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ABSTRACT

Coleoptera, or more commonly known as beetles, are the largest and most diverse order within the arthropods. This study aimed to categorize the diversity between diurnal and nocturnal beetles as well as determine if there are distinguishing phenotypes between the two groups. For a total of five days, I collected diurnal and nocturnal beetles throughout the tropical cloud forests of the Monteverde zone in Costa Rica and identified to Family. For each species, I recorded the size, coloration (number of colors visible and light versus dark tones), pattern, texture, and iridescence and compared between diurnal and nocturnal beetles. With respect to diversity, my results revealed that while diurnal beetles are more diverse within the leaf beetle family, Chrysomelidae, nocturnal beetles have the highest diversity within the scarab beetle family, Scarabaeidae, indicating that diurnal or nocturnal activity may be more correlated the taxonomic category than to individual species phenotypic traits. This is further supported by the presence of exclusively diurnal as well as exclusively nocturnal families in my data. T-test analysis proved size to be not correlated with beetle diurnal or nocturnal activity. The only phenotypic significant difference I discovered was that nocturnal beetles are darker in coloration than diurnal beetles, indicating that the darker tone is possibly an adaptation for nocturnal beetles to remain hidden while active in the darkness of the night. Other phenotypes may instead show more correlation to crypsis, aposematism, and sexual selection than diurnal and nocturnal activity.

Diversidad y fenotipos de coleópteros diurnos y nocturnos en el bosque nuboso de la zona de Monteverde

RESUMEN

El Orden Coleoptera contiene los comúnmente conocidos como escarabajos y son el grupo más grande y diverso dentro de los artrópodos. El objetivo de este estudio fue categorizar la diversidad entre los escarabajos diurnos y nocturnos y determinar si hay fenotipos distintivos correspondientes a cada grupo. Durante un total de cinco días, colecté escarabajos diurnos y nocturnos en los bosques tropicales de la zona de Monteverde en Costa Rica y los identifiqué a nivel de Familia. Para cada especie, registré el tamaño, la coloración (en dos categorías: número de colores visibles, tonos claros y oscuros), los patrones, la textura y la iridiscencia y comparé estos datos entre escarabajos diurnos y nocturnos. Con respecto a la diversidad, mis resultados revelaron que los escarabajos diurnos presentan mayor diversidad dentro de la Familia Chrysomelidae, mientras que los escarabajos nocturnos tienen la diversidad más alta con la familia Scarabaeidae, indicando que la actividad diurna o nocturna puede estar más correlacionada con su categoría taxonómica que con rasgos fenotípicos independientes. Este resultado es apoyado por el hecho de que hubo familias exclusivamente diurnas o exclusivamente nocturnas en mis datos. Una prueba de T-student demostró que el tamaño no está correlacionado con la actividad diurna o nocturna de los escarabajos. La única diferencia fenotípica que descubrí fue que los escarabajos nocturnos son de coloración más oscura que los escarabajos diurnos, lo que indica que el tono más oscuro podría ser una adaptación para que los escarabajos nocturnos permanezcan ocultos mientras están activos en la oscuridad de la noche. Otros fenotipos pueden mostrar más correlación con la cripsis, el aposematismo y la selección sexual que la actividad diurna y nocturna.

INTRODUCTION

In the animal kingdom, the class that occupies more than 75% of the population is Insecta (Daly 1978). Within this large group, the most diverse group of insects is the Coleoptera Order, or more commonly known as the order of the beetles. Beetles undergo a complete metamorphosis, meaning that they morphologically change as they grow from eggs to larvae to adults (Crowson 2013). Beetles occupy a wide range of habitats ranging from within plant material or rotting wood to carrion or dung. Most are terrestrial but there are some species are known to be are aquatic. Features that characterize these organisms are compound eyes, compact bodies, mandibles designed for chewing, and sclerotization of an exoskeleton (White 1983). The key feature that separate beetles from other orders are the modified front pair of wings that form the elytra. The elytra's main function is to protect the membranous wings that are used for flight. In most beetles, the elytra extends to the end of the abdomen, but in some coleopteran families, the elytra goes past the abdomen or only covers the thorax. Most of beetle coloration, design, and shape, come from the elytra and helps in identification of different species.

Like other animals, different species of beetles are active at different intervals of the day. While majority of beetles are diurnal, meaning that they come out during the hours of sunlight, there are select nocturnal species that are active when the sun disappears and their world is encased in darkness (Crowson 2013). One of the main factors why most species are diurnal is that they require the sun to stay active (Rosenthal 2006). Whether it is because it supplies them with thermoenergy to forage, or allows them to see more clearly, the sun is an important component in controlling diurnal activity. Nocturnal species are active during times of colder temperature and darkness. These conditions are best for organisms with the ability to see in the dark or those with other heightened senses that help them to maneuver while with poor sight. There are also few species that are crepuscular, or active during twilight hours between day and night or vice versa. Crepuscular species are awake during the in between hours either because their prey also come out during this time or to forage before other competitors are awake (Thery 2008).

