2do Simposio de Investigación Subgraduada en BIOLOGIA

Universidad de Puerto Rico • Recinto Universitario de Mayagüez

Departamento de Biología

Sábado, 5 de mayo de 2012

8:00 am – 6:30 pm

Biología
Universidad de Puerto Rico Mayagüez

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

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¡Bienvenidos al Segundo Simposio de Investigación Subgraduada en Biología!

El Departamento de Biología de la Facultad de Artes y Ciencias en el Recinto Universitario de Mayagüez, de la Universidad de Puerto Rico, celebra y reconoce la labor investigativa de sus estudiantes subgraduados. Actualmente, nuestro Departamento cuenta con un promedio de 1,400 estudiantes; el por ciento de estos que esta envuelto en proyectos científicos cada vez es más alto. Podemos entender que el método científico ha sido completado cuando se divulgan los resultados dentro del contexto de la comunidad cívica y científica.

El Segundo Simposio de Investigación Subgraduada tiene como propósito crear el espacio para consolidar el conocimiento adquirido durante la experimentación en el laboratorio y practicar las destrezas de comunicación oral científica.

El comité organizador agradece el apoyo del Departamento de Biología-Recinto Universitario de Mayagüez, *Role Model-H.H.M.I.* (subvención de *Howard Hughes Medical Institute)* & *Puerto Rico Science, Technology and Research Trust.*

Agradecemos al Dr. Carlos Muñoz por el diseño del logo utilizado en el Segundo Simposio, y a la señora Sandra Zapata Torres por la preparación del catálogo de los resúmenes de las presentaciones estudiantiles. Nuestro agradecimiento también a la Dra. Dimaris Acosta por sus comentarios sobre la versión final del libro de resúmenes.

Comité Organizador:

- David Logue, Presidente
- Sandra L. Maldonado Ramírez
- Luis Ríos Hernández
- Inés Sastre De Jesús
- Jarrod Thaxton
- Lucy B. Williams

Itinerario Segundo Simposio de Investigación Subgraduada

5 de mayo de 2012

8:00	Registro
8:30	Bienvenida : B-392
9:00	Dr. Dwayne Elías – B 392
9:30	Merienda
9:45	Sesión I
	B-180 Ecología I B-181 Medicina B-182 Evolución
12:00	Almuerzo
1:00	Dra. Elvia Meléndez – B 392
1:45	Sesión II
	B-180 Ecología II B-181 Genética I B-182 Biotecnología
3:30	Merienda
3:45	Sesión III
	B-180 Ecología III B-181 Genética II B-182 Fisiología y Ecología
5:00	Premiación y Clausura (B-392)
6:00	Foto, Grupo de Participantes y Entrega de Certificados

ABSTRACTS

Song performance in a species with a divided song repertoire

Isamarie Acosta Morales, Daniel A. Pereira, Orlando Medina, and David Logue

Department of Biology, University of Puerto Rico - Mayagüez Campus

Like other wood warblers, Adelaide's warbler (*Setophaga adelaidae*), has a split song repertoire consisting of type A and type B songs. Song type A is considered to be used during the day all year long whereas type B songs are used at dawn during the breeding season. We compared the performance of A and B song types to determine whether the pattern observed in other warbler species was also true for Adelaide's warbler. Therefore, we have studied the variation of the performance between both song types in five banded birds. Song performance is mostly defined as the ability of a bird to give information of their quality or condition through singing different types of songs to influence in a female's choice. The Adelaide's warbler is different from any other studied warbler species since it sings all year long and both song types have very similar patterns. It has been found in previous studies with other species, clear differences based on the purpose of the song performance, whether it is for male competition or female choice. Song repertoires have been divided in order to feature the song with the highest vocal performance they might have in that repertoire. Type A songs tend to be used at the highest performance limit compared to the type B songs. Given this, we have studied the Adelaide's warbler to find and prove such findings.

The local genome diversity studies: frequency of the CCR5 delta 32 deletion in Puerto Rico

Liz Marie Albertorio Sáez, Taras Oleksyk and Juan Carlos Martínez Cruzado

Department of Biology, University of Puerto Rico - Mayagüez Campus

The ultimate goal of the Local Genome Diversity Studies at the Laboratory of Genomic Diversity at UPRM, is to create a DNA repository of 96 saliva samples from each of the 78 municipalities in Puerto Rico. This collection can serve as a resource for future projects that study genetic variation across the island, and identify local polymorphic genetic variants related to human disease. We plan to use our growing collection to study the distribution of evolutionarily and disease-relevant markers as well as diversity in Puerto Rico. As we collect the samples, we have started to estimate the frequency of the CCR5 delta 32 deletion, since it is easily identified in an agarose gel because of the size difference between the two alleles. This deletion, if present in both chromosomes, has been shown to confer resistance against HIV infection by making the protein non-functional. Up to date, our students have collected 927 samples in 15 municipalities and tested for CCR5 in 169 samples. The saliva samples are collected using 8mL of water as mouthwash and 2mL of ethanol are added to prevent bacterial growth. We then extract the DNA using an ethanol extraction procedure. We amplified a 210bp long segment through PCR and run it in a 3% agarose gel. Preliminary data suggests that the deletion has a frequency of 1.5% in Western Puerto Rico.

Depression and anxiety behaviors are influenced by sex and estrous cycle stage

John K Alvarado[1], Esther A Jimenez[3], Dinah L Ramos[2], and Annelyn Torres[2]

Department of Biology, University of Puerto Rico, Ponce Campus[1], Pharmaceutical Sciences[2], Nova Southeastern University[3]

It is well-documented that as compared to men, women experience higher levels of mood disorder and depression during the reproductive years. However, it has not been fully elucidated how variations in gonadal hormones are linked to depression and anxiety disorders. During eight weeks, adult females and males Sprague Dawley rats were exposed to a mild stressor consisting of housing isolation (HI) and were compared to paired-housed controls. Vaginal smears were taken to determine the estrus cycle stage. At the end of the eight-week period all animals were tested on the open field (OF) followed by the forced swim test (FST) for measuring anxiety and depression-like behaviors. Myeloperoxidase activity in the colon of female rat showed a significant increase in HI females as compared to the paired housed females, suggesting underlying inflammatory processes produced by stress. However, the stressor (HI) only produced mild effects on the animal's behaviors. There were significant effects of sex and estrous cycle on OF and FST. During the OF the males presented reduced locomotor activity as compared to females. Females in proestrus showed less depressive behaviors as compared to males, confirming previously reported antidepressant effects of estrogen. These experiments demonstrate a significant role of gonadal hormones on the expression of depression-like behaviors.

Mesenchymal stem cells as nanoparticle delivery vehicles for cancer

Melissa Alvarado-Vélez, Delva Rivera Chacón, Surinder P Sing, and Jaime Ramírez-Vick

Department of Engineering Sciences & Materials, University of Puerto Rico-Mayagüez Campus

Multifunctional nanomedicine is emerging as a highly integrated platform that allows for targeted drug delivery, and simultaneous monitoring and treatment of cancer. We synthesized a multifunctional core/shell nanoparticles (M-NPs) system consisting of a magnetite (Fe3O4) core and a ZnO shell capable of generating singlet oxygen generation when excited with electromagnetic radiation and monitoring of this treatment through magnetic resonance imaging (MRI). The ability of mesenchymal stem cells (MSC) to specifically home tumors has suggested their potential use as a delivery vehicle for cancer therapeutics. To assess their potential as a multifunctional nanomedicine we studied the effect on M-NP uptake on MSC viability. The core-shell nanoparticles produced a significant reduction in the MSCs viability while ZnO nanoparticles do not had a significant impact in cells viability. The results suggest that the magnetic core of the core-shell nanoparticles may reduce the nanoparticles solubility contributing to its cytotoxic effect. Currently, we are trying to reduce this cytotoxic effect by modifying the core-shell nanoparticles surface to promote its solubility. In the future, we want to quantify the nanoparticles uptake and retention by the MSCs.

Identification and description of the yeasts cultivated by the fungus-growing ant *Cyphomyrmex minutus*

Karla M Antonetti, Mariely Medina-Rivera, and Matías J. Cafaro

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Attini ants cultivate a specific fungus (Basidiomycota: Agaricales) as their principal source of food. To protect the cultivar from the specific parasite *Escovopsis (*Ascomycota) or any other opportunistic pathogen; the ants engage in special grooming behaviors and live in association with an antibiotic-producing Actinobacteria. *Cyphomyrmex minutus* is the only species reported from Puerto Rico, which belongs to a group of *At*tini ants that practice yeast agriculture. We have little information about yeast agriculture ants and especially about this symbiosis in the island. The principal objectives of this project were: identify *C. minutus* cultivar and the yeast associated to it. All the samples were taken from *C. minutus* nests at the Cambalache Forest. We select four nests and described the cultivar and yeast associates morphological characteristics through light and/or SEM microscopy. We used culture independent methods to identify the ant cultivar. To identify the yeast associates we isolated and cultivated the yeast using minimal media and Potato Dextrose Agar with antibiotic. The DNA isolation products of both samples were amplified using specific primers for the D1/D2 18s region. The cultivar was identified as a *Leucoprinae fungus* similar to *C. minutus* symbiont 950106-03 from Trinidad and Tobago with 98%. At the moment we are on the process of sequencing all the yeast morphotypes isolated from the cultivar. With these results we want to establish the identity of the yeast community associated to *C. minutus* cultivar from PR and study in more detail this symbiosis.

Identification of fungi associated to *Nasutitermes costalis* termite nests

Alexander E. Benítez Rodríguez, Emanuel Méndez Morales, and Matías J. Cafaro

Department of Biology, University of Puerto Rico- Mayagüez Campus

In the arboreal nests that are constructed by *Nasutitermes costalis* we found diverse fungi that are usually associated with tropical environments in which the termites live. This species travels through moist soils and leaf litter to consume dry wood, hence becoming in contact with microorganisms that are carried to their nests. Most of the organisms are harmless, but some could be potential pathogens. Some microorganisms are degraders that modify complex compounds, like cellulose, making the food more palatable for the termites. On the other hand, there is evidence for glandular secretion suppression of fungal pathogens. Despite this protective mechanism, *Aspergillus* sp. is prevalent in *N. costalis*, and is believed to be an entomophagous species. Our goal is to characterize fungal communities associated with *N. costalis* nests. Six samples of nest material were obtained during dry season from Miradero Mayagüez. Suspensions of nest material in water and 10-6 dilutions were made and aliquots were placed in Potato Dextrose Agar (PDA). Isolates were processed for DNA extraction, PCR and sequencing of ITS region. A total of 39 fungal cultures were obtained and 23 of these cultures presented different morphotypes. We identified *Penicillium, Fusarium, Aspergillus, Pestalotiopsis, Trichoderma, Curvularia*, and *Aureobasidium*. The predominant fungi isolated from the termite nest are *Trichoderma (31%), Penicillium (23%),* and *Fusarium (13%)*. This confirms that most of the fungi within the nest are characteristic of tropical soils and plants. The identified potential pathogen was *Aspergillus niger*. This potential pathogen may adapt to *N. costalis* to maximize its reproduction and fitness.

