
Q. gravesii
Percent Loss Conductivity
0.0
0.4
0.8
−6
−4
−2
0
Xylem tension (MPa)



Tailor Brown

I have thankfully completed three years at Texas Tech, and going on the fourth. I am majoring in Biology and minoring in Chemistry. When I am not working, schooling, or researching I enjoy gardening, writing, cooking for friends, and admiring art. I have absolutely no idea what I will do after graduation, but God willing I will spend some time traveling.

Economic Sustainability and Global Climate Change

Alexander Norton
Mentor: Dr. Mark McGinley
Texas Tech University - Honors College

Human emissions of greenhouse gases are believed to have immense and devastating impacts on the Earth's ecology through climate change. Some of these ecological impacts include increased global average surface temperature, increased sea levels, ocean acidification, increased drought, and increased severity and frequency of extreme weather events. Naturally, any impacts that climate change may have on the earth's ecology may also impact the global and national economies of its inhabitants. Crude estimates of the economic damage caused by global climate change range from billions of dollars per year to 5% of the global gross domestic product (GDP). As scientists begin to better understand climate change, it is becoming increasingly important to understand how various ecological services will be affected, how much the economy might be damaged, and to try and create and implement incentive-based economic public policy to slow down climate change's devastating impacts.

I hypothesize that anthropogenic greenhouse gas emissions can be decreased and the economic impacts of climate change can be reduced with the economic cooperation of large energy companies and their governments through financial incentives that have mutual long-term benefits for both the ecology and economy of national and international areas. During the course of my research and the writing of my thesis, I would like to answer to answer the following questions relating to ecological services: 1) What is an ecological service? 2) How can we measure the economic value of ecological services? 3) What are some "success stories" and failures of using the concept of ecological services to try and help create policy that positively affects both the long-term ecology and economy of local, national, or international areas?

I would also like to answer some questions more directly related to understanding climate change from an ecological economic perspective: 1) Is climate change being accelerated by the anthropogenic emissions of greenhouse gases? 2) What is the estimated economic damage that climate change's implications could cost our species by the year 2100? 3) Have any global or domestic public policies been effective in sufficiently decreasing greenhouse gas emissions? 4) What incentive-based public policies might help slow down the rate at which climate change is occurring without significantly disrupting local economies? 5) If nothing significant is done to reduce global greenhouse gas emissions, what is the future global economic outlook given current and predicted levels of climate change commitment?

My methodology for this research will primarily be an extensive review of the literature. I will review literature that describes the concepts of ecological services, ecological economics, the atmospheric science of climate change, and the economic aspects of climate change. I will also read and review databases and sources that cover past public policies regarding climate change and economic incentive-based policy relating to ecological issues. I intend to interview Dr. Kathryn Hayhoe of the IPCC and Robert Costanza, a leading ecological economic scientist, in order to gain a more complete and comprehensive understanding of the subject and my research-related questions. I also intend to interview a couple of other Texas Tech University faculty that are familiar with ecological economic policy. This is the appropriate methodology for my research because I need to have an adequate understanding of ecological services, ecological economics, climate change, and past economic ecological public policy relating to climate change.

I hypothesize that the global climate change due to the anthropogenic emission of greenhouse gases has an immense impact on the earth's economy. I expect that some of the implications of climate change, such as rising average global surface temperature, rising sea levels, and more extreme weather patterns, account for billions of dollars of losses to the global economy every fiscal year. I hope to research and create hypothetical government-funded economic incentives to reduce greenhouse gas emissions that could benefit the world's long-term economic outlook.

Alexander Norton

I am a senior biology student from Spring, Texas, and I am writing an Honors Thesis through the Texas Tech Honors College under Dr. Mark Mcginley and Dr. Keira Williams. My general academic interests include political science, chemistry, medicine and evolution. I am currently applying to medical school, and after I graduate from Texas Tech University, I plan to pursue a career in medicine and journalism. My other interests include writing, reading, and sports.

