

**THIRTEENTH ANNUAL
STUDENT RESEARCH
POSTER SYMPOSIUM**

MAY 15, 2009

1-4 PM

HOLT HALL

**DEPARTMENT OF BIOLOGICAL SCIENCES
CALIFORNIA STATE UNIVERSITY, CHICO**

POSTERS

LOWER DIVISION CLASSES

HOLT 227

**UPPER DIVISION CLASSES
UNDERGRADUATE RESEARCH**

Holt 225

GRADUATE CLASSES, THESIS RESEARCH

Holt 235

ENTOMOLOGY CLASS DISPLAY

Holt 235

HERBARIUM OPEN HOUSE

Holt 129-131

12:00 – 4:00 PM

*Stop in and see the showing of submissions to the
Plant Photo Contest and the winners (Holt 129).*

California State University, Chico
Department of Biological Sciences

**Recipients of the
Tom Rodgers
Outstanding Researcher Award**

1997 Ronald E. Loggins

1998 Maura Eagan

1999 Moaiad A. Kanaan

2000 Carina M. Jung

2001 Cary Coburn

2002 Glen Lubke, Jack Campbell, Gary Lechner

2003 Julia Terry

2004 Colleen Martin, Michelle Sopoliga, Michelle Ocken

2005 Alan Raetz

2006 Khang V. Do, Chris L. Clifford

2007 Adam M. Ferris

2008 Nicole J. Huber

Lower Division Class Projects

Antimicrobial capacity of different reagents on gram negative and gram positive bacteria

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BIOL 152 - David Wood

We tested the effects of 8 different types of soaps and cleaning agents on gram negative and gram positive bacteria. We did this by plating *E. coli* and *S. epidermidis* and sterile disks dipped in each reagent. After incubation, we measured the zone of clearing around each disk. This experiment was repeated 3 times to find the average antimicrobial capacity of each reagent. Not only did we compare the reagents, but we also compared the differences between the effects on gram positive and gram negative bacteria. Our findings show that there is no difference in antimicrobial capacity of antibacterial hand soap and antibacterial dish soap, even though there is a 0.035% difference in the concentration of the active ingredient. We also found that there is a large difference between the effects of antibacterial soap and non-antibacterial soap, especially in gram negative bacteria.

Effects of carbon dioxide emission on *Pittosporum tobira*

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BIOL 152 - David Wood

We were interested in the effect of increasing traffic on stomatal density. We choose the species *Pittosporum Tobira* because of its natural abundance. We hypothesized that the plants closer to high traffic areas would have an increased stomatal density due to the increased carbon fixation by Rubisco. We took two leaves from trees in 3 different positions, one with high traffic (32), one with medium traffic (holt), and one with little or no traffic (alumni glen). We took 3 stomatal density readings for each leaf and in the end found an increased stomatal density for the tree in alumni glen, compared to the relatively similar concentrations of Holt and hwy 32. These surprising results indicate that perhaps less stomata are needed in co2 rich areas vs. co2 deficient areas.

The evolutionary history of flight

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BIOL 152 - Christopher Ivey

Flight in birds has been an interest to biologists since Darwin. Some birds are completely flightless and some are limited in flight ability and range. The problem that we investigated was the evolutionary history of flight in birds. To investigate this, we selected birds that can fly, are flightless, and are limited in flight, then used the Mega 4 program and GenBank to

download 12s rRNA sequences for the birds we chose. We used these data to create a phylogenetic tree. Our results portray that flight evolved first, followed by limited flight and no flight, of which flight then re-evolved in the case of the goose and gull. Our results support our alternate hypothesis that flight evolved more than once.

Stomata density in manzanita

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BIOL 152 - Shelley Kim

Manzanita leaves from the genus *Arctostaphylos* at low elevation (200 ft) to high elevation (4800 ft) were collected to determine the density of stomata. Due to difference of precipitation, temperature, and oxygen level at different elevation, Manzanita at lower elevation have less stomata density compare to those at higher elevation. The results concluded that Manzanita at higher elevation does have more stomata density (P-value = 5.65E-05).

A comparison of species richness in area with high vegetation and no vegetation

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BIOL 152 – Christopher Ivey

In this experiment we compared the ecological gradient of species found in areas with high vegetation and areas with no vegetation. The areas were tested using pitfall traps we placed in the ground and left for a week. After collecting the traps we then counted and grouped the species based on their taxon. From this we were able to calculate the Shannon diversity, standard error, standard deviation, and t-test. We then tested for species richness and concluded that areas with high vegetation have greater speciation than areas with no vegetation.

Soil dwelling taxa and soil compaction

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BIOL 152 - Michael Marchetti

Our intent was to search out a new method to collect species around us in our every day lives, which we had never seen before. The Berlese Funnel is an easy and cheap way to collect species that live within a soil matrix. Our hypothesis was that in the three soil types of varying compaction (High, Medium, and Low), the biodiversity would differ. In the end only the Medium and Low compaction soils yielded any taxa. When an ANOVA test was run on the Medium and Low data sets, a P-Value of 0.31

was indicated: not supporting our hypothesis. Using the excess sample dirt, we then sifted through looking for the most abundant taxon: Nematodes. The Medium sample yielded an

average of 25.33 Nematodes while the Low sample average was 0.66 Nematodes; both, with a standard error (SE) of 0.67: Indicating a significant difference between the Nematode populations of the samples.

Allelopathic effects of tannic acid and juglone on tomato seed germination

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BIOL 152 - Ailise McEnteggart

In this study, the effects of the naturally occurring toxins tannic acid from the valley oak (*Quercus lobata*) and juglone from the black walnut (*Juglans nigra*) were tested on tomato seeds. These substances are known to adversely affect the germination of a variety of seeds including members in the Solanaceae family. Soil samples from two oak and two black walnut trees from separate locations in Bidwell Park in Chico, California were taken as well as two non-contaminated control groups. Tomato seeds were placed in the soil samples in conditions that promoted growth. After a period of five days, the samples were analyzed and the effects of the toxins on germination success were evaluated.

No difference in stomatal density of *Eucalyptus obliqua* leaves in areas with different weather patterns

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BIOL 152 - Michael Marchetti

Is there a significant difference in stomatal density between non-deciduous *Eucalyptus obliqua* leaves found in areas with different weather patterns? The expectation was that the leaves collected from *E. Obliqua* trees in Corning, California would have a higher stomatal density than the leaves collected from Bayliss, California because the Corning area has more extreme weather than the area around Bayliss. Using clear nail polish impressions and a compound microscope, the average stomatal densities of the leaves from both areas were collected and analyzed. With a p-value of 0.05, it was determined that there is no significant difference between the stomatal densities of the leaves.

There is a significant difference between insect communities at 10ft, 35ft, and 60ft from the Sacramento River

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BIOL 152 - David Wood

We found that there is a significant difference between insect communities at 10ft, 35ft, and 60ft from the Sacramento River. Are motivation for this experiment was to see if there was a relationship between rivers and the abundance of insects and to study the

differences between insect diversity between grassy sandbar and riparian forest along the Sacramento River. We hypothesized that there be a greater abundance and diversity closer to the river and in the riparian forest compared to the grassy sandbar. Two plastic cups was set at 10ft, 35ft, and 60ft from the river and in both the grassy sandbar and riparian forest. After collecting the cups and sorting through the insects we found that there was a greater difference between the distances from the river than there was between the different habitats. We found the greatest difference to be between the 10ft and 35ft distances. This is significant because it enforces the relationship between water and life on our planet.

Arthropod species diversity in Upper Bidwell Park between the north facing slope and the south facing slope of Big Chico Creek canyon

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BIOL 152 - Ailsie McEntegart

Our project investigating insect diversity in upper Bidwell Park was designed to provide information about whether or not two qualitatively different environments supported different diversities of insects, and to a lesser extent to examine the types of insects found in both of the contrasting environments. The North facing side of Big Chico Creek was more wooded, while the South facing side was open grasslands. Our hypothesis was that the more wooded environment would hold a more diverse population of insects. We calculated the diversity of several sub-habitats on each side of the creek using the Shannon Index, then found averages for both sides. Our statistical analysis determined that there was not a statistically significant difference in the two diversities, with the North facing side average diversity being 1.595 with a standard deviation of 0.2758, and the South facing side average diversity being 1.465 with a standard deviation of 0.04950. Overall, the two sides of the creek seemed to basically share the same species of insects, although some were more common on one side or the other.

Pollutant effects on stomatal density on *Photinia sp.* and *Nerium oleander* plants

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BIOL 152 - Ailsie McEntegart

One of the major structures plants have for gas and water exchange is stomata. Plants regulate the opening and closing of their stomata depending on various factors. Motivation for this experiment was to discover the pollutant effect among plants. Samples of *Photinia sp.* and *Nerium oleander* were collected from high and low traffic locations. After statistical analysis, a t-value of 2.11 for *Photinia sp.* indicated that there was a greater stomatal density in high traffic environments. A t-value of 2.99 for *Nerium oleander*, however, indicated that stomatal density was greater in low traffic environments. The results show opposite responses to levels of traffic emissions, and other factors clearly affect the stomatal density.

Further studies would be necessary to determine whether traffic emissions have a direct effect on stomatal density.

There is no difference in insect biodiversity between riparian areas and areas that are completely exposed in upper Bidwell Park

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BIOL 152 - Ailsie McEnteggart

The purpose of the experiment was to determine whether or not slight changes over an ecological gradient within upper Bidwell Park in Chico, California has an effect on insect biodiversity there by directly measuring the species richness of insects in two different areas of the park: a grassy field open to frequent ecological disturbances and in a Riparian area down by the stream. However, $p > 0.05$ and thus the results failed to support the alternate hypothesis, therefore we were forced to accept the null hypothesis that a difference in insect biodiversity does not occur between these two areas due to slight changes over an ecological gradient in this particular ecosystem. Because the theory dealt with did not hold true for all situations other possibilities must be considered. One possibility is that insects inhabiting upper Bidwell Park could be what are called generalists, organisms able to adapt easily to ecological disturbances. Thus, taxonomic richness isn't necessarily the only factor involved in the stability of an ecosystem.

Stomatal density of *Juglans regia* leaves suggests edges receive more sunlight

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BIOL 152 - Michael Marchetti

Stomatal density is affected by a variety of environmental factors. The following experiment attempted to illustrate that plants located near the edge of an orchard receive more sunlight and as a result have a higher stomatal density than plants in the interior of an orchard. A compound microscope at 400X magnification was used to determine the average number of stomata per field of view. The results indicated that a plant's distance from the edge of the orchard was statistically significant (α value = 0.05, $f = 36.8$, f -critical = 3.7, P -value = 1.6E-06, degrees of freedom = 17). As a result, we rejected our hypothesis that tree placement within an orchard would have no effect on stomatal density. These findings suggest that plants in an orchard are subject to an edge effect, where the plants on the interior of an orchard receive less sunlight than plants located on the perimeter of the orchard.