Comparison among three different PCR fingerprinting techniques for identification of actinobacteria associated with *Nasutitermes costalis* (Isoptera:Termitidae) nests in Western Puerto Rico

Katiria Bonilla Miranda, Carolina Riascos, Emmanuel Méndez, and Matías J. Cafaro

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Nasutitermes costalis are fully social insects, composed of three castes: reproductive, workers, and soldiers that live in colonies. They create their nests with soil, wood, fecal secretions and saliva on the trunk or branches of trees above soil level. This condition makes them vulnerable to pathogen attacks. We believe that like in other insects such as ants and wasps, *N. costalis* could have symbiotic relationships with actinobacteria. These are Gram positive bacteria that are found mostly in the soil; they are excellent antibiotic producers and also participate in complex substrate degradation. Samples of *N. costalis* nests were collected from three different parts of Puerto Rico: mangrove (Boquerón), rainforest (Mayagüez) and dry forest (Guánica) to determine the presence of actinobacteria. Fingerprinting techniques (BOX PCR) were implemented to determine their usefulness in characterizing actinobacteria isolates associated with *N. costalis*. This technique was tested using three different primers: BOXAIR, which corresponds to the boxA subunit of the BOX elements in the bacterial genome, ERIC corresponding to the enterobacterial repetitive intergenic consensus regions and Rep, the repetitive extragenic palindromic region. The purpose of this study was to compare the effectiveness of each of these primers in identifying actinobacteria. We identified 53, 38 and 21 morphotypes of actinobacteria associated with termite nests in the rainforest, dry forest and mangroves, respectively. BOX primed fingerprints were the least taxonomically discriminating of the three primers tested.

Evolution of duet singing in songbirds (Passeriformes, suborder Passeri)

Wildeby Borges Díaz, Noelia A. Nieves Colón, Michelle L. Hall, and David M. Logue

Department of Biology, University of Puerto Rico - Mayagüez Campus

Songbirds are a diverse taxon with more than 4,000 species all over the world. Many songbirds sing duets, in which pair mates alternate syllables to produce a synchronized song. This behavior is hypothesized to have various functions. Among the best supported functional hypotheses are the 'mate guarding' and 'joint territory defense' hypotheses. These hypotheses state that duetting is used to produce more threatening songs to intimidate intruders and that individuals sing in duets to guard their mates, respectively. Our objective was to study the evolution of duet singing in birds for the first time. We were interested in understanding how duet singing evolves, and testing the hypotheses that duetting tends to evolve in the tropics and is associated with sexual monochromatism. A total of 464 species were randomly selected from a large phylogeny of songbirds. Several characters were scored for each species, including the presence of duetting, sexual dichromatism, and breeding latitude. We found that duetting has evolved many times among the songbirds. We ran 'Pagel 1994' tests of correlated evolution to test our research hypotheses. These tests showed that duet singing is more likely to evolve in tropical areas, and is associated with sexual monochromatism. These findings are consistent with the longstanding hypothesis that duetting is evolutionarily associated with similar sex roles.

Characterization of sub-cellular compartments and their link to neuromuscular plasticity in vivo

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Regarding synaptogenesis we unfortunately do not know enough about why and how is the synapse put together. We use *Drosophila* as a model system because of its simplicity, well-characterized developmental genetic tools and single cell identification of the neuromuscular junction (NMJ). We have identified novel sub-cellular compartments called myopodial clusters that are composed of actin based filopodial aggregates. These structures have been previously identified in embryonic stages and now we have identified them in later developmental larval stages. We are characterizing the appearance of this structure and exploring potential links to plasticity events by means of high-resolution confocal microscopy and laser micro-dissection of fixed and live tissues. Preliminary results show that the structure consistently appears in some muscles while not in others. We have also performed laser micro-dissection to further explore sub-cellular behavior when this cellular compartment is ablated. Ablation results demonstrate the behavior of the partner axon. Further analysis of these structures could provide enough information for neuromuscular junction manipulation in vivo in order to fix damaged tissues by leading the neuron to its correct partner. Having the ability to perform this would help prevent neuromuscular junction degeneration and instead propel its regeneration after the tissue has undergone injury.

Puerto Rico population study: pediatric obesity

Cristina M. Castro Rivera, Tarás K. Oleksyk, and Juan C. Martínez Cruzado

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Obesity is one of the leading causes of death in the US and PR. Pediatric obesity [PO] in particular has increased significantly in recent years and has a debilitating effect throughout life. Studies have pondered at the possibility of certain hereditable causes of obesity including abnormalities in reward and addiction pathways. This study in particular aims to ensemble a list of candidate genes and variants to better understand the metabolic pathways related to obesity. Thus far, genes related to PO and obesity in general have all been found to be related with pathways related to feeling satisfied, anxious/sad, metabolic intake, and even addiction. Low levels of dopamine usually provoke binging and further caloric intake. Additionally, some pathways related to lipid metabolism are often faulty in obese individuals. This suggests that a myriad of interrelated biochemical aspects must be studied and understood to assess the hereditary cause of PO. Future goals of this project are to use this student-compiled library of genes and variants related to pediatric obesity to study their frequency and distribution across the Puerto Rican populations.

Using sequences of the mitochondrial D-Loop evaluate the success of the eradication project on Don Luis Cay, Puerto Rico

Javier Chévere del Río, Israel Rivera, Verónica Seda, and Taras Oleksyk

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Invasive pest species have caused major ecological damages across the globe, the famous examples being rats in New Zealand and rabbits in Australia. Puerto Rico is not an exception. Rat populations have been observed thriving on a series of cays off the southwest coast of the Island. These cays have been recognized by the US Fish and Wildlife Services (US FWS), as an important breeding and nesting area of the endangered brown pelican (*Pelecanus occidentalis*). The Montalva project was carried out through 2008 and 2009 to eradicate the invasive black rat (*Rattus rattus*) from Cayo Frío and Cayo Don Luis south off the coast of Lajas and Guánica municipalities. The eradication proved to be a success eliminating the rat presence, improving the conditions for nesting. In 2011 a new project also intended to monitor Don Luis Cay for possible re-infestation. The eradication effort was carried out using the same method as the Moltalva project. In order to determine possible migration or repopulation events between both eradication projects, DNA samples were collected, and two different fragments of the D-loop region from mitochondrial DNA of a pre- and post-eradication population of *R. rattus* was amplified and sequenced. The sequencing results were examined to determine the genetic effects of eradication. Our analyses demonstrated the origin and relationship between the populations of both eradication projects. Based on our study, the US FWS will make recommendation for the design of extermination procedures in the future wildlife conservation programs on Puerto Rico.

Validation of epigenetic markers in endometriosis

Mariano Colón Caraballo Maricarmen Colon, Janice Monteiro, Perla Báez, Abigail Ruiz, and Idhaliz Flores.

University of Puerto Rico, Ponce Campus, Ponce School of Medicine and Health Sciences

Endometriosis is a gynecological disease that affects 1 in 10 women during their reproductive years, cause incapacitating pelvic pain and infertility. Endometriosis is characterized by the growth of endometrial tissue outside the uterine cavity. Endometriosis has no cure, its diagnosis can only be done via surgery, there are no non-invasive markers and its etiology is unknown. Epigenetic mechanisms may play an important role in the etiology of this disease. The modification of histones by acetylation and methylation of lysine residues has been shown to regulate gene expression by changing chromatin structure. We have shown by ELISA that endometriotic lesions have high levels of trimethylated histone 3 at lysine residue 27 (H3K27me3). The purpose of this investigation will be to measure H3K27me3 levels using immunohistochemistry (IHC). Using a Tissue Microarray (TMA) composed of 168 biopses (endometriotic lesions and control tissues) we will assess levels of H3K27me3 by IHC. Troubleshooting experiments identified the 1:100 as the appropriate dilution of the antibody. We expect that endometriotic lesions and endometrium of patients will express higher levels of H3K27me3 than controls. IHC will be able to show the cell specificity (i.e., glands vs. stroma) and will confirm the nuclear localization of this histone mark. In addition, we will be able to determine possible differences in this mark by lesion type (ovarian vs. peritoneal). These results will help understand how genes are regulated in endometriosis, and may support the use of novel treatments for this incurable disease.

Primer catastro de hongos endófitos asociados a *Oplonia spinosa*

Edsy Z. Cruz Pérez, y Sandra L. Maldonado Ramírez

Departmento de Biología, Universidad de Puerto Rico – Recinto Universitario de Mayagüez

En esta investigación se estuvo trabajando con la identificación de hongos endófitos asociados a tejido foliar sano de *Oplonia spinosa*. Las hojas de esta planta endémica del área norte de Puerto Rico e incluida en el listado de especies en peligro de extinción es la fuente de alimento para la mariposa *Atlantea tulita*. Para el aislamiento de los hongos endófitos asociados a esta planta se utilizó el procedimiento de esterilización superficial para tejido foliar descrito por Santamaría y Bayman en el 2005. *Colletotrichum y Gloeosporium* fueron los dos hongos aislados de hojas sanas de *O. spinosa*. *Colletotrichum spp*. fue el hongo aislado con mayor frecuencia durante el estudio. Este hongo está frecuentemente asociado a materia orgánica de origen vegetal y es causante de antracnosis en diversos cultivos como el gandul y el mango. Actualmente se desconoce si la presencia de *Colletotrichum spp*. en tejido foliar de *O. spinosa* representa algún peligro para esta planta endémica en peligro de extinción.

Comparison of virulence factors presence and antibiotic resistance in clinical and environmental isolates of *Enterococcus* spp. from Puerto Rico

Mara F. Cuebas Irizarry and Luis Ríos-Hernández

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The Enterococci are the golden standard organisms used to assess recreational water quality in marine habitats; it's also the leading cause of nosocomial infections in hospitals around the world. In this study we characterized the population of non-pigmented Enterococci present in natural habitats in Puerto Rico and compared them with clinical isolates. The isolates were compared based on a molecular analysis using a multiplex PCR to amplify five different virulent genes (gelE, asa1, hylA, esp and cylA) and their resistance pattern to three different antibiotics (Vancomycin, Piperacillin, and Rifampicin). Our preliminary results suggest that 67% of the population of the environmental isolates contain a dominant virulent genotype with gelE and asa1. Surprisingly, the clinical isolates also share the same genotypic dominance with 60% of the population. The antibiotic resistance pattern was different among the two populations; in the environment 83% were resistant to Rifampicin and 72% were susceptible to Piperacillin. In contrast, less than 30% of the clinical isolates were resistant to all of the antibiotics tested. Furthermore, we found that only 50% of the clinical isolates were capable of expressing the gene product (gelE), in contrast to 100% of the environmental isolates. Our preliminary results suggest that the non-pigmented Enterococci with gelE and asa1 are dominant in both environments. Further studies are needed to determine if both populations share the same host, humans, and if the environmental isolates could potentially colonize the GI tract of swimmers and cause a nosocomial infections in the future.