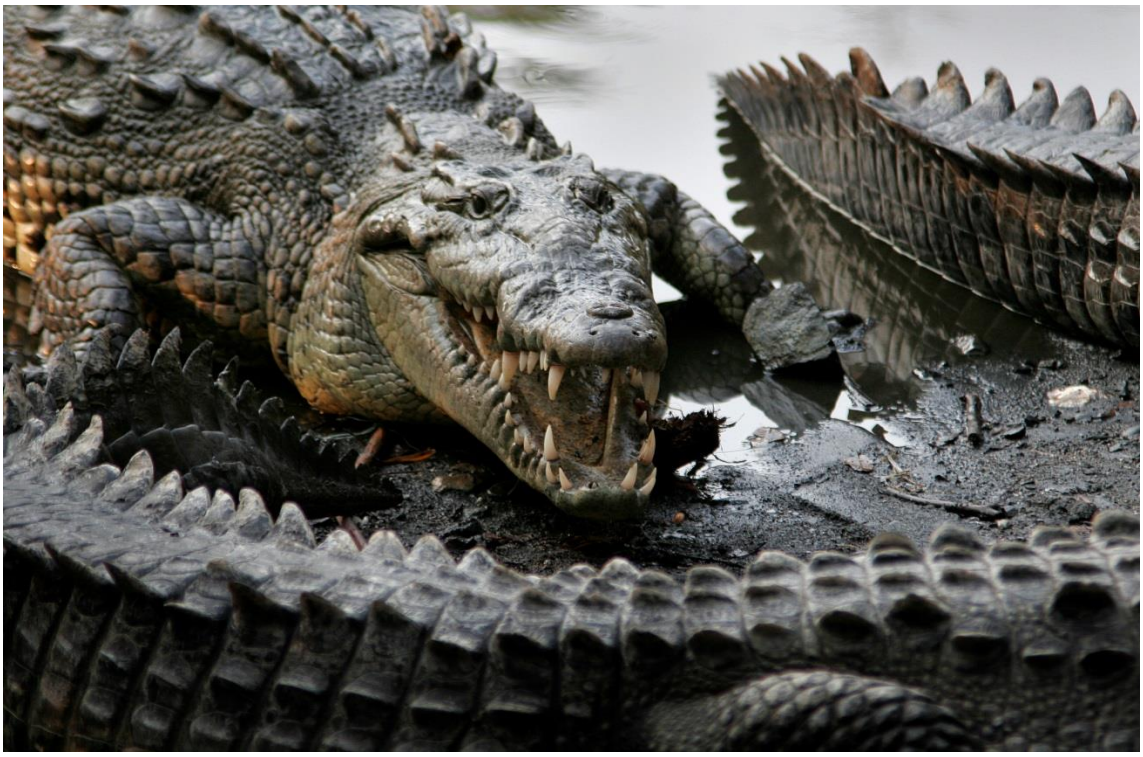
Using Nuclear and Mitochondrial DNA to Identify Captive Crocodylus rhombifer Individuals in Zoological Institutions

Mia Perkins
Mentor: Dr. Lou Densmore

Crocdylus *rhombifer* (Cuban Crocodile) is considered to be endangered due to anthropogenic driven effects such as habitat loss and alteration, hunting, and competition with introduced exotic animals. Their survival depends on being able to differentiate between purebred C*.rhombifer* and hybrids. Institutions interested in the management and conservation of this species rely on genetic screenings that weed out genetically compromised crocs through the examination of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). nDNA coincides with classical Mendelian inheritance in that there's information from both the male and female parents, unlike mtDNA which adheres to matrilineality (inherited maternally). Separately, both pieces of genetic material only partially illuminate a specimen's genetic lineage ;especially when hybridized creatures are being considered. Due to chromosomal similarities between C.*rhombifer* and C.*acutus,(*American Crocodile*)* as well as the species' frequent sympathy with one another, identifying hybrids based on morphology alone presents a problem. There have been documented cases of C.*acutus* and C.*rhombifer* hybridization; suggesting that the genetic integrity of C.*rhombifer* is at risk. Using both mtDNA and nDNA can provide a much more accurate view of an individual's genetic background. The accuracy of this information is crucial when screening or considering candidates for breeding and eventually reintroduction programs; only purebred individuals should be used to ensure the success of such programs.

We are comparing two captive species samples from an institution in the UK to pure C.rhombifer and three C. *acutus* from Panama and the Atlantic and Pacific coasts. Whole blood samples were collected from the caudal or dorsal sinuses and stored in a cell lysis buffer. Total genomic DNA will be extracted using the PureGene isolation kit, electrophoresed in a 5% agarose gel and visualized with ethidium bromide under UV light. A partial cytochrome-b (cyt-b) fragment will be amplified from the captive individuals using crCYTB primer, as well as 0.5microliters of forward and reverse primers. PCR would then be performed using 0.5 microliters of total genomic DNA.

Our main objective is to use this information to facilitate the identification of purebred C.*rhombifer* for breeding programs and hopefully the eventual repopulation of areas where the species has been locally extirpated or is extinct.



Cuban crocodile

American crocodile

Mia Perkins

I am currently in my last semester at South Plains College and will be transferring to Texas Tech this coming spring. My major is Pre-Veterinary sciences, though I'm very interested in Biochem as well, and I hope to attend Texas A&M for vet school. My research interests are varied. I've greatly enjoyed my time studying crocodilians and would love to continue to do so but I'd also like to study other animals as well in a variety of labs if possible. Outside of the academic world I enjoy drawing, painting, sewing, as well as sculpting with clay. I love to play video games and download the sheet music from them to play on the piano.