Investigation of stomatal density in leaves at the highest and lowest height gradients on a tree

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BIOL 152 - Michael Marchetti

The motivation for this study was to see if leaves found higher up a tree have a higher stomatal density than those that are found lower down the same tree. This being known, the study was designed to test if leaves found closer to the sun and above any wind blocking buildings would cause leaves to have higher stomatal densities. We collected 16 leaves from a tree at different height gradients; 8 leaves from 8ft up the tree and 8 leaves from 38ft up the tree. Imprints of the stomata were then observed under a microscope, the average stomatal densities were calculated from the two locations and then a T-test was performed to analyze the results. The results showed that leaves taken from 8ft up the tree had an average of 155.475 stomata per field view and the leaves taken from 38ft up the tree had an average of 156.9 stomata per field view. The T-test was calculated with a p-value of .05 with 14 degrees of freedom. The results showed that the t-crit was greater than t-calc and therefore, we rejected our hypothesis that the leaves from higher up a tree would have higher stomatal density than those from lower down.

Phylogeny of cetaceans by means of protein gene coding

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BIOL 152 - Christopher Ivey

Life on Earth has been dated back 3.8 billion years from today, with the original single celled organisms originating in the vast oceans of the appropriately named, blue planet. Over those 3.8 billion years, life began to take shape into the millions of species humans know today, and the thousands scientists are yet to discover. The purpose of this project was to examine the evolution of one specific trait of aquatic organisms, the ability to breathe oxygen from the atmosphere without the use of gills, to determine whether the evolution of the cetaceans, or whales, forms a monophyletic group. The experiment consisted of thirteen organisms: ten organisms that have blow holes for breathing air from above the water surface, and three organisms that use gills to absorb oxygen from the environment. The software MEGA 4 was then utilized to form a phylogenic tree of all the organisms based on their complete protein gene coding provided by genbank. The findings indicated that the ten cetaceans do form a monophyletic group, showing the attribute evolved once from gilled organisms.

Investigation of biodiversity of terrestrial invertebrates at varying distances comparative to dry creek

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BIOL 152 - Michael Marchetti

A high diversity of insects is crucial to support a complex food chain in a particular area. If a community has a particularly low diversity, it will not be able to support a food chain and thus the particular community will be inadequate of its natural energy source. My question was where, in location to closer and further from the creek, does it have the highest biodiversity? My null hypothesis was that biological diversity of terrestrial invertebrates does not increase further away from the creek. My alternative hypothesis is that biological diversity of terrestrial invertebrates increase further away from a creek, as opposed to closer to the creek. This experiment was conducted by using pitfall cups as means to collecting the insects and then using a dichotomous key to identify them. My results indicated that there was a pattern of increase in biodiversity while going away from the creek. Using the Simpson's index of diversity, my results indicated that the diversity nearest the creek was .539 and the diversity furthest the creek was .745. I can be 86% confident that the average biological measurement means is increases farther from the creek (2SampTInt, n=2). I can reject my null hypothesis, which supports my alternative.

Stomata density in a poisonous and a nonpoisonous leaf

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BIOL 152 - Christopher Ivey

The purpose of this experiment is to differentiate the amount of stomata density in a poison oak leaf and the stomata density in a regular oak leaf. The results of the experiment will be used to learn why or why not these plants are so different when it comes to carbon dioxide intake. The amount of stomata in the poisonous leaves will be lower than the nonpoisonous leaves because the poison oaks oil secretion is a higher trade-off then the plants ability to take in a large amount of carbon dioxide. To begin this experiment, numerous amounts of the two leaf types were collected so the experiment would have consistent numbers. The stomata was then taken from the leaf and looked at through a microscope to determine the count. After the information is gathered, it was put into various analysis tests to determine the stomata density differences between the two plants. After calculating the stomata density on the two different plants, it was proven that the regular oak leaves had three times as many stomata per leaf than the poison oak leaves. These results prove that the carbon dioxide intake of regular oak leaves is higher because they have lower trade-offs than poison oak, meaning that do not have the extra defense mechanism that poison oak has allowing regular oaks focus on stomata to be higher.

Gradient analysis of aquatic bottom-dwelling animal diversity in Big Chico creek

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BIOL 152 - David Wood

Creek-bottoms are an extremely diverse landscape and conditions vary substantially based on elevation, geology, amount of sunlight, latitude, etc. Erosion caused by the constant flow of water forms diverse sections of creek-bottom in relatively small areas. These sections are unique and it makes sense that the life present in different sections may also be unique. This experiment was designed to test the difference in taxonomic richness between two creek-bottom types: Rocky Bottom with stones ranging in size from pebbles to large boulders in excess of 100 pounds (mostly small rocks ranging from 10-20 lbs), and Silt/Mud bottoms with few rocks. Four samples were taken from each of these environments by plotting out 1ft² areas and then collecting and counting all bottom-dwelling animals present. An analysis of the data was then conducted using a Shannon Index, which provided us with details regarding the existence of an ecological gradient between these two habitats. As predicted, very few animals were found in the muddy sections in comparison to the rocky environment. This finding was not surprising, as many insect larvae and mollusk species require rocks to cling to. It was surprising though how few animals were living in the muddy soil sections.

Insects on evening primrose vs. daisy

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BIOL 152 - David Wood

We were interested in seeing if insects preferred one species of flowers vs. another and whether color had any effect on the insects. We hypothesized that the white daisy with a dark purple center would have more insects vs. the pink evening primrose. To test this hypothesis we looked at thirty flowers from each species and counted the number of insects on them. After counting the number of insects on the two different species of flowers, it was clear that the white daisy attracted far more insects than the pink primrose. The insects were more sparse and spread out on the primrose, where as on the daisy the insects were all located near the center. We concluded that the insects were more attracted to the dark center on the white daisies vs. the primrose.

Stomata density of sycamore trees in urban vs. rural environments

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BIOL 152 - Christopher Ivey

How are plants affected by automobile emissions and everyday life in urban areas compared to rural environments where they would have little to no contact with pollutants from

automobiles and people. This research will show the effects of city life on stomata density of sycamore trees. Through our research we hope to show concrete evidence that our everyday lives are having an effect on the surrounding plant life. Our alternative Hypotheses is that the leaves retrieved from Bidwell Park will have a greater average number of stomata than leaves retrieved from downtown Chico. Our null Hypotheses is that there will be no significant difference in stomata density between trees in the two environments. Methods: We obtained 10 leaves (5 from Bidwell park and 5 from downtown Chico) to use to calculate average stomata density for trees in the two environments. Once abstracting the 3 census stomata samples from the each of the leaves we placed the sample under the microscope under 40 times magnification. At this magnification we counted the amount of census stomata in one field of view for each sample. If there is a significant difference of stomata density.

Insect diversity and richness in jeopardy due to community development

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BIOL 152 – David Wood

In the United States the most significant cause of biodiversity reduction is fragmentation due to loss of habitat. Some of the most detrimental factors that lead to loss of species biodiversity are climate change and human activities, which is what inspired this experiment. We examined two different insect communities, developed (where recent residential construction has taken place since 2004) and undeveloped, that run parallel to a drainage where Doe Mill road crosses East 20th street. The 48 pitfall traps were divided into groups of 6 and were spread out evenly dividing the developed and undeveloped communities each totaling 24 pitfalls. We confirmed our hypothesis when we determined there was a higher taxonomic richness in the undeveloped community with an average of 9.5 taxa while the developed community averaged 7.5 different taxa. We did not confirm our hypothesis regarding species diversity using the Shannon Index. We determined the average diversity for the undeveloped community was 1.34 and the developed community averaged 1.79. These results support the idea that taxonomic richness is affected by the developing area while the species diversity seemed unaffected.

Adjoining tank to detect color specific aggressive responses in the Siamese fighting fish (*Betta splendens*)

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BIOL 152 - Christopher Ivey

Betta splendens are known for their brilliant colors and aggressive behavior against conspecific males. Color may be of significance suggesting that a specific color, such as red is more aggressive than other colors among the species. Conspecific mature male *B. splendens* of

both different and the same colors were placed adjacent each other for one minute. Three colors (red, blue, yellow) totaling six *B. splendens* were trialed adjacent each other in an adjoining tank performing four replicates each. Results showed that red and blue were significantly more aggressive than yellow by 79% (significant at $p < 0.05$, $p = 0.0001$). It was also found that fish of similar colors were 48% more aggressive toward each other than to fish differing in color (significant at $p < 0.05$, $p = 0.038$). Therefore, color was a significant factor regarding aggressiveness in *B. splendens*.

The difference in elevation of a habitat has no effect on terrestrial insects' communal diversity through the use of pitfall traps in Upper Bidwell park

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BIOL 152 - Shelly Kim

If communities were diverse in accordance with their elevation, then new data could be discovered that certain communities prefer certain elevations in which to obtain their optimal habitats in. The null hypothesis was that there would be no significant difference in communal diversity throughout the different elevations. The alternative hypothesis was that the habitat that was located at approximately 297 feet would have the most species rich community. The experiment was conducted at Upper Bidwell where three different elevations were selected: 240 ft, 297 ft, and 358 ft, with three cups at each elevation. After analyzing the insects from each cup, an ANOVA table was used to compare our results; our p-value was .31. Our results displayed that there was no significant difference of diversity throughout each elevation, and that elevation had no effect on terrestrial insect diversity. Our results showed that the elevation had no apparent affect on terrestrial insects' communal diversity.

Effect of light on the development of Pacific tree frog tadpoles

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BIOL 152 – Christopher Ivey

Exposure to light and prolonged exposure to complete darkness affected the growth and development of pacific tree frog tadpoles. We found that the development of limbs was effected the most by the two different light exposure variations. 100% of the tadpoles kept in the dark had fully developed back legs after 6 weeks, while only 2 % of the tadpoles kept in normal light conditions had fully developed back legs. 4.5 % of the tadpoles kept in the dark began to develop front legs while 0% of the tadpoles kept in normal light conditions showed any sign of front leg development. This data was collected by keeping four one gallon tanks of purified water with 10 tadpoles in each and subjecting two of the tanks to normal light conditions while keeping the other two tanks in total darkness. The motivation for this

experiment was to see whether light played as important a role in the development of animals as it does in plants.