Elucidation of CXCR4-CXCL12 mediated angiogenesis mechanism in endometriosis

Martha Echevarría Andino, Abigail Ruiz[2], Perla Baez[2], Maricarmen Colón[2], Janice Monteiro[2], and Idhaliz Flores

Department of Biology, University of Puerto Rico - Mayagüez Campus, Ponce School of Medicine and Health Sciences

Endometriosis is defined as the presence of the endometrial tissue outside of the uterine cavity, primarily on the pelvic peritoneum, and ovaries. The main symptoms are chronic pelvic pain, pain during intercourse, and infertility. High CXCR4 mRNA levels were reported in endometriotic lesions in a rat model of endometriosis and protein expression is higher in human endometriosis tissues compared to control endometrium. CXCR4 is a G-protein coupled receptor, activated by its specific ligand, chemokine stromal cell-derived factor (CXCL12). CXCR4 is predominantly expressed by endometrial epithelial cells (EEC) and its ligand, CXCL12, by endometrial stromal cells. The CXCR4-CXCL12 axis is a potent inducer of angiogenesis, migration/invasion and cell proliferation. It has been shown that the CXCR4-CXCL12 axis can induce angiogenesis by inducing expression of vascular endothelial growth factor (VEGF). We hypothesize that CXCL12 can induce the expression of VEGF and its receptors, (VEGFR1 and VEGFR2) in EEC. The aim of this study is to determine if CXCL12 induce the expression of the angiogenic factors, VEGF and its receptors, in EEC. Cells were incubated with hrCXCL12α, and the expression of VEGF and its receptors was assessed by Western blot. Preliminary results showed that EEC only expresses VEGFR1. We expect to determine the effects of CXCL12 in the expression of the angiogenic factors studied in EEC and endometriotic epithelial cells. Our findings may have implications for future therapeutical strategies that may target the angiogenic components of endometriosis.

Testing six analogs of natural anti-cancer metabolite (2-methoxyestradiol) in neurofibromatosis type 1 cell lines

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Department of Biology, University of Puerto Rico – Mayagüez Campus [1], University of Michigan Medical School[2]

Neurofibromatosis type 1 *(NF1)* is the most common autosomal dominant disorder of the nervous system. Loss of the NF1 protein product, neurofibromin, is associated with benign neurofibromas, comprised primarily of non-myelinating Schwann Cells (SC) that can form along nerves and under certain conditions transform into malignant peripheral nerve sheath tumors (MPNST). Treatment options for NF1 are very limited. The experiments described here tested a promising class of drugs that are based on a natural anti-cancer metabolite, 2-methoxyestradiol *(2ME2),* which has been used in human trials for a decade. However, *2ME2* must be given at high doses because bioavailability is low due to degradation in the liver and the gut. This problem has been solved by synthesizing sulfamoylated variants of this molecule, all of which have 1) 100-1000fold better bioavailability and low degradation rates in vivo, 2) act at 100 times lower concentration with much greater effect on apoptosis, angiogenesis and microtubule disruption than *2ME2*, and 3) have low toxicity in vivo. The *2ME2* drug analogs were tested on NF1 human tumor cells and on non-myelinating SC derived from mouse embryonic stem cells (mESC). Cell proliferation was assayed using the Cell-Titer96 (Promega) proliferation assay. Absorbance was measured by microplate reader (Fisher) every 24 hours over 4 days. Absorbance is directly proportional to the living cells in the plates. Most of the *2ME2* analogues worked very effectively against a human NF1 cancer cell line ST (derived from a malignant peripheral nerve sheath tumor) and also against a benign human plexiform neurofibroma cell line (PNF). Cell proliferation was significantly reduced in the PNF and ST cell lines by selected *2ME2bisMATE* analogues. If tumor burden can be reduced or the transition to malignancy affected, then these compounds could be administered orally in patients to alleviate symptoms of *NF1*.

Diversity of bacteria associated with the hindgut of *Uca rapax* fiddler crab in Puerto Rico: their role and ability to degrade cellulose.

Verónica Figueroa Negrón, Angélica M. Olmo-Fontañez, and Matías J. Cafaro;

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Mangroves are one of the most important ecosystems in the tropics because they protect coastal areas from erosion. The fauna in the mangroves is varied; we can find the fiddler crab, *Uca rapax*. These marine invertebrates are detritivores; therefore they feed on detritus or suspended organic material in the sediment of mangroves. Because fiddler crabs do not have the required enzymes for the degradation of some organic materials we assume they have a gut microflora, responsible of secreting enzymes that may assist in degradation of these materials. This study aims to identify the bacteria associated to the fiddler crab hindgut that may have a nutritional symbiotic relationship, especially in the degradation of cellulose because it is the main component of the organic matter immobilized in the mangrove sediment. Crabs were collected in the area of Boquerón, Puerto Rico and bacteria were isolated from their hindgut and from the mangrove soil. Isolates were identified morphologically and molecularly. We amplified and sequenced the 16S rDNA of bacterial cultures and conducted a phylogenetic analysis. When comparing the hindgut microbiota with the soil microbiota, we want to evaluate the presence of any potential symbionts, which are only present in the hindgut, not in the mangrove soil. These bacteria were: *Aeromonas* sp., *Exiguobacterium* sp., *Photobacterium* sp., *Enterobacter* sp., and *Proteus* sp. We tested to all the bacteria found in the hindgut to evaluate their ability to degrade cellulose. The genera with positive results for the test were: *Aeromonas* sp., *Exiguobacterium* sp. and *Proteus* sp.

Morphological description of microbial community associated with the nest material, exoskeleton and gut of *Nasutitermes costalis*

José J. Gay Fagundo, Emanuel Méndez Morales, and Matías J. Cafaro

Department of Biology, University of Puerto Rico - Mayagüez Campus

Diversity of niches exists where the microbial communities exercise fundamental functions for the movement of nutrients and the stability of said habitats. Currently, we understand some of the ecology of microbial communities associated with social insects such as ants, bees, wasps and termites. Many have protective roles against pathogens and some help to break down materials consumed by the host. Such symbiotic relationship has been extensively studied in the fungus-growing ants of the tribe Attini. On the other hand, information about microbial communities associated with termites is scarce. Some studies have shown communities of bacteria and archaea associated with higher termite intestine involved in cellulose degradation, their primary food source. Observation of microbes associated with nest material and exoskeleton of higher termites has not been attempted. In this study we want to characterize the microbial community associated with a very common termite in Puerto Rico: *Nasutitermes costalis*. Samples of the material of the nest and of two castes (workers and soldiers) were taken. Nest material, exoskeleton and intestine of both castes were studied under scanning electron microscopy (SEM). Preliminary analyses of SEM images indicated an abundant microbial community associated with the nest material. We mostly observed filamentous forms associated with the wall material of the nest that most likely corresponded to fungi. Also we observed round shaped bacteria in the gut and the nest material.

Isolation and diversity of actinobacteria morphotypes from Río Grande de Manatí and Río Cupeyes

Félix N. Gómez-Claudio. Laritza Sanabría-Maisonave, and Carlos J. Santos Flores

Department of Biology, University of Puerto Rico-Mayagüez Campus

The search for new antimicrobials has focused on terrestrial actinobacteria, traditionally called actinomycetes, due to their well-known ability to produce of natural antibiotics. It has been demonstrated that many of them are producers of unique metabolites with antimicrobial, antiparasitic, antiviral, antitumor and cytotoxic activity. Actually it is known that many important pathogens have created resistance against many antimicrobial agents such as antibiotics and their variants. This research aimed to the isolation of actinobacteria from freshwater systems, particularly from Manatí River (Río Grande de Manatí) and Cupeyes River, to assess the species richness on these systems and evaluate the antimicrobial capabilities of the isolated species. Three samples of water and sediments were taken from each river. Actinobacteria were first isolated using a selective chitin medium supplemented with antifungals. Then, the morphological features were observed using yeast-malt extract agar (YMEA). A total of 111 actinobacterial morphotypes were collected from Río Grande de Manatí and 32 morphotypes were obtained from Río Cupeyes. Preliminary results suggest that there is a relatively high diversity of actinobacteria in both rivers. The significant difference in the number of isolated morphotypes between these two rivers might be related to the human disturbances in the Río Grande de Manatí, in contrast to the pristine waters of the Río Cupeyes. Further studies are needed to elucidate this statement. The rivers of Puerto Rico seem to be promising sources for the discovery of novel actinobacteria and new antibiotics.

The kinetics of the TLT-1/moesin interaction in human platelets

Magdalis González Vega, Mónica Fernández, and A. Valance Washington

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Decades ago, platelets were considered a non-entity, whereas now, we struggle to understand the intricacies of this suddenly important component in human hemostasis. Activation of these tiny cells is a key element in coagulation as well as the inflammation response. Activation consists of a series of signals leading to the expression of molecules not normally found on the platelet surface. Amongst these molecules we find the TREM-like-transcript-1(TLT-1). Current evidence suggests that it is a significant player in the inflammatory response and platelet aggregation. TLT-1's mechanism, however, remains unidentified. Here we strive to decipher the mechanism of TLT-'s role in platelet activation. We have previously demonstrated that TLT-1 interacts with moesin, a cytoplasmic scaffolding protein. This is TLT-1's only known intercellular interaction partner. Little is known about this interaction, representing a gap in knowledge. To better understand TLT-1 function a first step would be to decipher the kinetics of TLT-1/moesin interactions. We hypothesized that TLT-1/moesin interactions increase after platelet activation. Using immunoprecipitations and western blot analysis we completed a time-course of TLT-1/moesin interaction after platelet thrombin activation. TLT-1 was captured by antibody, and immunoblotted. Results show that TLT-1 co-immunoprecipitated with moesin in both thrombin-stimulated and resting-platelets. Furthermore, volume analysis on the density of the immunoblot-bands permits us to quantify the levels of this protein/protein interaction. Our hypothesis was unsupported when the kinetic interaction graph presented a decrease of protein expression around the one-minute mark of thrombin-activation, suggesting a release and recapture mechanism of TLT-1/moesin interaction.

"Comparative chromosome painting of chicken autosomal 1-9 conserved paints in *Amazona vittata*"

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This year, the first draft of the Puerto Rican parrot (*Amazona vittata*) genome was completed using Hi-Seq Illumina Sequencing Platform. In order to help the assembly of the genome, we are using bioinformatics tools to design chicken probes that will be hybridized to the chromosome spreads of the Puerto Rican parrot using the fluorescence in situ hybridization technique. Each probe is 30 bp long and corresponds to an evolutionarily perfectly conserved region among terrestrial vertebrates. They are distributed every 10Mb along chromosomes 1 to 9 in the chicken. Sequence alignments of parrot genome scaffolds to the chicken and zebra finch chromosomes suggest extensive chromosomal rearrangements after their divergence. Thus, we expect to be able to describe the different chromosome rearrangements that have occurred after the divergence of psittacidae and phasianidae. Finally, to further describe the evolution of the chromosomes of the *Amazona* genus, we will perform karyotypes of different *Amazona* species from across South and Central America and the Caribbean.