Stomata density of blue oak trees at varying elevations

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BIOL 152 - Christopher Ivey

Environmental influences have been shown to affect the stomata density of plants. The purpose of this project was to determine the affect elevation had on the stomata density of blue oak tree's of Northern California. We gathered three different samples, one from the north side of a hill, another from the south side of the hill, and one from the top of the hill. We then used light microscopy at 400x to approximate the stomata density. The sample taken from the north side of the hill had on average 39 stomata per leaf while the sample taken from the south side had on average 38 stomata per leaf and finally the top of the hill had on average 48 stomata per leaf. The findings indicated that elevation played little, or no, role at all in the number of stomata per leaf.

The evolution of bufotoxin production as a synapomorphy

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BIOL 152 - Michael Marchetti

Bufotoxins are the neurotoxins that are produced from parotoid glands, usually found on the dorsal section of the organism, for defense. Since bufotoxins are present in such a variety of toads and some other amphibians, we wanted to find how these toxins developed during its evolution. Our hypothesis was that bufotoxins developed in a monophyletic pattern through its evolution. We used 12s RNA genetic sequences, found in Genbank, and inserted the sequences into the program MEGA 4. This program analyzes sequences in comparison to a common ancestor to find the interrelatedness between each species' genetic code. A phylogenetic tree is then constructed to display the ancestry between all selected species. The tree illustrated that the bufotoxin trait evolved from a common ancestor, but not all of the descendents demonstrate the production of bufotoxins. This is evident to show that bufotoxins are paraphyletic.

Germination success as a product of depth of planting

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BIOL 152 - David Wood

Important factors in the life and death of any annual plant species such as Rye Grass, are environmental signals such as water and light availability which may trigger certain events

within the life cycle of individual plants, such as germination or senescence. Depth of planting has an obvious and well documented correlation to both of these factors as well as many others, which may or may not contribute to the germination success of seed plants. In an experiment conducted at California State University Chico, twenty rye grass seeds were planted at 6 depths in 5 replicates and allowed to sit for 1 week and then tallied according to which individuals exhibited growth beyond the soil surface. Germination was most successful at two and three centimeters below the soil surface, and was null at a depth of six centimeters. According to the results of a single factor ANOVA test, depth of planting played a significant role in the germination success of Annual Rye Grass seeds.

The effects of golf course maintenance on biodiversity

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BIOL 152 – Michael Marchetti

Human activities have an impact on nature. The use of pesticides, fertilizers and herbicides are all associated with golf course maintenance. Pesticides affect the type of insects that are found while the combined use of fertilizers and herbicides affect the type of vegetation that can be found on and near a golf course. The effects of golf course maintenance on insect diversity along Big Chico creek were investigated using pitfall traps. Ten traps were set in two ecological zones, one bordering Big Chico creek and Bidwell golf course, and the other bordering Big Chico creek and Bidwell park. The insect diversity in each area was analyzed using both the Simpson and Shannon index of diversity. A total of 1654 insects from 13 distinct taxa were collected over a period of five days. The t-test analysis showed no statistical difference between the means of insect diversity for the two ecological zones at a p value of .05. These results indicate that golf course maintenance has little to no effect on creek-side insect diversity.

Ecological gradient analysis between Salmon Hole creek in Upper Bidwell and the Big Chico Creek east of the CSUC campus

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BIOL 152 - Christopher Ivey

The Salmon Hole location consisting of two habitats (which are near/alongside the creek and far creek spots) in Upper Bidwell Park showed no significant overall taxonomic diversity difference from the Big Chico Creek location consisting of two habitats (near/alongside the creek and far creek spots) just east of the CSUC campus after a total of 24 cups were distributed evenly in both locations in a total time span of 7 days. Six samples were taken from each habitat from both the Salmon Hole and Big Chico Creek locations. The habitats near/alongside the creek were about 1 foot-1 meter in distance from the creek and the far creek habitats were about 4-5 meters in distance from the creek. After counting plus

identifying all our data/specimens collected in our cups from both locations, we then input our data collected in excel and then used the ANOVA software which gave us the results of a F-crit of 2.816 and a P-value of 0.539. With a P-value of 0.539 we weren't able to reject our null hypothesis. Our null hypothesis stated that the habitats from the Salmon Hole location don't have a significant higher taxonomic diversity than the habitats in the Big Chico Creek location; even though human trafficking/obstruction is far greater in the Big Chico Creek area than in the Salmon Hole area. Therefore this disclosed the idea that human trafficking/obstruction is not a factor nor affects the taxonomic diversity in the Salmon Hole area.

Similar stomatal densities among plant species indicates relatedness

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BIOL 152 - Christopher Ivey

Stomata are critical to plants because they facilitate the plant's absorption of carbon dioxide to be used in photosynthesis, as well as to facilitate the release of photosynthesis' byproduct, oxygen. The rate of the absorption and release of gasses is relational to the amount of stomata a plant has. In our experiment, we observed the stomatal densities of seven various plants and constructed a phylogenetic tree. We did this to determine if similar stomatal densities indicate relatedness among plants. The plants used in our investigation were *Theobroma Cacao*, *Tacca Integrifolia*, *Musa Acuminata*, *Musa Velutina*, *Musa Coccinea*, *Piper Aurtium*, and *Piper Betle*. After counting the stomata of the plant samples under light microscope, we compiled the data into a bar graph. A phylogenetic tree indicating genetic distances was constructed using the rbcL gene, and the genetic distances of the seven plant species and assembled a scatter plot. From the scatter plot, a P value of .0512 was calculated, which allowed us to reject our null hypothesis, and accept our alternate hypothesis that genetic distance, as determined from a phylogentic tree, will have some correlation with stomatal densities. With an R value of .043091, the results led to our conclusion that there is a relationship between genetic distances and stomatal densities; as genetic distance between pairs of plants increases, the difference in stomtal densities increases as well.

Ecological diversity of insect populations in forest and meadow habitats of Lovelock, CA found to be not significant

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BIOL 152 - Michael Marchetti

We wanted to expand on a previous experiment performed on Big Chico Creek where the ecological diversity of insect populations was found to be not significant, so we decided to perform the experiment on a different environment. We chose an area with forest and meadow using pitfall traps to collect insects. Data was analyzed using ANOVA, and was found to be not significant (F=0.55, df=8, p=0.60).

Micro invertebrate diversity in kept & unkept lawns

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BIOL 152 - Ailsie McEnteggart

In this study, we researched the diversity of micro invertebrates in lawns that were kept and unkept. Kept lawns were lawns that were regularly cut, watered, and fertilized. Unkept lawns were lawns that were not maintained or watered. Some variables could be the use of pesticides near the kept lawns and the frequency of watering. Our hypothesis was that unkept lawns would have a higher diversity because the species were left uninterrupted due to lower amounts of traffic and less frequent trimmings. We placed twelve pitfall traps-three in each of two kept lawns and two unkept lawns. After five days the traps were collected and the numbers of micro invertebrates were counted. Using the Shannon Index to determine diversity we were unable to determine a significant difference in the two habitats. This may have been due to an extreme amount of ants collected in a few of the lawns. If repeated, we suggest using more lawns to collect samples from.

Species diversity in grazed vs. ungrazed land

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BIOL 152 - Christopher Ivey

The Intermediate Disturbance Hypothesis states that species residing in areas with moderate levels of disturbance will have greater productivity than species residing in areas with high or low disturbance levels. Grazing cattle will certainly cause disturbance to the plant species in a field, affecting the species richness which in turn, will affect the biomass levels in each area. In order to measure the significance of grazing on species richness and biomass, we collected every plant within twenty separate one square foot areas; ten from an ungrazed field and ten from a grazed field. Upon statistical analysis using a t-test, we determined that there was indeed a significant difference in species richness between grazed and ungrazed land ($P = 0.009$)

but no significant difference in biomass ($P = 0.1$). Although we can say that our data supports the Intermediate Disturbance Hypothesis for species richness, it does not support our prediction that species richness and biomass are related.

Variation in stomatal density across a natural gradient

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BIOL 152 - Michael Marchetti

Stomata are microscopic pores on the undersides of leaves that allow CO₂ to enter, and water and free oxygen to leave. An increase in the number of stomata per unit area allows the plant

to take in more CO₂ and to release more water. The stomatal density can vary within a plant or individuals as well as between species. This variation is caused by many factors, including changes in sunlight and atmospheric CO₂. The stomatal density of leaves found near or away from water should be different because more precipitation results in more stomatal density. In this experiment we examined this difference by collecting leaves near water and away from water. We collected leaves from trees at three sites in Lower Bidwell Park. The first sample of leaves came from a tree that's leaves were over Big Chico Creek, the second sample of leaves was collected twenty three feet away from the creek, and the last sample was collected forty six feet away from the creek. After the leaves were analyzed underneath a microscope, we found that leaves collected directly over Big Chico Creek had more stomata than leaves collected from further away from the water. Using an ANOVA test we found a P-value of 0.00001, which indicates a significant difference between the three collection sites. The results of the data showed that leaves found near the Big Chico Creek had higher stomatal density than leaves found further away.

Effect of highway 99 on valley oak stomatal density in Bidwell Park

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BIOL 152 - Michael Marchetti

Various environmental conditions including sun-exposure, humidity, and atmospheric CO₂ concentrations can account for differences in stomatal density between and within species. Anthropogenic effects such as increased CO₂ and other greenhouse gas emissions, especially in heavily populated areas, can have a significant impact on nearby biological habitats. According to the literature, when CO₂ is more abundant, stomatal density falls. When CO₂ concentrations are high, plants need fewer stomata in order to obtain an equal amount of carbon required for photosynthesis. This study investigates the affect of freeway pollution on stomatal density of oak trees in Bidwell Park, Chico, California. Five leaf samples were collected from three separate Valley Oak trees at 0, 1 and 2 miles from Highway 99 and an average number of stomata per field of view at 400X was compiled for each tree. Averages were analyzed using Microsoft Excel and ANOVA. Results indicate a significant difference between averages, however, no gradient in stomatal density was observed. Although we expected the tree closest to the freeway to have the lowest stomatal density, this was not the case—the near Valley Oak tree actually had the highest average density at 62.28 stomata per field of view. Possible future work may include utilizing a larger distance between samples and more than simply one tree to represent a single location.

Sunlight reduces stomatal densities of live oak leaves in Upper Bidwell park

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BIOL 152 - Michael Marchetti

Stomata are microscopic pores that permit movement of gases between the atmosphere and the intercellular space of leaves. A leaf's stomatal density inherently affects its rate of gas exchange, and consequently, since carbon dioxide and water are necessary for plants, atmospheric carbon dioxide concentration and water availability are two obvious factors affecting stomatal densities. However, another possible stomatal density determinant is sunlight exposure. Some research has explored this possibility, but it has given confusing results. For example, Aleric found fewer stomata in shady environments, whereas Hovenden found more stomata in shady environments at certain altitudes. To help clarify the confusion, we sought to analyze the effect of sunlight on the stomatal densities of leaves of live oak trees in Upper Bidwell Park. In pursuit of this goal, we found two environments in the park with extremely different sunlight exposures. We collected leaves from each of these two environments and determined their stomatal densities by creating impressions of the leaves' stomata using nail polish and tape, and employing light microscopy. Our results showed that the leaves from the sunny environment had fewer stomata ($P = 0.0004$). One plausible explanation for these results is that the leaves in the sunny environments had fewer stomata to decrease their likelihood of desiccation from their intense sunlight exposure. However, since there were many variables that could not be standardized and the results disagree with most previous studies, we are timid to extrapolate our findings into an exact relationship between sunlight exposure and stomatal density.

A study of variance of stomatal density relative to location and light intensity on *Hedra helix*

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BIOL 152 - Christopher Ivey

After conducting two separate, in class experiments comparing light intensity (Sun vs. Shade) and plant location (near or far from Big Chico Creek) and receiving inconclusive results, we felt that our hypothesis needed further investigation. We designed a new experiment in which our sample size was increased significantly and allowed us to do a cohesive investigation comparing the effects of location and light intensity on *Hedra helix*, commonly called English Ivy. We believed that plants in the dry, sunny locations would have a higher average stomatal density due to the potential for increased desiccation via water loss from the stomata. The plants with more stomata would have more control over water retention, and be able to survive in more arid climates. To test this hypothesis, we collected samples from plants close to the creek in sunny and shady locations, as well as samples from plants farther from the creek in sunny and shady locations. After collecting, we

returned to the lab and proceeded to take impressions of the leaves to study under the microscope, recording our observations and calculating average stomatal density per mm². After running a statistical ANOVA, we found that there was no difference in density relative to light intensity, but a very high level of density for plants near the creek. Although our conclusions do not support our hypothesis, they are significant for understanding adaptive evolution in *H. helix*.

Testing the allelopathic effects of Tree of Heaven (*Ailanthus altissima*) on seed germination of radish seeds (*Raphanus sativus*)

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BIOL 152 - Shelly Kim

In this experiment we tested the effects of an invasive species of plant known as Tree of Heaven on radish seed germination. We decided to perform this experiment because we were interested in seeing if the leaves contained the same allelopathic tendencies found in the root as shown in other experiments. We muddled leaves of the Tree of Heaven with water creating 3 different concentrations that we poured on seeds covered with paper towels inside of labeled petri dishes. Six days later we collected the seeds and statistically compared the germination ratios for each treatment. All though there were less germinated seeds in the treatments, an ANOVA test was conducted for all 4 treatments and no significant difference was seen (f-value of 1.697.) These results can be used to direct further studies into the allelopathic effects of Tree of Heaven. Further studies can be conducted to show that the allelopathic chemicals need to be secreted through the roots in order to affect germination of other plants. Perhaps the allelopathic chemicals need to have other chemicals present or chemical reactions in order to thwart germination of other plants. A more extensive study of the allelopathic properties of Tree-of-Heaven may show more significant results.

Biotic diversity: open meadow vs. wooded area (Upper Bidwell park, Chico CA)

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BIOL 152 - Shelly Kim

Biotic diversity is noticeably influenced by many factors, including, but not limited to, moisture level, amount of direct sunlight, soil type, and temperature. All of these factors are addressed when considering biotic diversity of an open meadow versus a wooded area. The differences in biotic diversity between the two environments were presumed statistically different. In an attempt to quantify biotic diversity of the differing environments, a series of bug traps was set in Upper Bidwell Park- 15 traps in a meadow area and 15 traps in a wooded area. Collection sites were approximately 30 meters apart with similar flora. The contents of the traps were collected after four days and the macroscopic invertebrates were cleaned, sorted and identified. Both environments contained large numbers of species, mites

being the largest group by far (a population estimation of over 7000 evenly distributed throughout both collections). Of the remaining specimens the most abundant taxa were flies, ants, beetles, and dragonflies. The samples from the wooded environment contained a larger number of species and a larger population in each common species. (Shannon Diversity Index wooded= 0.30, meadow= 0.19) This isn't surprising because the wooded area receives less direct sunlight, protecting it from wind so the soil retains more moisture. Additionally, a layer of decaying leaves approximately 2-3 inches thick covers the ground in the wooded area creating an environment rich in nutrients. The lack of direct sunlight and the layer of leaves also serve to keep the average temperature lower during the hot season.

Upper Division Class Projects

Bacteria reaffirming what we already know: there's nothing like a good cup of joe

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BIOL 321 - Gordon Wolfe

Caffeine is a natural stimulant for humans but is found to be toxic to many other organisms. Coffee pulp waste produced from coffee plants and the solvents used to remove the caffeine are expensive and harmful to the environment. Some microbes have been found to naturally degrade caffeine in a more bio-friendly way. Some known species have been studied for their caffeine degrading pathways, such as *Pseudomonas putida*, *Serratia marcescens*, and *Klebsiella* species from the phylum Proteobacteria, and *Rhodococcus* species from the phylum Actinobacteria. These bacteria are typically found in moist environments. In attempt to isolate bacteria capable of utilizing caffeine, a soil sample was taken from the CSU, Chico campus and a bacterial suspension was made. This was used to inoculate a medium composed of TSB and coffee grounds. A series of sequential dilutions were made in order to wean the bacteria off other carbon sources and grow solely on caffeine. Several phenotypic tests that were carried out include motility, oxygen requirement and a Gram stain. A PCR was run on the sample to determine the genotype of the bacteria. Once determining that the bacteria isolated was a Gram positive, endospore-forming, motile, obligate aerobic bacillus, it was concluded that the successfully isolated bacterium belongs to the genus *Bacillus*.

Glutamic acid induced excitotoxicity: Cyclosporine A is neuroprotective.

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BIOL 418 - Jonathan Day

Glutamate (Glu) binds to ionotropic receptors on the postsynaptic membrane which allows cations like calcium to enter the cell; this is the basis of membrane depolarizing. High concentrations of Glu can induce excitotoxicity. Under these conditions high calcium concentrations within the neuron can disrupt mitochondrial function and lead to cell death. It has been shown that CsA substantially improves cell viability after excitotoxic insult *in vivo*. Whether or not CsA affects neurons directly or if the neuroprotective effects are mediated by non neuronal cell interactions is unknown. If CsA acts directly on neurons then the application of CsA to neurons in culture should increase viability of neurons exposed to an excitotoxin. We hypothesize that CsA is neuroprotective, and the application of CsA to neurons prior to excitotoxic insult should result in higher neuronal viability compared to controls. To test the neuroprotective actions of CsA, cells were pretreated with 25 μ M CsA for 15 minutes and then 100 μ M Glu was added for an additional 15 minutes. Cells were rinsed and incubated for 24 hours prior to the viability assay. The data were analyzed by one way ANOVA and Dunnett's post hoc analysis. Results showed that CsA was neuroprotective. Almost 60% of the cells treated with CsA survived Glu insult, compared to 10% survival for 100 μ M Glu alone ($P < 0.002$). These data support our hypothesis and demonstrate the direct action of CsA on neurons. These results suggest that CsA could be evaluated as a pharmacological intervention for neurodegenerative disease.

Characterization of five *E. coli* bacteriophages isolated from sewage

Ryan Berryman, Brandy Butler, Julia Canders, Keerthana Chandrakuma, Matthew Cheung, Alena Chincurtis, Lisa Dietrich, Amanda Farley, Corey Henderson, Seth Jones, Trisha Lund, Sarah Montez, Catherine Neutze, William Reeder, Audrey Sharp, Matthew Vakili, Schuyler VanBuskirk, Mee Yang
BIOL 476 - Larry Hanne

Bacteriophage (phage) are viruses that infect bacteria. As with other viruses, phage are very specific with respect to the host species they will infect. We enriched for phage in sewage that could replicate in various strains of *E. coli* (MM294, DH5 α , C91, or B). Based on plaque morphology, we each isolated different phage. Each phage was tested for envelope, temperature stability, single hit kinetics, host specificity, burst size, and size of structural proteins. Generalizations that we can make about these 5 phage isolates are: All are non-enveloped; most could tolerate temperatures of 60 to 80°C; most followed single hit kinetics; average burst size was approx 500 viruses per infected host cell; and, attachment did not seem to be completely host specific. We are presenting examples of some of the data collected from these experiments.

Impacts of cattle grazing on fish communities in a California stream

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BIOL 404 - Michael Marchetti

This study investigated the effects of cattle grazing on the biomass and abundance of fish in a small California stream by comparing stream sections that were heavily grazed,

intermediately grazed, and non-grazed. Cattle grazing pressures are known to negatively affect fish by altering stream nutrient concentrations, riparian vegetation, and channel morphology. Fish communities in three sections of Willow Creek were electroshocked to obtain estimates of biomass and abundance. Results indicate no significant difference of average biomass per meter and abundance among the three sampling locations. However, both total abundance and average biomass per meter appears to be negatively correlated with turbidity at each site. These findings suggest that cattle grazing may not impact fish biomass or abundance within Willow Creek, or that cattle grazing is only occurring in the intermediate site and not the heavily grazed location. Results in the intermediately grazed site may also be explained by possible sediment contribution from the Eagle Lake outflow channel. The negative relationship between turbidity and fish parameters supports the potential for grazing to negatively impact fish communities. Further research may better explain whether cattle grazing alters fish biomass and abundance or if these parameters are primarily influenced by turbidity.

Niche partitioning of two species of ants

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BIOL 350 - Shelly Kim

The dietary preferences of two species of ants found on the California State University Chico Campus were analyzed and compared. Four sites, two for each species, were used. Three different types of food: honey, tuna, and nuts, were placed by each of the four sites and the weights of each food type before and after were recorded. It was hypothesized that there would be a statistically significant difference in the dietary preferences of the two ant species due to the fact that both species appeared to be coexisting successfully, leading to the conclusion that they partitioned the local resources differently. According to statistical data measured using two trials it was found that there was a mathematically significant difference between the dietary preferences of the two ant species. An ANOVA test comparing the food type consumption of each species was used to determine if there was a statistically significant difference. A resulting P-Value of 0.000137 allowed for the null hypothesis that there was no statistically significant difference to be rejected.