Cellulose-degrading ability of actinobacteria associated with the termite gut of *Nasutitermes costalis*

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Actinobacteria are known for their ability to produce secondary metabolites that can inhibit growth of other microorganisms. Also, they are capable of degrading complex structural polysaccharides such as chitin and cellulose. The association between actinobacteria and insects has been extensively studied. The actinobacteria function in protecting the host from pathogenic microorganisms and providing access to nutrients that the host cannot degrade. In this study we want to determine the cellulose-degradation ability of actinobacteria isolated from the gut of the termite *Nasutitermes costalis*, which is a common, xylophagous termite in Puerto Rico. Actinobacteria were isolated and identified from the termite gut in a previous study. We screened all actinobacteria isolates for cellulose degradation using a qualitative plate assay method. We detected 42 isolates capable of degrading cellulose, which were further analyzed. We used a quantitative method to characterize the degradation process. Cell growth was determined through optical density to standardize inoculum. Isolates were cultivated in cellulose agar for 72 hours at 25 °C. Iodine staining was used to observe the hydrolysis halo and recorded. The qualitative test revealed that 90.5% of the bacteria tested were positive for cellulose degradation. Quantitatively, 4% of isolates were poor cellulose degraders (<20 mm degradation halo), 92% were intermediate degraders (20-40 mm degradation halo), and 4% produced a halo of degradation >40 mm in diameter, being excellent cellulose degraders.

Using molecular markers to determine gender in avian species

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Some bird species like chickens, peacocks, and mallard ducks exhibit sexual dimorphism where males and females can be distinguished phenotypically, while others, like parrots, cranes, and emus do not. In such cases, gender can be determined using molecular techniques. However, there are currently no protocols for determining bird gender in Puerto Rico and the analysis needs to be sent to laboratories in the United States. We adopted a genetic test based on the CHD gene marker exhibiting different lengths on the W and Z chromosomes. PCR amplification results in two bands for the female (WZ) and only one band for the males (ZZ). Using this technique, we successfully determined gender in 25 Puerto Rican parrots (*Amazona vittata*) and our results were verified against the test from a commercial lab. We further tested the methods in eight different species of parrots and in African crowned cranes (*Balearica regulorum*), and successfully determined their gender. The method did not work in ratite birds, specifically the emu (*Dromaius novaehollandiae*), and a different set of primers (w1 and k7) had to be tested in the Wpkci gene marker. Recommendations from this experiment will be used to establish a genetic service for the Puerto Rico Zoo Juan A. Rivero and for the commercial aviaries around the island.

Frozen zoo: organization of DNA collection for conservation genetics and comparative genomics programs at the Department of Biology, UPRM

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Conservation of DNA from different species can help us preserve genetic material of a variety of animals, some of which are in danger of extinction. The first frozen zoo was founded in 1972 at San Diego Zoo's Institute for Conservation Research as a repository for skin-cell samples from rare and endangered species. We have started a similar project at the University of Puerto Rico at Mayagüez in collaboration with Puerto Rico Zoo Juan A. Rivero. Blood, feathers and hair of animals are collected during routine veterinarian procedures. Different protocols are applied for DNA extraction of the different tissue samples. Tubes containing genetic material of different species are sorted with respect to class, order, family, genus, and species. Subsequently, stored at very low temperatures (-85oC) for optimal long-term preservation. Our objective is to create a scientific and educational collection of animal DNA from a wide variety of species available either from the Puerto Rico Zoo, or other collaborative sources for the future studies in comparative genomics. In addition, these samples will preserve genetic variation of rare and endangered species that may contribute for their conservation. In order to make our collection available for other researchers, we are designing a web page containing information about specimens where visitors could search for samples and the associated information about the animal species using our browser. This initiative is a first step in the collaborative effort to develop a research direction at Puerto Rico Zoo Juan A. Rivero.

Does Adelaide's warbler (*Setophaga adelaidae*) have a dawn chorus?

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During their reproductive period, certain passerine birds exhibit a behavior known as the "dawn chorus" in which their singing activity is elevated. According to previous work, Adelaide's warbler (*Setophaga adelaida*) has a distinct dawn chorus. Our objective was to confirm these prior findings. We went to the Cabo Rojo National Wildlife Refuge and recorded singing behavior of four territorial males from 30 minutes before dawn until three hours after dawn. When analyzing the recordings we scored the following behaviors: song rate, song-type switch rate and fight rate during the pre-dawn and post-dawn periods. We found that the song rate and switch rate were much higher in the dawn chorus than in the following three hours but the fight rate was similar. These results are consistent with previous findings, allowing us to confirm that Adelaide's warbler does indeed have a dawn chorus. Future work will build on these finding to determine the different functions of pre-dawn and post-dawn singing.

Differential toxicity assessment of nanomaterials relevant to conventional products and biomedical applications in *Drosophila*

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Advances in nanomaterials synthesis and characterization offer an extensive array of potential biomedical applications. Establishment of guidelines regarding the use of nanomaterials is therefore required. There is growing demand for well-characterized, low cost toxicity assays for the validation of these nanomaterials. Here, we use the fruit fly *Drosophila* as a cost-effective model organism for the evaluation of novel nanomaterials. *Drosophila* offers a well-characterized repertoire of genetic tools, a rapid reproduction rate with short lifespans, and a panel of efficient molecular techniques in a system amenable to imaging. We assessed toxicity effects of nanomaterials present either in everyday-use products or those that are employed in biomedical applications, and from which traces have been found in the environment. We tested 8 different nanomaterials single-wall carbon nanotubes and multi-wall carbon nanotubes; Ag, TiO2 and Au nanoparticles; and IO nanoparticles coated with (a) aminopropylsilane, (b) carboxymethyldextran (CMDx) synthesized by thermal decomposition and (c) CMDx synthesized by co-precipitation. We exposed appropriately staged, intact live embryos to different nanomaterial concentrations through two different delivery methods. First, through micromanipulation and microtransfer for stereotypic microimplantation in segments A5/A6 with microneedle entry at the postero-dorsal end. Second, by placing embryos in direct contact with nanomaterial solution. Our results suggest that some nanomaterials have the ability to cross biological barriers and thereby aggregating in the visceral musculature. Our work presents a cost-effective experimentation with the possibility of being conducted as a high-throughput screening methodology of nanomaterials.

Confrontations between actinobacteria isolated from *Cyphomyrmex minutus* and microfungi with pathogenic potential

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In the fungus-growing ant symbiosis, the ants cultivate a fungus (Basidiomycota) and protect their cultivar from specific parasites (Ascomycota) using the antibiotic production capacity of actinobacteria. Three different genera of fungus-growing ants are known from Puerto Rico. Of these, *Cyphomyrmex minutus* is the only species that cultivates its fungus in yeast form. The cultivar pathogen *Escovopsis* has not been described for any yeast agriculture ant species. The main objective of this research is to study potential antibiotic producing actinobacteria isolated form *C. minutus* against other fungus-growing ant's cultivar pathogens. We selected five of the most frequent actinobacteria previously isolated and identified from *C. minutus* for bioassays and confrontations. Seven microfungi known for their pathogenic potential against the cultivar of others fungus-growing ants were used in the bioassays. Actinobacteria were inoculated in yeast malt extract agar (YMEA) on the center of the plate and incubated at 30oC for 30 days. When the actinobacteria reached an approximate diameter of 2 cm the microfungi was inoculated at the edge of the plate and incubated for two weeks. At this point the zone of inhibition (ZOI) was measured. We report the interaction between the actinobacteria and the fungi. *Streptomyces* sp 8-1 and *Kitasatospora* sp. look like good candidates for producing a zone of inhibition against other microfungi. We will be testing the production of secondary metabolites and exploring their antibiosis potential.

Isoflurane-induced impairments in rodents exposed to a Novel Object Recognition task

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Previous work has shown that general anesthesia results in post-surgical cognitive deficits. In addition, previous work has shown that isoflurane increases inflammatory cytokine expression and causes cell injury in the hippocampus, which may contribute to isoflurane-induced cognitive impairment in rats. Others found that a single isoflurane exposure does not affect Morris Water Maze performance in mice. Although the possible role of isoflurane on hippocampal plasticity has been previously assessed, the role of an acute exposure of isoflurane on novel object recognition has not been described. Here, we will compare the acute effects of isoflurane anesthesia and ketamine anesthesia in a rodent model of hippocampal learning known as the Novel Object Recognition (NOR) Task. Male Sprague Dawley rats (3 mo. old) were exposed to either isoflurane anesthesia (1-1.5%) or ketamine anesthesia (100 mg/kg) 11 days prior to training on NOR. Our results clearly indicate that animals anesthetized with ketamine display typical learning of the novel object recognition task whereas isoflurane administered animals display distinct impairments ($p < 0.05$). Our results support the notion that an acute administration of isoflurane anesthesia results in hippocampal-dependent spatial memory impairments. Future work should be aimed at developing neuroprotectants to diminish isoflurane-mediated learning deficits in the hippocampus in surgical patients.

New reports on monogenean parasites of the Almaco Jack (*Seriola rivoliana*) from the North coast of Puerto Rico

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The Almaco Jack is a game fish that belongs to the family Carangidae. It is an internationally important natural resource due to its commercial and recreational value. In previous studies, a single species of *Monogenea*, parasites of the gills, was found. For the present parasite assessment, the whole fish was examined including stomach, intestines and gills. Parasites were collected and fixed. Nomarsky microscopy was employed to aid identification of parasites. During this investigation fish examined from the north coast of Puerto Rico did not possess the previously reported gill worm, but were parasitized by two monogenean parasites; one species of *Kuhnia* and a member of the Capsalidae family. *Kuhnia* spp. are known to be host specific for the fish family Scombridae and possibly restricted to the genus *Scomber*. There are many mechanisms by which gill worms may infect different hosts. Although the families Scombridae and Carangidae belong to the order Perciformes, they are not closely related. This finding suggests that host switching might be taking place.

Using mitochondrial intergenic regions to characterize *Beauveria bassianna* isolates from Puerto Rico

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Coffee is cultivated in more than 60 tropical countries and is the 5th most important agricultural economic crop in Puerto Rico. Production of coffee has been limited worldwide due to plagues with the coffee berry borer (*Hypothenemus hampei* Ferrari) being the most destructive. Control of *H. hampei* has been accomplished utilizing biocontrol traps, insecticides and most effectively, the natural entomopathogenic fungi *Beauveria bassiana*. Spraying a *B. bassiana* suspension in coffee plants has proven to infect and consequently kills up to 30% of the plague when the insect is outside of the coffee berry. Studies have shown that different isolates of *B. bassiana* in Puerto Rico have varying pathogenic levels. This study attempts to genetically characterize 6 isolates of *B. bassiana* collected from different parts of the island. Previous analysis of *B. bassiana* isolates have shown that mitochondrial intergenic regions are more effective than nuclear intergenic regions to characterizing this specie. We successfully amplified NADH dehydrogenase subunit 3 (nad3) and ATP synthase F0 subunit 9 (atp9) genes from the 6 isolates and, after sequencing, compared them to known isolates as well as to each other by sequence alignment using t coffee software. Preliminary results suggest that four of the six samples belong to the same *B. bassiana* strain since no difference in nucleotide sequence was observed. When compared to previously known sequences, samples group in the same main cluster but identification of a strain is not possible.