Isolation and identification of radiation resistant bacteria from a Wilbur Hot Springs sample

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BIOL 321 - Gordon Wolfe

Radiation resistant bacteria are typically gram positive, non-motile, aerobic extremophiles. Their genotypic characteristics allow them to thrive in extreme conditions. They are commonly found on the human skin which is continuously exposed to the sun's rays, as well

as irradiated foods. The purpose of this project was to isolate radiation resistant bacteria, mainly *Deinococcus radiodurans*. Mud was used from three different Wilbur Hot Spring samples and plated on TSA plates. These plates were then exposed to gamma radiation for forty-five minutes and incubated at 28° C until growth occurred. Once colonies were present the plates were re-streaked and exposed to the gamma radiation once again and incubated as before. A pure culture was obtained from the Wilbur Hot Springs Sample 08 plate #1. In order to determine the species of bacteria we were culturing we conducted a series of tests including fermentation, motility, oxygen utilization and two gram stains. Our isolate ferments sucrose and glucose, is non-motile, a facultative anaerobe, and a gram positive cocci. A PCR was performed and was submitted to Genbank for analysis and it was determined that our sample was not the hoped for *Deinococcus radiodurans*, but another radiation resistant genus *Kocuria*. *Kocuria* is part of the Actinobacteria phylum and is a skin bacterium.

Aestivation patterns of *Helix aspersa* in varying relative humidities

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BIOL 350 - Shelly Kim

California's prevalent terrestrial land snail, *Helix aspersa*, commonly goes into a period of dormancy, or aestivation, to overcome hot and dry conditions in its Mediterranean environment. In order to prevent water loss, the snail secretes a mucosal membrane, which then dries into an epiphragm, protecting it from potential desiccation. With only a tiny hole to breath through, the snail becomes a recluse from the environment, in a state of arrested activity that lasts sometimes for months at a time. Of the environmental conditions affecting the snail, our study focuses on relative humidity as the prime factor influencing aestivation. From an ecological perspective, identifying a relative humidity range in which aestivation no longer occurs among snails, a relation to feeding periods may be found. Using saturated solutions of manganous chloride, sucrose, and pellets of dry potassium hydroxide, the humidities in three glass terraria were varied in order to develop an understanding of when snails de-aestivate. It was found that the critical relative humidity in which snails are found to begin de-aestivation ranged between 75% and 80%, with a mean de-aestivation percentage of 84%. Above the critical relative humidity value there tended to be a greater percentage of de-aestivation amongst snails, while below this value there tended to be a greater percentage of aestivation.

Decomposition rates in woody plants

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BIOL 350 - Shelly Kim

The activity of detritivores plays an integral role in nutrient cycling and the overall rate of ecosystem energy flow. Riparian environments produce large amounts of leaf litter that rely on these decomposers to break down the organic matter. Some factors influencing the decomposition rates depend on the type of leaf material, the amount of nutrients in the water, and the abundance of invertebrates, fungi, and bacteria present in the stream. Data was obtained by measuring out four samples of leaves at a known weight for each of the three tree species examined. A sample of each species was collected from Big Chico Creek every week for four weeks, where they were allowed to dry for a day before the weights were documented. We tested the hypothesis that there would be a significant difference in the rates of decomposition between Common Manzanita (*Arctostaphylos manzanita*), Bigleaf Maple (*Acer macrophyllum*), and Valley Oak (*Quercus lobata*) leaves. After four weeks, both the Valley Oak and Maple leaf matter decomposed over 80% compared to Manzanita leaves, in which only a little over a quarter was decomposed. The Manzanita had physically tougher leaves than compared with the Oak or Maple, which corresponded to a lesser decomposition rate.

Characterization of radiation-resistant bacteria isolated from the Great Salt Lake, Utah

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BIOL 321 - Gordon Wolfe

Radiation resistance is due to very efficient DNA repair systems, which make them able to sustain harsh conditions such as UV radiation and drying. In order to isolate radiation resistant bacteria, specifically *Deinococcus radiodurans*, we exposed TSA agar plates inoculated with water samples from The Great Salt Lake to {time period} of shortwave UV radiation. After growth of several diverse cultures, we attempted to isolate three pigmented colony types, labeled p3A, p3B, and y4A. We characterized these three strains morphologically and genetically. We attempted to test their resistance to drying (using salt media) and different length exposure to UV radiation. Although we were unsuccessful in our attempt to isolate *Deinococcus radiodurans*, 16S rRNA analysis showed that all three strains of our radiation resistant bacteria were Actinobacteria: *Cellulosimicrobium* sp., *Arthrobacter* sp., and *Staphylococcus epidermidis*.

Interactions between acorn woodpeckers (*Melanerpes formicivorus*) and both the California ground squirrel (*Spermophilus beecheyi*) and the western grey squirrel (*Sciurus griseus*) in the One Mile Lower Bidwell park recreation area.

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BIOL 350 - Shelly Kirn

After the oaks have dropped all of their acorns and they become scarce, the squirrels and woodpeckers alike turn to their stores and granaries for food. The Acorn Woodpecker bores holes in the trunks of trees and forces acorns in for storage; the squirrels dig numerous holes in the dirt at the base of the trees and bury their acorns. For areas with high squirrel populations there can be instances of squirrels robbing the Acorn Woodpecker granaries for food when their ground stores have become empty. The question we proposed was which squirrel species would risk injury by raiding the woodpecker's granaries more, the ground squirrel or the grey squirrel? Our hypothesis was that the larger and bolder grey squirrel, which relies on acorns for its diet more than the ground squirrel, would be more apt to fight the woodpeckers and steal from their acorn stores. Our observations, however, came to the conclusion that neither squirrel stole from the granaries. There could be many reasons for this, including the time of day we observed the squirrels, the time of year, and the fact that last year had an extremely large acorn yield so the squirrels could have buried all they needed and were not wanting of food enough to risk injury by pilfering from the woodpeckers.

Size-assortive coupling in a hermaphrodite, the *Physa* snail: size does matter

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BIOL 350 - Shelly Kirn

Hermaphrodites are commonly found in the animal kingdom. The *Physa* snails used in this experiment exhibit this trait, with the smaller of the mating pair acting as the male donor and the larger acting as the female recipient. The null hypothesis is that there will be no statistical distinction between the average difference in snail size of the mating pair in successful versus contested mating. The alternative hypothesis is that there will be a statistical distinction between the average difference in snail size of the mating pair in successful versus contested mating. In a successful mating the larger female is on the bottom of the pair and the male is on the top. If the opposite occurs the mating is often contested and is illustrated by behavior such as: the bottom snail waving its shell or biting the genitalia of the top snail. A comparison of successful versus contested mating yielded a t-calc value of 0.010308 and a t-crit value of 1.68 when measuring differences of snail shell sizes. Due to these values the null hypothesis was not rejected. In a revised experiment the expected results would support the rejection of the null hypothesis.

Giving up densities of western gray squirrels in urban and rural environments

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BIOL 350 - Shelly Kim

Giving up density (GUD) is the point at which density of available food is too low for foraging efforts to continue. Important determinants for habitat selection by the western gray squirrel includes food, nest-site, shelter, and mate availability. The variation in these over an urban-rural gradient can have an effect on squirrel populations (Bowers, 1996). In order to evaluate the GUD's of western gray squirrels in urban and rural habitats 10 pie pans containing sand and 200 raw sunflower seed were placed in each habitat type where gray squirrel activity had been identified previously. Pie pans were left for 48 hours and the remaining seeds were counted in order to estimate GUD's. Urban sites were located in Chico, CA in backyards and on the CSU Chico campus. Rural sites were located on CSU Chico's Big Chico Creek Ecological Reserve (BCCER), and Butte Creek Ecological Preserve (BCEP) in the foothills surrounding Chico, CA. GUD's were found to be higher in urban habitats than in rural habitats. This result seems to be correlated with the availability of food in selected habitats.

Functional genomics of AT2G34060, class III peroxidase gene, in *Arabidopsis thaliana*

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BIOL 409 - Kristopher Blee

In *Arabidopsis thaliana*, 73 class III peroxidases have been identified; however, the functions of these peroxidases are not fully understood. It is believed that they function in cell wall structure, lignification, and plant defense. Previously, a peroxidase with a short C-terminus was observed to be excreted to the cell periphery, and conversely, a peroxidase with a long C-terminus was observed to be exported to the cell vacuole. AT2G34060, peroxidase 19 of *A. thaliana*, has a short C-terminus. In this study we investigated the function of AT2G34060 by studying its subcellular location. To do this, two reporter constructs tagged with Yellow Fluorescent Protein (YFP) were made. One construct contained AT2G34060 with its native C-terminus. The other construct had the native AT2G34060 C-terminus replaced with the long C-terminus of AT1G71695. Both constructs were successfully inserted into the pMN20GW cloning vector, which was transformed into *Agrobacterium tumefaciens*. Electrophoresis of products of *Agrobacterium* colony PCR using YFP primers produced bands around 700 bp, indicating successful bacteria transformation. *Arabidopsis thaliana* was then inoculated with the transformed *A. tumefaciens*. Microarray data on peroxidase expression within *Arabidopsis* shows that AT2G34060 is expressed in the cotyledon, hypocotyledon, and hypocotyl during early development. If plant transformation is successful, subcellular location of our construct can be observed, which may provide insight into the function of peroxidase.

Peroxidase gene AT4G33870 expression in *Arabidopsis thaliana* leaves

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BIOL 409 – Kristopher Blee

AT4G33870 is a class III secretory peroxidase and is one of over 70 genes in the family. Class III secretory peroxidases are present in all land plants, and are responsible for many physiological processes such as generating reactive oxygen species, polymerizing cell wall compounds, and regulating hydrogen peroxide levels¹. Calculations based on von Heijne mathematics predicted that the AT4G33870 protein is unlikely to enter the cellular secretory system. This is significant as it suggests one will find the protein in the Golgi apparatus. This research introduces the question of: where AT4G33870 will be expressed, on both a cellular and sub-cellular level, and what is its function? AT4G33870 will be expressed in juvenile leaf tissue inside the Golgi apparatus, and based on where it is expressed a likely function is regulation of hydrogen peroxide levels. This hypothesis will be tested by isolating the AT4G33870 gene from *Arabidopsis* genomic DNA, tagging it with yellow fluorescent protein, cloning it into *Agrobacterium tumefaciens*, and then finally by analyzing micro-array data to determine likely expression locations. Results from this experiment show that a reporter construct was generated, successfully transformed into *Agrobacterium*, and then micro array data analysis showed expression of AT4G33870 in juvenile leaves. These results support the initial hypothesis about the location of AT4G33870 expression, and allow, in the future, analysis of *Arabidopsis* leaves for the determination of the cell type that AT4G33870 is expressed, and where it is being expressed on a sub-cellular level in order to determine a more specific function.