Census comparison of actinobacteria populations in live and dead nests of *Nasutitermes costalis*

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As eusocial insects, termites live in colonies composed of several million individuals. *Nasutitermes costalis,* an arboreal species of great ecological distribution, is found on the bases, branches and hollowed trees. Nests are made with the termites' own feces and glandular secretions, forming a thin layer of carton known as the outer wall. Being porous, these hold the only contact with the exterior, which exposes the termites to pathogens; hence we think that the termites could form a symbiotic relationship with antibiotic producing actinobacteria. We hypothesized that the actinobacteria flora would differ greatly, in colony numbers, between live (light brown) and dead (dark brown) nests once the termites are gone. Samples were collected in the rainforest area behind the Biology building in Miradero, Mayaguez. We sampled six nests, taking material from three live and three dead nests with a sanitized saw. Each sample was crushed and suspended in sterile water; after serial dilutions, they were plated into a selective chitin medium. Colonies were counted after a period of incubation of five to six days with the purpose of calculating colony-forming units (CFU). We performed a correlation analysis between nest capability (live vs. dead) and actinobacteria CFUs. Our analysis (significance level 0.05) showed significant variation, resulting in greater CFUs for dead nests in comparison to live nests. Our results do not sustain our hypothesis; this could probably be an effect of constant nest material restoration in the presence of the termites, which maintain a stable microbiota and homeostatic conditions.

Comparison of the efficiency of different biomass: marine algae and tropical climbing vines for the anaerobic bioconversion to a renewable energy source

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Scientific research to find an efficient biomass to create biofuels has expanded to investigate on the use of lignocellulosic terrestrial biomass and marine macro-algae. Tropical climbing vines, although abundant, could contain up to 43% of lignin making its degradation difficult due to this complex polymer. On the other hand, marine algae contain very low concentrations of lignin and cellulose making them relatively easier for anaerobic bioconversion. The purpose of this study is to compare which biomass, either the tropical climbing vines *Mucuna pruriens* or *Dioscorea bulbifera*, or a mixture of marine algae, has the highest potential to be converted through anaerobic degradation to methane. Microcosms were created with the different biomass, different terrestrial sediments and incubated at 35°C under anaerobic conditions. The product of metabolism, methane, was used as an indicator of microbial activity, and was measured by gas chromatography. All calculations for the direct comparison were based on the dry weight of the different biomasses. Among the climbing vines, *D. bulbifera* produced the highest amount of methane with 0.50g, whereas the marine algae produced an average of 1.10g. The energy obtained in the highest production of methane with the mixture of algae was equivalent to 18.37W which, can light an 8-watt light bulb for 2.20 hours, meanwhile the same biomass from the climbing vines could produced the amount of energy to do it for 1.20 hours. In conclusion, under our experimental conditions, the marine algae are more suitable for the anaerobic conversion to methane than the tropical climbing vines.

Biomass allocation and leaf moisture of native and exotic grasses in a subtropical dry forest

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Invasive exotic grasses capable of increasing the frequency, spread and intensity of anthropogenic fires have invaded subtropical and tropical dry forests worldwide. Since many native dry forest tree species are susceptible to fire, this can result in loss of forest cover and native biodiversity. In some dry forest landscapes, native grasses are also an important component of the ecosystem. Little is known about how the ecological characteristics of these native grasses compare to those of invasive non-native grasses. Within a subtropical tropical dry forest reserve in Puerto Rico (Guánica Commonwealth Forest), we asked whether the three dominant grass species: native grass *Uniola virgata* and exotic grasses *Megathyrsus maximus* and *Cenchrus ciliaris* differed in their patterns of biomass allocation and leaf moisture. We measured biomass analysis by randomly choosing and excavating individual clumps of each species, separating aboveground and belowground parts, drying at 70°C for 3 days and weighing each sample. Leaf moisture was measured by randomly marking 10 individuals of each grass species and collecting leaf samples every 3 weeks for 5 months. These samples were weighed wet, dried at 70°C for 3 days, and weighed after drying to calculate moisture percentage in each sample. Results indicate that native grass *Uniola* had greater aboveground biomass and rooting depth than exotic species. During the transition from wet to dry season, leaf moisture of *Uniola* decreased less rapidly than that of exotics species. These patterns suggest different ecological strategies among the dominant native and exotic grass species in this forest.

Diversity of actinobacteria associated with the yeast-agriculture ant *Cyphomyrmex minutus* exoskeleton and cultivar

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The fungus-growing ants have a symbiotic association with fungi (Basidiomycota: *Leucoagaricus*) that serve as their food source. The fungal cultivar is attacked by pathogen fungi in the genus *Escovopsis* (Ascomycota: Hypocreales). To protect their cultivar from pathogens the ants use actinobacteria, well known for their ability to produce antibiotics. There is little information about the interaction between the yeast agriculture ant *Cyphomyrmex minutus* and their symbionts in the Caribbean. The purpose of this study was the identification and description of the actinobacteria community associated with *Cyphomyrmex minutus* exoskeleton and their cultivar. We collected samples of *C. minutus* and its cultivar in Cambalache Forest. In the laboratory we inoculated the samples into chitin media (CHA) plates. The colonies were purified and maintained in Yeast Malt Extract Agar (YMEA). The actinobacteria isolates were classified and characterized based on macro- and microscopic morphology. We isolated genomic DNA and amplified 16S rDNA using bacteria universal primers (27F and 1492R). We identified 153 isolates in four different genera of actinobacteria (*Nocardia 1%, Tsukamurella 1%, Kitasatospora 3%,* and *Streptomyces 95%*). The most frequent isolate from the nest and the exoskeleton was *Streptomyces* sp. 8-1. These results suggest that *Streptomyces* sp. 8-1 has an important role in the association of this fungus-growing ant. We are using this information to perform bioassays. In the future we want to investigate the role of the most frequent actinobacteria in this community.

Inventory of fungi associated to feces of the butterfly *Atlantea tulita* (*Lepidoptera: Nymphalinae*)

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Atlantea tulita is an endemic butterfly of Puerto Rico. So far, there is hardly any research regarding fungi associated to the intestinal tract of this insect. The present study is the first inventory of fungi associated to the feces of this species. Fungal specimens were obtained from fresh feces of captive butterflies collected from localities near Playa Jobos in Isabela, Guajataca, and Quebradillas. A modification of the moist chamber technique and the serial dilution method using phosphate buffer were used to process the samples for fungal isolation. Fungal isolates with mycelial growth were identified using the slide culture technique and the API 20C AUX system was used to identify yeast isolates. Common fungi isolated from the samples included *Absidia, Aspergillus, Candida, Cephalosporium, Cladosporium, Curvularia, Geotrichum,* and *Mucor*.

Electrospun chitosan-Ag membranes for wound healing applications

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Chitosan is a biocompatible and biodegradable polymer derived from the natural biopolymer chitin, the most abundant amino polysaccharide in the world. Besides its proven antimicrobial properties for a wide variety of organisms, ranging from bacteria to fungi, its non toxicity and processing capabilities make chitosan an especially suitable candidate for potential applications in the biomedical industry. Furthermore, its antimicrobial properties can be enhanced by forming composites with diverse materials, such as silver nanoparticles (Ag-NPs) which have also demonstrated antibacterial effects. This combination is particularly desirable for applications such as human wound dressings, which aim to enhance wound healing. An electrospun chitosan-Ag composite was synthesized in the form of membranes with varying Ag concentrations ranging from 2 to 40 % wt. To validate the antibacterial effect of the membranes, common pathogens including the genera of *Escherichia*, *Staphylococcus, Klebsiella,* and *Pseudomonas*, were exposed to the composites for 24 hours at 37oC. Preliminary results confirm growth inhibition for all the bacterial genera exposed to the membranes, validating the antimicrobial effects of the chitosan-Ag composite. Morphological characterization of the membranes was conducted using Scanning Electron Microscopy (SEM) and J-image software. The average diameter of the resulting fibers was found to be in the range of 73 to 273 nanometers (nm). Experiments conducted with raw or electrospun chitosan did not exhibit any antibacterial properties suggesting a synergistic effect between the Ag and the chitosan polymer. Current efforts involve the optimization of chitosan-Ag ratios and the antimicrobial effects obtained from these variations.

Isolation of calcium binding protein interaction peptides using T7 phage display

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Calcium Binding Proteins (CaBP) play relevant roles in processes such as mitosis, microtubule-based cytoskeleton formation among organisms such as yeast, algae, mice and humans. It is involved in cell motility, cytokenesis and segregation of chromosomes. The use of combinatorial chemistry strategies, such as Phage Display, allows the identification of interaction partners among proteins in a rapid and effective manner. In this research, four Calcium Binding Proteins (CaBP), were used as target ligands, to determine and isolate interaction partners from T7 Phage Display cDNA library from Human Colon. The CaBP targets were first adhered to the ELISA plate and after performing the blocking reaction, three rounds of biopanning were developed and the presence of interaction phages was determined using phage lift. The putative phages displaying interacting peptides were isolated, and tested for the presence of an insert by PCR, to further determine the identity of the fragment by *in silico* analysis. The data confirms the presence of interaction peptides in all four targets with a differential affinity. Identification of proteins, such as attractin, a glycoprotein involved in cell adhesion, and vimentin, important for microtubule formation in mesenchymal pluripotent cells. From a total of 1.1 x 107 of T7 phages in the library, the number and diversity of the phages cloned inserts was reduced approximately to 4.0 x 103 PFU/mL. The specificity assay is in progress to confirm recognition among partners interaction. These findings have shown a series of CaBP interaction peptides can be applied in biomedical research as cellular biosensors.

Morphological description of actinobacteria associated with *Nasutitermes costalis* (Isoptera: Termitidae) nests in three ecosystems in Western Puerto Rico

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The phylum actinobacteria is the largest and most diverse of the Eubacteria. Their morphology is complex; they present different shapes from bacilli to aerial filaments with associated spores producing different pigments. These microorganisms can produce antibiotics; participate in symbiosis with different roles. We believe that because these microorganisms are susceptible to pathogen attacks there is a symbiotic relationship between actinobacteria and *Nasutitermes costalis*, which is an eusocial species of arboreal termite in the neotropics. We isolated actinobacteria from nests of rainforest, dry forest and mangrove. These zones were selected in order to identify the actinobacteria present in the nests throughout the range of the termite. If the number of morphotypes recovered in all nests were to remain constant, this would indicate a more specific association, possibly a symbiotic relationship. We recollected nest material from three nests per sampled zone. In the laboratory, the material was crushed and suspended in sterile water and plated in a selective chitin medium. Later, colonies were transferred to YMEA. We identified 122, 84 and 66 morphotypes in the dry forest, mangrove and rainforest, respectively. We found that there were no significant differences ($p = 0.05$) in the amount of morphotypes isolated for each zone. We believe that due to the little exposure to the outside environment the termites manage to maintain a specific community of actinobacteria in their nests. Hence, the number of morphotypes would remain constant. In the future, it is necessary to confirm through molecular analysis the identity of these morphotypes.

You should eat the seed

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Thromboembolism is a formation of a platelet clot in the blood vessels that detached and is transported by the blood stream to plug another vessel. Thromboembolitc events are commonly associated with cardiovascular disease, cancer, and long periods without movement as advertised on long flights call deep vein thrombosis or DVT. Comparisons of thromboembolitic events and other coronary heart diseases by Continent show that the European continent has fewer deaths from thromboembolism even though their diets are high in fat. This difference, often called the French Paradox, is attributed to a healthy intake of grape wine over their lifetimes. The French Paradox suggests that grapes may have anti thrombolytic properties. To test this hypothesis we investigated common natural grape products on platelet aggregation. Platelet poor platelet rich plasma of humans were used to measure the effect of grapes seed extract (GSE) and grape skin extract (GKE) collagen induced platelet aggregation in vitro. We evaluated several concentrations of GSE and GKE alone and in combination. We identified that 50 mg/L GSE was the lowest effective dose to fully inhibit collagen induced platelet aggregation. We next hypothesize that GKE may be a treatment for diseases such as DVT. To test this model we used a mouse collagen/epinephrine thromboembolism model. The current status of these studies will be presented here. Our preliminary results suggests that GKE had a protective effect against thromboembolism increasing survival time of the mice by almost 10 minutes and that it may be advantageous to, "eat the seeds".

Evaluación parcial de la micoflora en el Edificio de Biología, UPRM

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Actualmente los seres humanos pasamos la mayor parte del tiempo en el interior de un edificio. Sin embargo, no todos los edificios tienen un ambiente apropiado para permanecer en ellos por largos periodos de tiempo. Durante la investigación realizada en febrero de 2012 se quiso determinar la calidad del aire en el interior del edificio de Biología. Se tomaron muestras de áreas afectadas anteriormente por *Stachybotrys chartarum*, un hongo tóxico y altamente patogénico utilizando un muestreador Anderson de dos etapas conteniendo platos Petri con Malt Extract Agar. En adición se recopilaron datos ambientales incluyendo la temperatura y la humedad relativa, los cuales nos ayudan a determinar si las variables ambientales de alguna manera tienen efecto sobre la cantidad y diversidad de hongos recuperados en comparación con el ambiente externo. Luego del análisis de datos se determino que el hongo más abundante en el interior del edificio fue *Cladosporium* spp, un hongo común en los ambientes de interior. Para el exterior *Fusarium* spp fue el hongo recuperado en mayor frecuencia. Sin embargo, en ninguna de las muestras se detecto la presencia de *S. chartarum* sugiriendo que los procesos de remediación que se llevaron a cabo en el las áreas afectadas fueron efectivos. La detección temprana de condiciones de humedad, cambios en temperatura y otros factores relacionados al crecimiento de esta micoflora nos permite tomar medidas correctivas antes que se produzcan daños importantes o epidemias.

Development of a system to evaluate platelet affect on neutrophil signaling

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Hemostasis and inflammation are intimately linked. Platelets play an indispensable role in mediating this relationship; however, the mechanisms that regulate this interaction are poorly understood. Our laboratory studies the Triggering Receptor Expressed in Myeloid-Like Transcript-1 (TLT-1), a receptor found in the platelet α-granules. Oddly enough, characterization of treml-/- mice demonstrates a severe hemorrhage associated with lipopolysaccharide administration in mice. This hemorrhage seems to be mediated by neutrophil dis-regulation. We hypothesize that TLT-1 may preemptively regulate neutrophil function as a mechanism to control bleeding. As a preliminary step to this understanding, we are developing a new in vitro system using flow cytometry and western blot analysis to measure neutrophil activation in the presence of platelets from mice. Our experimental design consists of isolation of neutrophils through density gradient centrifugation. After isolation, the population is incubated with platelets from wild type and treml1-/- mice and subsequently lysed and analyzed by western blot to confirm the phosphorylation levels of the map kinases p38 and p42/44. In our first experiments we were having problems getting the signal on the western blot even though we were getting neutrophils by flow cytometry. Recently, with improved methodology, the neutrophil concentration has increased; this will be confirmed by western blot. Here we demonstrate the current state of this project. Further studies of the interaction between the TLT-1 and neutrophils will allow us a broader understating of the physiological processes occurring in the hemostatic and immunological systems.

The frequency of the obesity gene MC4R rs13447331 polymorphism in Isabela, Puerto Rico

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The project "Adopt a Gene" is a collaborative work of a group of students from the Laboratory of Genomic Diversity. The objective is to use the information collected about the most common disease-causing polymorphisms in Puerto Rico to guide the synthesis of a chip for the early diagnosis of these diseases in newborns. To achieve this, the students choose a gene and one polymorphism associated with a disease whose early diagnosis would help for prevention or to increase treatment effectiveness, using available bioinformatics tools, and reading scientific papers. The student designs a pair of primers that will amplify a DNA segment of approximately 400 bp that can be sequenced in full in a single Sanger reaction and that will include the identified polymorphism and other polymorphisms that could yield information about its continental origin. The analysis is being performed using unrelated samples collected in all the municipalities of Puerto Rico. I selected the MC4R (melanocortin 4 receptor) gene, which is related to autosomal dominant obesity among Europeans, and its rs13447331 polymorphism, which causes the replacement of serine for leucine, probably affecting the capacity of the protein it codes for. This is a receptor on neurons in the hypothalamus region of the brain that receives signals about the status of the body's fat reserves. I am currently sequencing from samples obtained in Isabela. The data collected will give information about the frequency of each polymorphism and haplotype, the haplotype geographic distribution in Puerto Rico, and its possible continental origin.

The UPRM Genomes Browser: integrating genome-wide data and bioinformatics analysis into an open-source web page at the Biology Department

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Bioinformatics has been a new field in biological research that has paved the way towards new directions in science. It main goals are to process the biological database and provide new way of displaying the results. In our project, we focused on designing an approach to easily access the genome-wide results from the recent sequencing and genotyping projects. In order to assess the immense amount of data available in the Laboratory of Genomic Diversity in UPRM, and for academic purposes, a website was developed based on the open-source Drupal platform, incorporating a database for the "Puerto Rican Parrot Genome Project" and a genome browser for the "Taíno Genome Project". The webpage has been recently launched where both the research databases and genome browser are readily available for use by our and other research groups in the worldwide scientific community. The web page we created can serve as a model of other online projects at the Biology department, and an example of undergraduate contribution to the dissemination of research in genomics and bioinformatics.

Hongos asociados a la corteza de mangle rojo (*Rhizophora mangle*) de la Isla Magueyes en Lajas y la Bahía de Jauca en Santa Isabela, Puerto Rico

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Los hongos asociados a los mangles poseen un papel fundamental dentro de este ecosistema pues llevan a cabo funciones importantes como el reciclaje de nutrientes. Sin embargo, a pesar de su importancia los estudios dentro de la micología marina enfocados en este tema son muy pocos. En Puerto Rico, existen 4 especies de mangle, siendo *Rhizophora mangle* o el mangle rojo, la más conocida. Esta investigación se concentró en determinar preliminarmente la diversidad de hongos asociados a la corteza de *R. mangle*, provenientes la Bahía de Jauca en Santa Isabel y la Isla Magueyes en Lajas. En el estudio se encontraron similitudes en la diversidad y abundancia de hongos presentes en ambas regiones, así como algunas variaciones en la micoflora asociada a las raíces adventicias, tronco y ramas de cada individuo de mangle estudiado. Algunos hongos comúnmente encontrados fueron *Aspergillus, Penicillium, Cladosporium, Alternaria* y *Pestolatia*. En total se aislaron 25 especies de hongos, agrupadas en 10 géneros, 32% no pudieron ser identificadas y solo *Cytospora rhizhophorae* se pudo identificar a nivel de especie. Los datos obtenidos sobre las especies y géneros en este estudio nos brindan una idea sobre la diversidad de hongos en mangles de Puerto Rico.

Differences in specific leaf area and leaf dry matter content in shade tolerant species growing in shade versus in the sun in a tropical dry forest in Puerto Rico.

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Differences in specific leaf area (SLA ,m^2/kg) and leaf dry matter content (LDMC, mg/g) in plants of two species (*Cocoloba diversifolia* and *Eugenia foetida*) growing in the shade versus plants growing in full sunlight were compared. These two traits correlate well with a species' potential relative growth rate or mass-based maximum photosynthetic rate, as they can reflect a fundamental trade-off for plant functioning. Mature leaves from three plants of each species growing under both light conditions were collected in the Guanica Forest. The SLA and LDMC averages for *Coccoloba diversifolia* growing in sunlight were 7.61 and 478.139 respectively, and for plants growing in shade 9.34 and 425.837 respectively. The SLA and LDMC averages for *Eugenia foetida* growing in sunlight were 9.649 and 459.375, and for plants growing in shade, 11.013 and 406.632 respectively. Although, SLA was higher in the shade for both species while LDMC was lower in the shade for both species, these differences were not significant at $p<0.05$. More data is needed to improve the statistical power of these results.

Functional assessment of bacterial α-amylases in *Arabipdopsis thaliana*

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Ethanol has been identified as a potential solution for the current deficit of fossil fuels. Ethanol is produced by fermenting sugars, mainly glucose, obtained from different biomass sources. Each biomass source has a different processing pathway depending on the form for glucose accumulation. Starch storing crops such as cassava are being explored as potential bioenergy crops for the future. Starch is a polysaccharide that has two glucose polymers, amylose and amylopectin. A treatment with heat or starch hydrolyzing enzymes is required to convert starch to glucose; this is a key step on the biomass processing pathway. The goal of this project is to introduce a bacterial α–amylase, a starch hydrolyzing enzyme, into *Arabipdopsis thaliana*, a model plant, to analyze the enzyme conversion efficiency of starch to fermentable sugar. By increasing the biomass digestibility (glucose yield per dry biomass), the ethanol yield would also increase. The DNA of three bacterial α–amylases have been isolated, sequenced and their enzymatic activity has been analyzed. The gene of the α–amylase with highest activity (line 5366) was cloned into a binary T-DNA vector and introduced into *Escherichia coli*. Currently the potential transformed colonies are being confirmed through PCR. After confirmation, the recombinant vector will be introduced into *Agrobacterium tumefaciense* through electroporation. Using *Agrobacterium*-mediated transformation *A. thaliana* will be transform with each α–amylase to further study the activities of the three α–amylases *in planta*.

Natural agents against digestive *Candida*

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The yeast *Candida albicans* is part of the human micro-flora. However, it is also an opportunistic pathogen in high concentrations causing infections, especially in those subjects with a weak immune system. *C. albicans* pathogenesis includes skin tissue and mucosal surfaces; it has been shown to live in a symbiotic relationship with humans in mucosal regions of the digestive system. Furthermore, this yeast has been linked to overweight. Our research consists in studying the effects of natural antifungal agents on digestive *Candida*. Products, "Yeast-Cleanse"© and "Candida Plus"©, were studied at concentrations of: 25%, 50%, 75%, and 100%. To compare the effect of the yeast at different conditions similar to the acidic pH of the stomach, plates containing Saboureaud Dextrose Agar (SDA), at pH 3.98, 4.57 and 5.2, were incubated. Inhibition halos were compared for each treatment. Results indicate that "Candida Plus"© was not successful against digestive *Candida*, since no inhibition was detected. In contrast, "Yeast-Cleanse"© showed an area of inhibition in all tests performed throughout the experimentation, reflecting that this product is more efficient. Results provide "Yeast-Cleanse"© as a possible alternative to treat digestive *Candida*, and further on helping people to their weight loss. We expect that some of the active ingredients of these products, such as basil and grapefruit, will show inhibition halos through a new experimentation. Further research on metabolism studies of digestive *Candida* will provide more understanding of how this yeast affects weight. Carrying out statistical analysis of human subjects whose diet includes the researched products, will confirm efficiency.