Use of the AT2g34060 peroxidase:YFP construct to transform *Arabidopsis thaliana*

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BIOL 409 - Kristopher Blee

Class III peroxidases are secreted glycoproteins known to be involved in the mechanism of cell elongation, in cell wall construction and differentiation, and in the defense of the plant against pathogens. In order to determine the function of our Class III peroxidase from the AT2g34060 gene in *Arabidopsis thaliana*, we developed a construct of the AT2g34060 peroxidase and yellow fluorescent protein (YFP). Through cloning and subcloning using plasmid vectors by Tri-template PCR and electrophoresis, followed by transformation of *Agrobacterium tumefaciens* and colony PCR and electrophoresis screening, we had hoped to insert the construct into the developing seed of the *A. thaliana*, thereby allowing for the expression of the YFP::peroxidase product. We were unsuccessful in verifying the presence of the construct in the *A. tumefaciens*, therefore we used the construct from a previous isolation from a collaborative group preparing the same construct. The bacterium was then

¹ Passardi. F. et al. "Peroxidases Have More Functions than a Swiss Army Knife." Plant Cell Rep. 2005.

applied to the *Arabidopsis thaliana*. This transformation of the *A. thaliana* will allow us to see the expression of the peroxidase-YFP construct in leaves, as the peroxidase produced from the AT2g34060 gene is secreted to the cell wall, which was determined from analysis of on-line data of the *A. thaliana*.

Allelopathy in plant species show 100 percent germination of cucumber seeds

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BIOL 350 - Donald Miller & Shelly Kim

This study involved analyzing the effect of allelopathy of five potentially allelopathic plant species on cucumber (*Cucumis sativus*). Allelopathy is the inhibition of plant growth by chemicals released from a nearby allelopathic plant. As a result, germination of cucumber seeds in the experiment would indicate that the plant is not allelopathic and lack of germination would indicate that the plant is potentially allelopathic. The five studied plants were obtained from the CSU, Chico campus and used to prepare chemical extraction filter papers that were used to plant cucumber seeds on. After a growing period of two weeks, results showed 100% germination in all five plant species studied and in one trial of the control. Trial 2 of the control had 80% germination. Therefore, we are unable to reject the null hypothesis that there would be no difference in germination between the control and five studied plant species. From our results, the plant species studied does not show characteristics of allelopathy.

Isolation of *Bdellovibrio* as plaques from soil

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BIOL 321 - Gordon Wolfe

Bdellovibrio and like organism (BALOs) are predatory bacteria that feed on other microorganisms. *Bdellovibrio* can be useful as a bio-control agent for plant, animal, and human pathogens. Our objective was to isolate and enrich BALO specimens contained within the Chico's soil outside Holt Hall. The sample obtained was passed through different procedures: The sample was vortex, rest for 30 min., and then centrifuged for 5 min. at 500 x g. The sample was centrifuged again for another 40 minutes at 27, 000 x g leaving only BALOs in the supernatant. To obtain pure culture of *Bdellovibrio*, the supernatant was passed through a 0.45 µm Millipore filter to exclude possible microorganisms. The media used to grow *Bdellovibrio* included life prey *Escherichia coli*, *Proteus vulgaris*, and *Enterobacter aerogenes*. The media were incubated at 28°C. After 3 days of incubation, positive plaques were observed in the media containing *E. coli*. In later inoculations, plaques were also obtained in the media containing *E.aerogenes*. Liquid media was prepared to obtain higher numbers of *Bdellovibrio*. Wet mounts were prepared and observed using phase microscopy and Gram stains were also performed. The data collected suggested possible presence of

BALOs. 16S rRNA tests indicated presence of two families, (Oxalobacteraceae and Comamonadaceae) in the same class Betaproteobacteria and order Burkholderiales, which is known to contain some BALOs.

Testing the river continuum concept: Butte Creek watershed to the Sacramento river, Butte County, California

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BIOL 404 - Michael Marchetti

The River Continuum Concept of Vannote et al. (1980) produced a fundamental model for understanding lotic systems in the field of aquatic ecology. The RCC model describes predictable changes in producer and consumer communities along a continuum based on changes in the processing of organic matter as stream morphology changes. To test the RCC in a Northern California Watershed, the composition of benthic macroinvertebrate communities were analyzed at four sites along streams within the Butte Creek Watershed of Butte County, CA. Comparisons were made between the communities at each site using functional feeding groups and stream orders. The variation in taxonomic richness and diversity were significant among the four sites sampled ($p = 0.002617$, $p = 0.0023$), but some site specific trends in diversity may indicate interference from dams and diversions along the main channel of Butte Creek. Functional feeding group composition correlated to the RCC,

especially the groups important to the breakdown of allochthonous material (Shredders) and uptake of downstream FPOM (Collector Gatherers and Collector-Filterers).

Foraging activity of *Helix aspersa* directly decreases with relative humidity

Lisa McKowan, Clint Nelson, Melissa Ng, Breann Peck

BIOL 350 - Donald Miller & Shelly Kirn

Helix aspersa's ability to keep their foot and mouthpart extended is dependent on relative humidity (Arad, 2001). Its effect will be determined by recording snail activity in a sealed terrarium as the relative humidity is gradually reduced. This will test hypothesis that snail activity will decrease and eventually stop as relative humidity decreases. The null hypothesis is that relative humidity will have no effect on snail activity. In addition, the hypothesis that light will affect *H. aspersa's* response to humidity will be tested against the null hypothesis that activity in lit and unlit conditions will be the same. Two replicates were conducted on separate days, observing a total of 21 snails. In each replicate, half of the snails were placed in brightly lighted terrarium, while the other half was tested under normal lighting. The relative humidity was decreased in small increments by removing saturated paper towels and venting. Both lamp and no-lamp best fit lines suggested that snails became more inactive as relative humidity decreased, but the correlation coefficients were quite low

($R^2=0.2447$ and $R^2=0.3966$). While this trend was clearly more distinct in a lit terrarium, an Avova test showed that lit and unlit activity levels were not statistically different ($p=0.208$).

The study of aquatic invertebrate activity using light traps in Eagle Lake, CA

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BIOL 404 – Michael Marchetti

The littoral zone of most lakes contains a diverse amount of copepods, cladocera and other zooplankton. A general lake pattern for these organisms is upward movement from the deep at dusk and vertical movement downward at dawn. In Eagle Lake, California the question was asked, do active zooplankton move when introduced to a light source? If they do become more active when attracted to light then numbers of zooplankton would increase when it gets dark. This study supposes by hypothesis that zooplankton will be more responsive to light on the surface after dark, than during the day. Four light traps were employed using glow sticks and sampling jars attached. Traps were placed near the shore of Eagle Lake, spaced twelve feet apart and three time periods were sampled for one hour each during, light, dusk and dark periods. The p-value for the copepods was 0.292, for cladocera the p-value was 0.321 and for other zooplankton the p-value was 0.281. Four aquatic insects were also collected in the light traps along with the zooplankton; one Corixidae and three Naucoridae from the order Hemiptera. The results show that the hypothesis could not be supported. There was no significant difference in activity during each sampling period

Investigation of the expression and function of the peroxidase class III gene AT4G33870 in *Arabidopsis thaliana* using yellow fluorescent protein

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BIOL 409 - Kristopher Blee

Class III secretory peroxidases are a set of diverse enzymes common to all plants. They are involved in a broad range of physiological process and vary in their pattern of expression. There are 73 multifunctional types of Class III peroxidases in *Arabidopsis thaliana*, each with a potential different expression pattern that would help identify respective function. Several molecular biology approaches have been developed to isolate, characterize and study the expression of peroxidase genes in plants. This study utilizes yellow fluorescent protein (YFP) to tag peroxidase gene AT4G33870 in order to locate expression and determine function of the enzyme. Successful construction of a tri-template reporter construct enabled the cloning, subcloning, and transformation of our tagged peroxidase gene into *A. thaliana* to observe its expression and elucidate its cellular function. Based on micro-array data available in the public domain, we discovered expression in the juvenile leaf of *A. thaliana*. Future investigation of the expression of our YFP tagged reporter construct will reveal the specific cell wall location and subcellular function of our peroxidase.

Foraging behavior in high-risk versus low-risk environments of the Western Grey Squirrel (*Sciurus griseus*)

Chelsea Sheppard, Rebecca Holden, Eric Slusarenko, Trevor Antonizio
BIOL 350 - Donald Miller & Shelly Kim

Giving-up density in high-risk and low-risk environments was studied to further understand the foraging behavior of the Western Grey Squirrel (*Sciurus griseus*). The study examined if squirrels would leave more food behind (the giving-up density, or GUD) in high-risk food patches compared to low-risk food patches. Food trays were placed out during each trial. Each feeding tray was collected after a 24-hour period. Four locations were used. Two trials were done at each location giving a total of eight trials for high-risk and low-risk environments. Environments were represented by open spaces without vegetation cover, and covered spaces close to tree trunks which provided a route of escape. Foraging behavior was quantified by examining the number of seeds taken from one risk environment compared to another. This would represent the amount of time squirrels spent foraging in each environment. The compiled data showed no conclusive results. There was no correlation between the giving-up densities in high-risk and low-risk feeding zones.

The use of microbes to degrade petroleum for further use in bioremediation

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BIOL 321 - Gordon Wolfe

Organisms in the genus *Pseudomonas*, *Rhodospirillum*, and *Rhodobacter* are common oil degrading microbes. These microbes break down long chain hydrocarbons for energy and food. Oils occur naturally in nature; however certain human actions pollute the environment to an unhealthy extent. The use of these microbes could be beneficial in the remediation of that very problem. Our project was centered on the enrichment and isolation of these oil degrading bacteria from common soils to see if in nature there is naturally occurring microbes that can fight problems of pollution in the environment. We suspended a small portion of soil, which we collected from the lawn of Holt Hall, in a solution of water and petroleum from Professor Wolfe. We made plates of the same materials but with added agar as a solidifying agent. With these plates we try to isolate colonies of different oil degrading bacteria. After only a few days we were able to see growth on the first plates and we were ready to isolate and enrich one of these. We were then able to isolate a single colony and perform multiple tests to acquire identification. The tests include: motility, denitrification, fermenting, oxidase, and a subsequent PCR and Genbank analysis. Gram stain proved to be gram-positive and microscopically looks to be cocci. Our goal is to acquire only one type of bacterial species and we are currently in the process of classification.