Functional characterization of novel cassava cysteine synthase gene using *Arabidopsis thaliana* mutants

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Cassava (*Manihot esculenta* Crantz) is a perennial shrub cultivated in the tropics and sub-tropics and is consumed worldwide. This root crop shows important advantages such as the ability of growing in marginal conditions of drought periods as well as in poor acidic soils. Despite the advantages of the plant, it is known to accumulate toxic cyanogenic glycosides in all of its tissues except seeds. These are a group of nitrile-containing secondary compounds in plants that yield free cyanide upon their enzymatic breakdown. Cassava is also equipped with a cyanide detoxification pathway, mediated by ß-cyanoalanine synthase (ß-CAS), which catalyzes the incorporation of cyanide into L-cysteine. ß-CAS shares a high degree of sequence homology and hence structural and functional similarities with cysteine synthase (CS), another enzyme belonging to the same family. Recently, two genes from the Bsas family, BsasA and BsasB, were isolated from cassava. Based on expression in *Escherichia coli*, BsasA apparently has more CS activity and BsasB has more ß-CAS activity. In this project BsasA gene is being expressed in the model plant *Arabidopsis thaliana* for its functional characterization. Also, *A. thaliana* mutants lacking a CS will be transformed with this gene to observe if it can compensate for the loss of function in the mutants. *Agrobacterium tumefaciens* carrying the gene is being used for plant transformation and enzyme assays will be done from crude protein extracts of *A. thaliana* leaves to measure protein activity. It is expected that a higher CS protein activity could lead to crops with a higher amount of amino acids.

Preliminary findings from analyses of ancient coprolites from the saladoide culture archaeological sites in Vieques, Puerto Rico

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Parasites have accompanied humans since the beginning of the species and through their migrations around the world. Paleoparasitology is the field for the study of ancient parasite infections and has developed over the last 100 years in many parts of the world. This is the first paleoparasitological work In Puerto Rico and adds knowledge to the few other studies conducted in the Caribbean. This particular research used coprolites, fossilized feces, found from a Saladoide culture archaeological site from Vieques, Puerto Rico. A subsample of each coprolite was rehydrated and spontaneous sedimentation allowed to occur. Ten replicates each of 8 samples were analyzed microscopically to detect, photograph, measure and identify parasite eggs. Nematode and other eggs were found including round worms and hookworms. Other eggs remain unidentified and could represent digenetic and cestode infections. Archeologists have suggested that this culture practiced farming and the parasites recovered support this contention as contact with animals and soil enhance parasite infection.

Crosstalk between ErbB 1/3 and IL-8 and GROα signaling in ovarian cancer

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Epithelial ovarian cancer (EOC) has the highest mortality rate of cancers that affect the female reproductive system. CXCR and ErbB networks have been found to play important roles in ovarian tumorigenesis. We focused on CXCR2 and ErbB1/3 and studied if autocrine signaling exists in serous epithelial ovarian cancer cell lines and if there is any crosstalk between these pathways. To study this, we used qRT-PCR, ELISA assays, proliferation assays, and quantitative Western Blots. We found that there are autocrine loops with CXCR2 and that possible crosstalk exists between this pathway and ErbB1 in serous EOC cell lines. These receptors may be affecting proliferation by activating pathways like ERK, AKT and JNK. Further experiments must be made to understand the role of these networks in cell behavior.

Análisis de fitoplancton de los embalses Guajataca, Cerrillos y La Plata

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Una base importante para determinar el tipo de contaminación de un embalse es conocer las especies del fitoplancton, que son los productores principales en la cadena trófica de estos sistemas. El objetivo de esta investigación es el estudio del fitoplancton en 3 embalses: Cerrillos, Guajataca y La Plata. Se busca una relación entre las especies de fitoplancton que habitan en dichos embalses con su estado trófico. Considerando la antigüedad y el estado trófico, reportado en otros estudios, el orden de riqueza de especies (S) fitoplanctónicas en estos embalses debe ser el siguiente: Lago Guajataca, seguido por el Lago La Plata y, por último, el Lago Cerrillos. Se realizó un muestreo en duplicado para cada lago utilizando una red de arrastre tipo Bongo, que colectó el producto de arrastre por 5 minutos a 2.7 millas/hr. El volumen final del filtrado (1L de agua en cada muestreo) fue preservado en formalina 5%. Según el análisis preliminar, el lago con mayor número de especies es el Lago Guajataca, dominando el grupo de las clorococales (Chlorophycea); luego el Lago La Plata, en el cual predominan las diatomeas (Bacillariophycea) y los dinoflagelados (Pyrrophyta); y en último lugar, el Lago Cerrillos, con su mayoría también de diatomeas y dinoflagelados. Preliminarmente, se observa una relación proporcional entre la antigüedad del lago y el número de especies encontradas.

Inhibition of cell proliferation due to trace elements released from oxidized Ti alloy orthopedic implant materials

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Titanium alloys are of great significance in orthopedic implants. However, the stability of the surface in terms of ion release in the human body is responsible for the durability of the implant. Our study tested two alloys Ti-6Al-4V, currently used for implants and γ-TiAl, a potential substitute. The experimentation is focused on analyzing the type and concentration of ions released in a simulated human body environment after the surface of these alloys are modified by oxidation at different temperatures. Studies have demonstrated that toxicity in implant recipients has been attributed to release of ions from implants. HFOB 1.19 cells were grown in culture media previously exposed for 72hrs at 33.5°C to these surface modified alloys. Cells were allowed to proliferate in this media for 72 more hours at 33.5°C followed by an indirect MTT assay. The results show that the Ti-6Al-4V alloy oxidized at 700°C did not sustain cell proliferation when compared to oxidation at 121°C and 500°C. The culture media exposed to the oxidized alloys were analyzed by Atomic Absorbance Spectroscopy using a Graphite Furnace to detect type and concentration of ions released from the alloys surfaces. During the analysis quality control samples were added as well as spiked samples to ensure accurate readings. The test results showed that increasing concentrations of vanadium are released as the oxidation temperature increases. Our hypothesis is that the release of vanadium ions from the oxidized Ti-6Al-4V alloys is the reason for the lack of cell proliferation.

Identification of actinobacteria associated with *Nasutitermes costalis* termite

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Actinobacteria are Gram-positive bacteria with high GC content, spores producers and have the ability to produce antimicrobial agents. They are capable of degrading structural polysaccharides such as cellulose, lignocellulose and chitin. Previous studies have found this group of bacteria associated with different groups of insects including ants, bees, wasps, beetles and termites. The bacteria have different roles in these associations (defense against pathogens, decomposers in the gut, etc.). In the exoskeleton of Attine ants and Beewolf wasps, actinobacteria protect them against pathogenic microbes. There has been no study of actinobacteria associated with the exoskeleton of termites. On the other hand, the termite gut has been extensively explored. Actinobacteria in the genus *Streptomyces* are believed to help degrade organic matter in the termite gut. In this study we characterize the diversity of actinobacteria associated with *Nasutitermes costalis*, a common arboreal termite in Puerto Rico. Two castes of termites, workers and soldiers, were collected in Miradero forest in Mayagüez. A sterile water suspension of extracted bacteria from the intestine and the exoskeleton of termites (workers and soldiers) was prepared and plated in selective medium (chitin agar). Colonies were transferred to nutrient media for maintenance (YMEA). Morphological and molecular techniques (DNA extraction, PCR amplification of 16S rDNA and sequencing) were later used for identification. We isolated 941 actinobacteria grouped in 331 different morphotypes. The analysis of sequences reveals that the most common genus found in this association is *Streptomyces* which presents great morphological variation.

Does the presence of a generalist predator and an omnivorous will minimize non-equilibrium dynamics in ciliate abundance? A test using the simple community of the phytotelmata of t

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Traditional ecological theory predicts that the stability of food webs declines with the presence of both, omnivory and efficient (specialized) predators. Moreover, omnivory and longer food webs have been associated with less stability in ecosystems. However, recent evidence suggests that the presence of omnivores and generalist predators are pivotal for reducing chaos (i.e., non-equilibrium dynamics sensu Mc Cann and Hastings, 1997) in simple communities. The purpose of this study was to test the effect of both, an omnivorous and a generalist species on the ciliate community within *Tillandsia utriculata*. If both, the omnivorous (nematode) and the generalist predator (flatworm) have a stabilizing effect, then there should be less abundance variation in ciliate abundance. After assembling 16 microcosms with four treatments, ciliate abundance was significantly higher in the presence of the generalist, followed by the omnivorous only treatment (ANOVA, $F= 6.84$, $P=0.0061$). The control and the omnivorous plus generalist treatment showed low abundances with no significant differences. Contrary to theory, there was high variation in abundance (CV%) under the omnivorous treatment; however, consistent with previous work, the generalist treatment and the omnivorous plus the generalist showed less variation (chaos) in abundance. The generalist species seemed to have a stabilizing effect possibly because it had less rigid interactions with other species and higher tolerance to environmental change. Is possible that less trophic levels will show less omnivory and the generalist could had a potential buffering effect on the ciliate community creating equilibrium.

Assessing microbial diversity in dry and tropical forest soil metagenomic libraries using DGGE

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The emerging discipline of metagenomics has become a powerful tool to evaluate the genomes present in any environment, unraveling the function and diversity present using culture independent approaches. Our laboratory generated two Metagenomic Libraries (MgL) with approximately 800,000 fosmids clones of up to 40kb inserts from El Yunque Tropical Forest (YTF) and Guánica Dry Forest (GDF). The main focus of this research is the determination of the microbial diversity present in the MgL using Denaturing Gradient Gel Electrophoresis (DGGE). The fosmid DNA from induced MgL from both forests was extracted, and a direct DNA extraction was done to the soil samples used to generate the MgL. The DNA extraction revealed positive discrete bands in the fosmid libraries, but direct DNA extraction from soil was not as effective. Then, a 16S rDNA PCR using domain-specific primers was performed. Amplicons were obtained from YTF and GDF soil samples and also from the MgL fosmids. When DGGE was performed, different band patterns were obtained from the MgL and from the soil samples, and were compared. All the bands found in RTF soil sample are present in the MgL-DGGE pattern. This MgL-DGGE contains other bands not present in the soil sample, suggesting the extraction methods used for the Fosmid generation possibly allowed the access to additional bacterial groups when compared to the not-as-effective genomic extraction. The GDF-MgL-DGGE showed the presence of a 9-band-pattern in the soil sample which was not detected in the Fosmid sample, suggesting that the diversity present in GDF is not represented in its corresponding MgL. Further optimization of the extraction method as well as the primer used is necessary.

Efecto de la intensidad lumínica en el establecimiento de esporas y el desarrollo de protonema del musgo *Neckeropsis disticha* sembrado en la madera de *Spathodea campanulata*

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La expansión agrícola y el desarrollo urbano han alterado la distribución de bosques Neotropicales nativos y a su vez incrementado el desarrollo de bosques secundarios. En Puerto Rico estos bosques secundarios mayormente están dominados por el árbol *Spathodea campanulata* P. Beauv. cuyo ciclo de defoliación crea condiciones variables de microclima como temperatura, humedad e intensidad lumínica las cuales son esenciales para el establecimiento de briofitos. Estudiando la germinación de esporas y el establecimiento de protonema del musgo *Neckeropsis disticha* (Hedw.) Kindb. podemos descubrir las condiciones de intensidad lumínica óptimas para llevar a cabo esfuerzos exitosos de reintroducción y conservación de briofitos en los bosques Neotropicales. En este experimento, sembramos las esporas de *N. disticha* en pedazos de madera con corteza de *S. campanulata*; tratándolos con un fotoperiodo de 12 horas luz día/noche con un sistema de riego automatizado proveyendo humedad. Se establecieron cuatro tratamientos de intensidad lumínica variando la cantidad de capas de sarán: exposición total a la luz, luz parcial (una capa), luz filtrada (dos capas) y sombra (tres capas). Estos tratamientos se correlacionan al ciclo de defoliación en los bosques de *S. campanulata*. El establecimiento de esporas y desarrollo de protonema se midió bajo las categorías de espora, espora engrosada, espora con tubo germinativo y protonema. Los datos preliminares sugieren que las esporas de *N. disticha* germinan y se desarrollan a protonema bajo condiciones de luz filtrada. Esto se correlaciona el momento donde bosques de *S. campanulata* regeneran su dosel.

Are there separate repertoires for A and B singing modes in Adelaide's warbler?

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Wood warblers possess two singing modes which they use at different times of day, and in different circumstances. These singing modes are referred to as Type A and Type B singing. In most species of wood warbler, A and B singing modes are characterized by very different song structures. Previous work indicates that in Adelaide's warblers (*Setophaga adelaidae*), A and B songs are structurally similar, but individual males maintain distinct A and B repertoires. In this species, B singing occurs before dawn during the breeding season, and A singing occurs later in the day throughout the year. We attempted to confirm that males have distinct A and B repertoires. We chose four males and recorded them during and after the dawn chorus. We then identified all of the song types that they sang. Preliminary data show the following: 1. Some songs are used exclusively during the dawn chorus, while others are used after the dawn chorus only. 2. Sharing of songs between singing modes occurs, but in low frequency and sometimes with a small degree of variation. Our findings are largely consistent with previous studies indicating that males have split repertoires, but also indicate that certain songs are used in both modes with only minor modification. Further studies are needed to better understand development mechanisms of A and B songs, as well as the communicatory functions of these singing modes.

Operant Conditioning Bird Cage

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For male birds, their song serves as a mating signal. Each bird has a repertoire of discrete song types and these repertoires can be further classified into different categories. It is known that there are structural differences between the different categories of song repertoires but it is not yet clear what the adaptive significance of these differences is, or what features of these different types of male songs are relevant to the female bird when she is making her mating choice. The Operant Conditioning Cage will be equipped with perches that will be configured with a different prerecorded male song each. Every time the female bird poses herself on any perch, the playback of the prerecorded male song assigned to that perch will be triggered and reproduced through speakers inside the cage. The system will monitor the behavior of the female bird, creating a data log, which the researcher conducting the experiment can access via application software, that allows him to determine which male song the female bird prefers based on how often it poses herself on any given perch, and how long it remains posed on any given perch based on data gathered by a subsystem in the box which measures the length of the time interval in which the female bird remains posed on the perch after she listens to any song triggered by that particular perch. The software will also allow the researcher to modify the configuration of the cage according to the needs of the experiment. The Operant Conditioning Cage will provide researchers with a practical way to test mating signal preferences in female birds.

Methane stimulation and searching of endemic methanogens in Montana coal beds

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The coal bed methane in Montana represents an important renewable energy source that could reduce the high dependence on coal to produce electricity in United States. The methane generation and accumulation in coal beds is controlled by several factors that include methanogenesis. However few things about microorganisms that create methanogenesis and their capacity to decompose organic complex compounds in coal to produce methane are known. The main purpose of this research is to evaluate the Montana coal potential to produce methane under different treatments and to determine the presence of endemic methanogens. A series of treatments with Montana's coal samples were developed. The treatments included the addition of a known microorganism's culture (WBC-2) with the presence of methanogens and the supply of certain conditions that benefit the microbial endemic activity in coal. It was observed that in all treatments methane was produced, though the treatments with the culture WBC-2 presented a higher production. When some organic complex compounds were added to the treatments with WBC-2, higher levels of methane was produced. The treatment with WBC-2 in groundwater no indicates the presence of mcrA gene. This study indicates that methane production in Montana coal can be stimulated adding some complex organic compounds, that exists endemic methanogens in Montana coal and that the groundwater can inhibit the mcrA gene expression in molecular analysis. The demonstration of endemic activity in Montana coal and the stimulation of methane production provide a platform for anthropogenic manipulation. Research is in progress to determine the microorganism biodiversity.

Enterobryus (Ichthyosporea: Eccrinales) associated with the gut of the millipede *Anadenobolus monilicornis* in Guanica Dry Forest

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Symbiosis is defined as the close association between two non-related organisms. The common yellow-banded millipede, *Anadenobolus monilicornis* (Diplopoda: Spirobolida: Rhinocricidae) and the protist *Enterobryus* sp. (Ichthyosporea: Eccrinales), a species of hair-like microorganism that inhabits its gut, form a commensalistic relationship. *Enterobryus* was once part of a fungal class Trichomycetes, but now it is classified as a protist. Other members of the genus *Enterobryus* have also been reported associated with other non-carnivorous arthropod hosts including beetles, crabs and millipedes. We intend to characterize morphologically the *Enterobryus* species that inhabits *A. monilicornis* through key structure descriptions and measurements. Also, we want to study the natural variation of morphological structures and their measurements through more rigorous statistical analysis. In the past, descriptions of new *Enterobryus* species have been based on few sample observations and even more scarce measurements. *Enterobryus* species are difficult to establish because intraspecies variation is sometimes higher than between species. We collected 60 millipede specimens in Playa Jaboncillo within Guanica Dry Forest to study the infestation with *Enterobryus*. Millipedes were dissected; gut linings with attached *Enterobryus* were removed. Carefully the hindgut was cut into smaller pieces and placed on a slide and hydrated with distilled water. After observation under microscope, slides were preserved utilizing 0.05 w/v lactophenol cottonblue. Mounted slides were used under a light microscope to measure structures. Morphometric data of thalli, spores and holdfasts were taken, specifically length, width and diameter. Based on these data we hypothesized that this is a new species of *Enterobryus*. Further electron microscopy and molecular data are needed to finalize the description.

Evaluation of antifungal production from actinobacteria associated with the exoskeleton of the termite *Nasutitermes costalis*

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The functionality of microorganisms associated with multicellular eukaryotes has been studied extensively. Currently, we know that actinobacteria exert antimicrobial functions in insects such as ants, bees and wasps. We also know that termites have actinobacteria in their gut and exoskeleton, although their exact role is still under debate. In the intestine, it is believed that actinobacteria function as degraders of cellulose, the primary food source of termites. In the exoskeleton, we suspect a defense function against pathogens as reported in other social insects. Pathogenicity of the fungi *Metarhizium anisopliae, Beauveria bassiana* and *Fusarium oxysporum* has been reported in many arthropods, including termites. In this research we want to study the antifungal capacity of actinobacteria associated with *Nasutitermes costalis*. The termite *N. costalis* is an arboreal, xylophagous and common termite in Puerto Rico. We have previously isolated and identified actinobacteria from the exoskeleton of *N. costalis*. Most of our isolates belong to the genus *Streptomyces*, well-known antibiotic producers. We selected 11 isolates based on their phylogenetic position in our previous analyses. We evaluated their antifungal capacity against entomopathogenic fungi through bioassays. *Streptomyces* isolates were cultivated in YMEA medium for 21 days so the secondary metabolites diffuse into the plate. Then the fungus was inoculated at the edge of the plate and allowed to grow for 30 days. Our preliminary results with *Metarhizium anisopliae* show that 45.5% of strains produce partial antifungal activity and 9.0% produces a total antifungal activity. Fungal growth is partially or totally inhibited after 12 days of incubation.

Identifying variation in genes involved in eye color determination in Puerto Ricans

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Genetic markers are recently used to predict phenotypic characteristics like eye, skin or hair color. However, in European Americans eye color is difficult to predict since many loci are involved. While eyes and hair of Puerto Ricans appear monomorphic (brown), their genetic ancestry is diverse and differences relating to both traits may be overlooked. It could be easier to determine eye or hair color in Puerto Rico due to the fact that the alleles come from different continents involved in the admixture. Therefore, we set out to determine variation in loci known to influence hair and eye pigmentation worldwide. We genotyped 4 SNP's from the OCA2 gene that are strongly associated with eye color, and two single nucleotide polymorphisms (SNP's) from the MC1R gene, associated with eye color but also with hair pigmentation. We used 64 samples from Santa Isabel and 8 worldwide samples and analyzed them for genetic variation. In our study, we report that there is a higher genetic variation in MC1R than in OCA2 in the Puerto Rican samples, and frequencies vary across the island. We are currently genotyping other SNP's in these genes to obtain a clearer image of the variation involved in phenotypic traits in Puerto Ricans. Despite the common misconception of Puerto Ricans having the same eye and hair color, genetic tests reveals the existence of variation in the associated genes. In the future, we plan to test our method by predicting human phenotypes. If successful, our research can be used in forensic investigation.

Effect of pruning on cassava (*Manihot esculenta*) post-harvest physiological deterioration

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Cassava is a cyanogenic crop and one of the most important source of carbohydrates in many countries. Despite this importance, cassava has a low storage potential due to post-harvest physiological deterioration (PPD). PPD is considered to be a wound stress response where reactive oxygen species (ROS) are involved. Interestingly, cyanogenesis in cassava causes ROS to accumulate and an oxidative burst follows which leads to PPD. This deterioration in cassava causes economic losses and restricts the capacity of the plant to become a commercial crop. A low cost method that has been associated with the reduction of PPD is pruning, but its cellular mechanisms are not completely known. In order to uncover and assess cellular mechanisms linking pruning to PPD, we analyzed cassava roots of unpruned and pruned plants grown at the Isabela Agricultural Experiment Station. Qualitative measurements of PPD percentage for the roots were evaluated visually 0, 5 and 10 days after harvest. Chemical assays were performed for linamarin and beta-cyanoalanine, which are involved in the cyanogenesis and cyanogen detoxification pathways, respectively. Also scopoletin, a hydroxycoumarin that has been previously shown to accumulate due to PPD, was analyzed. Gene expression analysis using Real-time PCR is on-going to identify transcripts up- or down-regulated as a result of PPD of cassava roots from unpruned and pruned plants.


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