Analysis of peroxidase gene AT4G21960 expression and function in *Arabidopsis thaliana*

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BIOL 409 – Kristopher Blee

Peroxidases are enzymes present in plant cells that catalyze the oxidation of substances by a peroxide. *The Arabidopsis thaliana* genome contains 73 genes from the Class III peroxidase gene family. The study conducted investigated where within the plant the peroxidase gene would be expressed and how that dictates function of the gene. Protein targeting was analyzed by studying peroxidase gene AGI AT4G21960 to test for targeting to the vacuoles or to the cell wall of *Arabidopsis thaliana*. The yellow fluorescent protein (YFP), which is a variant of the protein that was originally isolated from jellyfish, was inserted into the AT4G21960 gene as a tag through tri-template PCR reaction. This YFP tagging of the reporter construct allowed for tracking the location of the gene throughout the experiment. The reporter construct was then cloned into pDONR221, isolated and subcloned into the plant transformation vector pMN20GW for use in the transformation of *Agrobacterium tumefaciens*. Plant transformation used the *Agrobacterium tumefaciens* transformants to transfer DNA to the plant genome of *Arabidopsis thaliana*, producing seeds that can be screened for YFP, indicating the presence of the At4g21960 gene. With the microarray data, the AT4G21960 gene showed high expression on the hypocotyls, radicle, and petiole indicating possible functions in those areas. The transgenic seeds generated can then be planted to confirm the expressed location on *Arabidopsis thaliana*, but more importantly can help dictate further research regarding where within which type of cell the gene is expressed.

Investigating the function of Class III Secretory Peroxidase, AT4G21960 Gene from *Arabidopsis Thaliana* using YFP tagged Reporter Constructs

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BIOL 409 – Kristopher Blee

Class III plant peroxidases are members of a large family of genes that are present in all terrestrial plants. These genes encode catalytic enzymes that regulate many physiological processes such as lignification, cross linking of the cell wall components and pathogen defense. *Arabidopsis thaliana* encodes 73 class III peroxidases and these enzymes are active in many different tissues and at all stages of development. Research suggests that the c-terminus of the peroxidase protein may be responsible for its cellular targeting. The peroxidase that we selected for study, AT4G21960, possesses a short c-terminus which we believe will result in it being targeted to the cell wall. To test our hypothesis, we created a yellow fluorescent protein (YFP) tagged reporter gene using Tri-Template PCR. After a series of cloning, subcloning and transformation reactions, we used our reporter gene to create transgenic *Arabidopsis* plants. Our analysis of microarray data available in the public domain suggests that AT4G21960 is strongly expressed in the hypocotyl, cauline leaf, petiole and roots. In future work, we will use our reporter gene to identify where in these tissues

and possibly at what cellular locations our gene is expressed, which will help us answer our hypothesis about whether a short c-terminus results in expression in the cell wall.

Giving-Up Densities in Western Gray Squirrels

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BIOL 350 – Shelly Kim

Foraging ecology studies the foraging behavior of animals in response to their environment. In a heterogeneous environment, a forager decides between a variety of patches. Each patch may differ in terms of quality and quantity of resources. Food varies in value and any increase in the risk of predation could lead foragers to be more selective in deciding where, and how long to forage. The objective of this study is to predict the amount of risk the Western Gray Squirrel (*Sciurus griseus*) will take to forage for food. The critical point at which the amount of food available no longer outweighs the cost of feeding at that site is called the giving-up density (GUD), and is a useful way to analyze foraging risk. The site was located within lower Bidwell Park. Feeders were prepared that included a mix of two hundred sunflower seeds and about a half liter of sand. The first feeder was placed directly underneath a tree. A second feeder was placed 10 m from the tree and the third dish was placed 20 m from the tree. The feeders were left out for 24 hours. This process was repeated three times. After each 24-hour period, the remaining seeds were counted. An ANOVA was performed to compare the GUD's for the three areas. Assumptions could be made that the feeders further from the tree present a higher risk therefore will encounter less foraging. Once data analysis is completed, GUD trends may be found to validate our assumptions.

Undergraduate Research

Glutamate receptor 2 subunit containing AMPA receptors are neuroprotective

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Advisor: Jonathan Day

Studies have shown that B6 mice are resistant to excitotoxin induced neuron death, and that FVB mice display the typical neuronal loss seen in most mammalian nervous systems. Neurons cultured from B6 neonates show the same resistance as the adult mice indicating that some unknown property of the neurons confers resistance in B6. Using glutamic acid induced excitotoxicity, we asked whether the excitotoxicity was produced by a particular subtype of glutamate receptor. Ionotropic glutamate receptors are ligand-gated ion channels that open in response to glutamate, some allowing Ca^{2+} ions into the cell. Ca^{2+} plays a major role in normal cell function and excitotoxicity. To determine which ionotropic receptors could be involved in this process, B6 and FVB neurons were pretreated with glutamate receptor antagonists for 15 minutes, followed by sufficient glutamate to cause substantial cell death in the FVB neurons. None of the ionotropic antagonists improved FVB neuron survivability, except when all three ionotropic receptors were blocked at the same time. The antagonist combination used blocked the receptors that were permeable to Ca^{2+} . Since GluR2

containing AMPA receptors are impermeable to Ca^{2+} , a second experiment determined if a difference in GluR2 expression might account for the resistance in the B6 strain. B6 neurons had an average of 2.9 punctae/10 μm of dendrite length, while FVB neurons had an average of 2.3 punctae/10 μm (B6 > FVB, $p = 0.0005$). These data suggest that increased amount of GluR2 in the B6 neurons may be responsible for the excitotoxic resistance found in the B6.

Population densities of western gray squirrels (*Sciurus griseus*) in urban and rural environments of Northern California

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Advisor: Raymond Boggiatto

Small mammals like the western gray squirrel (*Sciurus griseus*) can be used as valuable indicator species, reflecting mast production and frequently suggesting the presence of other, larger organisms like mule deer (Healy and Welsh, 1992). In order to determine the densities of western gray squirrel populations in urban and rural environments of Northern California, eight study sites were established along Big Chico Creek and Butte Creek in Butte County, CA based on established criteria for urban and rural locations and the presence of tree species frequently used by western gray squirrels for food and shelter, including valley oak (*Quercus lobata*) and gray pine (*Pinus sabiniana*). Six surveys were conducted within each 5.5ha study area. For all gray squirrels observed the distance from the observer, location (tree or ground), and nearest point on each transect was recorded. Density of squirrel populations was estimated per hectare and compared between urban and rural sites, and creek systems. Along Big Chico Creek surveys found the density of squirrels to be greater in urban habitats, while Butte Creek surveys revealed a higher population density in rural habitats. The factors influencing squirrel populations include the availability of food and shelter, and tree species composition in a given habitat. The amount and type of development in an area also seems to effect populations.

Dendrochronology analysis of Monterey pine (*Pinus radiata*) occurring in a northern California coastal environment

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Advisor: Randy Senock

Dendrochronology is the scientific study of tree ring analysis and its application to determine temporal and spatial patterns of environmental conditions. Tree growth depends on annual precipitation as well as solar radiation. The northern California coast is known for its moisture and relatively stable temperature. The coast is known for its cool wet winters and warm dry summers. This climatic pattern is known as a Mediterranean climate. The Pacific Ocean stabilizes the annual variation in temperature and precipitation extremes. Tree species in this environment, like *Pinus radiata*, may grow continually, thus not producing distinct annual rings. This is more common if it is experiencing mild temperatures and no

drought conditions. This may make analysis of *Pinus radiata* rings more subject to error than other *Pinus* species found in the interior forests. The primary objective of this study was to examine the annual growth of *Pinus radiata* in relation to the prevailing annual environmental conditions of the coastal forest ecosystems. Is there a significant increase in growth during years of optimal temperature and precipitation? A secondary objective was to compare growth from the east and west azimuth directions for each tree. Is there a radial difference in growth based on azimuth location of east and west? Initial observations show rings on the west side and continual growth on the east side. Diameter at breast height (DBH) was measured for 10 trees and one core extracted from both the east and west azimuth direction. A 20-year annual weather dataset was obtained within 10 miles of the location of the sampled trees. Annual early and late wood was compared to annual temperature and precipitation totals.

Synthesis of a biodegradable plastic (PLA) from agricultural wastes

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Advisors: Larry Kirk, Joe Greene, Larry Hanne

Lactic acid can be polymerized and used to make a compostable plastic. A major drawback of commercial scale lactic acid production by microorganisms is the cost of nitrogen and carbohydrate sources. Our research uses pretreated agricultural waste as a nitrogen and carbohydrate source in order to reduce the cost of this process. We have previously found *Lactobacillus rhamnosus* (ATCC #9595) to be an efficient producer of lactic acid when growing on rice bran or hulls. Pretreatment with 0.2M sulfuric acid and heat for 45 minutes resulted in the highest conversion of complex carbohydrates to free glucose, with 48% dry weight converted to glucose. Bacterial fermentation using 20g of pretreated bran results in nearly 100% conversion of glucose to lactic acid. Lactic acid will be removed from the final bacterial culture using a multi-step process: centrifugation to remove solids, cation exchange chromatography to remove positively-charged impurities and finally anion exchange chromatography to isolate lactic acid from the remaining impurities. Currently these steps are in the proof of concept phase, using purchased lactic acid to optimize the interaction of these resins with lactic acid. Synthesis of the polylactate polymer involves a two-step process. We have successfully completed the second step of the synthesis with a 91% yield and average molecular weight of 8381g/mol for polylactate.

Effects of inbreeding in *Mimulus guttatus* on defense against parasitism by *Cuscuta europea*

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Advisor: Christopher Ivey

As primary producers, plants are under persistent attack by many enemies. The Plant Vigor Hypothesis suggests that vigorous plants are more likely to suffer attack by natural enemies.

Inbreeding is common in plants and typically reduces vigor. We tested the prediction that inbreeding in *Mimulus guttatus* would increase defense against a parasitic vine, *Cuscuta europea*. A single *C. europea* seedling was attached to nine-week-old experimentally self- or cross-fertilized *M. guttatus* and allowed to grow for up to 3.5 weeks. To measure inbreeding effects on host resistance to parasite attack, we compared self- vs. outcrossed hosts for (a) probability of host infection, (b) infection duration, (c) parasite survival, and (d) parasite biomass. Results suggested that inbred plants had increased resistance against parasites, which supports the predictions of the Plant Vigor Hypothesis; none of the differences, however, were statistically significant. To measure inbreeding effects on host tolerance, we counted flowers on inbred and outbred host plants growing with and without parasites. There was no effect of inbreeding on tolerance to parasitism. Parasite infection, however, reduced flower number by 80-92% in *M. guttatus* ($P = 0.0007$), indicating a substantial fitness cost to infection. Mid-experiment, we discovered that *C. europea* plants were preferentially attacked by thrips, which appeared to contribute to the unexpectedly high mortality that we observed. In conclusion, we found equivocal support for the predictions of the Plant Vigor Hypothesis. Inbreeding may limit the impact of enemies on host plants by increasing the range of defense phenotypes in natural populations.

Characterization of fungi isolated from Boiling Springs Lake, CA

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Advisor: Gordon Wolfe

Boiling Spring Lake (BSL)—a lake in Lassen Volcanic National Park (LVNP)—is an extreme environment: its mean pH and temperature are 2.2 and 52 °C, respectively, and it has very low nutrient and metal concentrations. Organisms from such environments could yield novel species with useful characteristics, such as the ability to digest cellulose. Research by a prior student, Marni Merrill, established fungal enrichments using BSL water and plant leaf and wood material at three temperatures: 20 °C, 40 °C and 52 °C. From the 20 °C isolates, she isolated five Ascomycetes, of which three were *Penicillium* spp. For our research, we worked with her 40 °C enrichments. We streaked these enrichments onto various plate media and incubated at different temperatures to determine growth optima. Isolates were identified by microscopy rRNA-ITS PCR. We also found only Ascomycetes, but different species than were isolated from the 20 °C enrichments, including *Ochrochonis gallopava* and *Aspergillus fumigatus*. Both are known components of other hot spring environments. Comparison of isolates from enrichments with autoclaved plant material suggests that all produce spores that can survive at 52 °C, but so far we have not found isolates that can grow above 40 °C. These temperatures occur only in the winter months, when snow inputs to the lake are high. For each of these organisms, we are currently characterizing C sources and T-pH optima.

Graduate Class Projects

Chromatin immunoprecipitation assay of Pdx1 binding sites in genes expressed in rat insulinoma cells

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BIOL 609 - Kristopher Blee & David Keller

The transcription factor Pdx1 binds to the promoter region of the insulin (*INS1*) gene of rat pancreatic beta cells. Pdx1 plays a crucial role in early pancreatic development and cell differentiation. Chromatin immunoprecipitation assay (ChIP) was carried out in order to confirm that Pdx1 binds to the promoter region of *INS1*. The goal of this study was to find additional genes with Pdx1 binding sites with implications for *diabetes mellitus* treatment. Polymerase chain reaction (PCR) on DNA samples recovered using Pdx1 antibody produced a 200bp product indicating Pdx1 binding to promoter region of *INS1*. In control reactions, *MYOD* primers in PCR of Pdx1 antibody recovered DNA did not produce a product indicating that Pdx1 does not bind to the muscle specific *MYOD* gene. The specificity of Pdx1 antibody was confirmed because similar reactions using preimmune serum did not produce PCR product. Additional experiments identified interaction of Pdx1 with the promoter of *JUP*, which plays a role in DNA replication and repair. Our experiment showed that the ChIP assay was performed successfully because PCR products were visible at 200bp on the agarose gel in both the ChIP and input samples. This confirms that the Pdx1 transcription factor binds to the promoter region of the insulin gene. A previous study identified potential new Pdx1 target genes, one of which was *JUP* (Keller et al. 2007, J. Biol. Chem., 282:32084-92). Our data suggests that insufficient Pdx1 would correlate with low activity of *JUP* and consequently DNA repair would be compromised.

Ecology and conservation of the introduced endangered Asiatic softshell turtle, *Palea steindachneri*, on the island of Kaua'i

Megan Masonek, Sarah Del Paine, William McCall, Sierra Sisk, Molly Thompson, Don Heacock².

BIOL 669 - Tag Engstrom

The wattle-necked softshell turtle, *Palea steindachneri*, is IUCN listed as Endangered in its native China and Vietnam where its survival is doubtful due to continued exploitation. *Palea steindachneri* was introduced to Kauai and O'ahu by Chinese immigrants during the mid 1800s, and appears to be established in Kauai. This creates an interesting conservation puzzle, as this species is both endangered in its native range and a potentially harmful invasive outside its native range. Little is known about the distribution, abundance and effects that *P. steindachneri* may have on Hawaiian aquatic ecosystems. In 2007 CSU Chico

² Kauai Department of Aquatic Resources (DAR) 3060 Eiwa Street, Room 306 Lihue, HI 96766.

biology classes began research with two goals: 1) to evaluate the Kauai population as a viable option to prevent the global extinction of this species; and 2) to understand the impact that this and other invasives have on Hawaiian aquatic organisms, which have no large native predators. Here we present preliminary results on the status and ecology of *P. steindachneri* on Kauai based on data collected by CSU Chico biology classes between 2007-09 and compilation of two decades of observations by Kauai DAR personnel. DAR observations and DNA sequences confirm the widespread establishment of *P. steindachneri*. CSU classes captured, PIT tagged and released ten turtles from three watersheds, recapturing two, and have collected tissue samples for DNA and stable isotope analysis and feces for diet studies. These limited data indicate that the turtle is locally abundant but has an uneven distribution, and feeds on a wide variety of invasive prey. Isotope analysis is ongoing.

Thesis Research

Migration and breeding distribution of Eurasian wigeon, *Anas Penelope*, wintering in the Sacramento Valley, California

Jeremy Ashe

The Eurasian wigeon (*Anas penelope*) is a regular winter visitor throughout the Pacific Flyway. Long-term data suggest that this population has been increasing for several decades. The Sacramento Valley serves as a wintering area for large numbers of these wigeon, and they are regularly observed at local National Wildlife Refuges and State Wildlife Areas. As it is likely that most if not all of these wintering birds nest in Eurasia, the potential for this species to transport the Highly Pathogenic Avian Influenza Virus (H5N1) from Eurasia into North America warrants investigation. There are currently no confirmed breeding records for Eurasian wigeon in North America, and therefore, an integral component of this species' natural history remains unknown. The initial phase of our research is designed to establish a link between the Eurasian source of H5N1 virus and wintering waterfowl populations of the Pacific Flyway. A single immature male Eurasian wigeon was captured in March 2009 using a rocket net at Lower Klamath National Wildlife Refuge, and subsequently tested for H5N1; test results were negative. Upon capture, we attached a 16 g back-mounted solar satellite transmitter (PTT transmitter) to the duck; we released the bird at the point of capture on the same day. The PTT unit transmits location data periodically, which allows us to track the bird during its spring migration, and during the subsequent nesting season.

Cyclosporine A increases synapse formation living rat hippocampal neurons *in vitro*

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In human neurodegeneration, like Alzheimer's disease, symptoms do not appear until there is a significant loss of neurons in specific brain regions. Early in degeneration, surviving

neurons are able to compensate for the ones lost by forming additional synaptic connections with cells downstream, but this response is limited. If we are able to promote new synapse formation in the surviving neurons, it might be possible to retard the progression of neurodegenerative diseases. Previous research by others showed that forskolin increased synapse formation through the second messenger, cyclic AMP. Cyclic AMP promotes the phosphorylation of synaptic proteins destined for new synapses. Through an alternative pathway, Cyclosporine A (CsA) acts to inhibit calcineurin. Calcineurin is a phosphatase that dephosphorylates synaptic proteins. The dephosphorylation of synaptic protein inhibits synapse formation. Thus by inhibiting calcineurin we might increase synapse formation. Since forskolin and CsA act through different pathways, they might have an additive effect when combined. Cultured rat hippocampal neurons were treated with forskolin, CsA, or both and then incubated for one hour. Active synapses in living neurons were identified using FM4-64 fluorescent dye. This dye illuminates sites of exocytosis associated with neurotransmitter release in axon terminals. In this preliminary study, our results showed that both forskolin and CsA increased synapse numbers comparably; in combination they produced an additive increase in synapse numbers. These results suggest that CsA might be a candidate for use in individuals with symptoms of neurodegeneration.

Natal origin of juvenile loggerhead turtles from foraging grounds in Nicaragua and Panama with newly suggested mitochondrial DNA primers

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Our study estimates the natal origins of juvenile loggerhead turtles (*Caretta caretta*) in coastal waters of Caribbean Nicaragua and Panama using a Bayesian mixed stock analysis. The analysis is based on newly suggested mitochondrial DNA dloop primers (LCM15382/H950 and LTEi9/H950), which amplify about 880 base-pair. These primers are designed to target longer mtDNA sequences because shorter primers (TCR5 and TCR6) do not provide the necessary resolution to categorize haplotype frequencies. For our project, we estimated natal origins from 74 blood or tissue samples of juvenile loggerhead turtles captured in foraging habitats in Caribbean Nicaragua. In addition, we reanalyzed 45 juvenile loggerhead blood samples from the Caribbean coast of Panama with new mtDNA primers to test the accuracy of previously published estimations. Further, we compared the stock composition between Nicaragua and Panama foraging aggregations. These two locations are not geographically distant, but do encompass different ocean current patterns. Thus, we compared the effect of ocean currents on the recruitment, migration patterns of juvenile loggerhead turtles, and tested the theory of juvenile loggerhead turtle homing to foraging habitats. Analyzing longer mtDNA fragments will increase precision of estimation of the natal origins of juvenile loggerhead turtles and their homing behavior. Further more, comparison of two geographically separate foraging grounds allows us to accurately analyze specific juvenile migration patterns with strong statistical power.

Propagule-size gradients in *Arctostaphylos* (Ericaceae) do not follow typical latitudinal or altitudinal patterns

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The latitudinal gradient in propagule size (fruits and seeds) is an unexplained pattern in phytogeography and paleobotany. Proposed causes often focus on differences in biome structure or mode of seed dispersal between tropical forests and temperate grasslands or savannahs. Here we compare effects of latitude and altitude on fruit size in *Arctostaphylos* (Ericaceae), an animal-dispersed and primarily Californian chaparral shrub genus, using both a meta-analysis of published geographic and morphological ranges, and field analysis of three species in the Sierra Nevada foothills. Stepwise linear regression on multiple measures variables reveals a weak but statistically significant negative correlation between fruit size and latitude and a similarly weak positive correlation between fruit size and altitude. Species with burls typically have low minimum latitude and high maximum fruit size. By contrast, intraspecific field data reveal a positive relationship between fruit size and both elevation and latitude. These results reveal that *Arctostaphylos* does not exhibit the global propagule size gradients exhibited by other angiosperms, even within California. Possible mechanisms responsible for these anomalous results involve the reliance of *Arctostaphylos* on animal dispersal, the disturbance ecology of chaparral shrublands, or the locally inverted latitudinal gradient in precipitation characteristic of Mediterranean-type climates. Latitudinal propagule-size gradients so far been predominantly investigated using floristic-scale literature-based meta-analyses. More studies are needed at smaller taxonomic scales in order to determine the complexity and mechanisms underlying the global pattern.