With about 750,000 known species of insects, 400,000 of them are beetles (Crowson 2013). In the tropical forests of Costa Rica, there are 110 out of the 178 identified families of beetles (Nadkarni 2000). The Monteverde zone is especially species abundant in animals including beetles. Monteverde is home to both the largest beetle in the world, the Hercules beetle, of the family *Scarabaeidae,* as well as the smallest beetle in the world, the "feather-winged" beetle, of the family *Ptilidae*. Beetles are extremely diverse in their morphology and behaviors that are characterized by different adaptive significance. Rosenthal (2006) sampled the abundance and diversity of diurnal and nocturnal insects of the montane rainforest in Monteverde, Puntarenas, Costa Rica. He discovered that the biodiversity of diurnal insects greatly surpasses that of nocturnal ones and hypothesized that it may be due to the additional “costs” that come with foraging in the colder temperatures of the night than the warmer ones of the day – this includes the need to use stored energy to be active at night as opposed to during the day when the heat from the sun helps regulate body temperature.

Immense diversity amongst beetles inspired me to propose two questions: What is the diversity of diurnal and nocturnal Coleoptera in the Monteverde zone cloud forests and are there distinguishing phenotypes between the two groups of beetles?

MATERIALS AND METHODS

During a total of five days, I collected beetles at three different locations within the cloud forests of Costa Rica. Two locations were in Monteverde, Puntarenas: the trail to the TV towers near the Estación Biologica with the highest elevation of 1575 meters above sea level, and the Crandell Memorial Reserve behind the Instituto Monteverde at 1,300 meters above sea level. The third collection site was the lowest elevation at 900 meters above sea level and surrounding Máximo Ramirez’s farm near San Gerardo – an area within the protected reserve Bosque Eterno de los Niños.

To avoid collecting beetles that are crepuscular, or active in the twilight hours, for diurnal beetles I only used specimens found between 9 am and 4 pm for diurnal beetles and between 6 pm and 10 pm for nocturnal beetles. I collected for about three hours in the day and three hours at night, estimating about 15 total hours of searching in the day as well as at night. I actively caught all specimens, either with an aspirator, plastic bag, plastic container, or with my hands. I determined beetles as active if they showed movement or were exposed to the environment. I avoided digging through soil or rotten wood, which are known habitats of many beetles, because I wanted to avoid capturing beetles that were inactive at that time of collection. With minimum disruption, I searched on and under all substrates of the forest.

After collection, I stored the beetles in a freezer. The cold temperature humanely killed the specimens to later identify them. Smaller beetles were left inside for a minimum of one hour while larger ones were inside for at least three hours. After an adequate amount of time in the freezer, the beetles were pinned. Once the beetles were pinned, they were categorized by the time of day they were active: diurnal or nocturnal. I identified each individual’s coleopteran family using *A field guide to the beetles of North America* by R. E. White. Then, I analyzed and categorized the following phenotypic characteristics: size, coloration, patterns, textures, and iridescence. Size was measured in millimeters from the tip of the eyes to the end of the abdomen. Some species have elytras that extend past their abdomen while others, such as weevils, have elongated snouts. Doing so ensured consistency in measuring beetle size. Other phenotypes were analyzed from only the dorsal side of the beetles since majority of the time, the underside of beetles are unexposed.

Coloration of coleopteran was split into two separate characteristics: number of colors visible and dark versus light coloration. I counted the number of colors easily visible on each species and categorized them as having one color, two colors, or three or more colors. To separate the species between light or dark colors, I held the beetles against a white background and then against a black background. If they were more noticeable against the light background they were placed in the dark category and vice versa.

Patterns were narrowed down to five different categories: no pattern, spots or blotches, speckles, stripes, or blocks. No pattern was present when beetles held only one color. I categorized spots and blotches as when multiple sections of the elytra had different coloration than majority of the elytra. Speckles were miniature dots of different colors that are difficult to differentiate. I indicated blocks of color when only two colors were visible in large distinct sections.

I created three categories of texture: glossy, smooth, and rough. A glossy texture holds reflectance on the surface of the beetle while a rough texture holds little to none. The smooth surface is the intermediate of the two other groupings. I determined texture visually rather than through touch.

Using the same categories of iridescence as Seago (2008), I separated the beetles by those without iridescence, multilayer refractors, three dimensional photonic crystals, and diffraction gratings. Iridescence is defined as “a change in the hue of the object exhibiting it as the angle of vision is varied,” meaning that different colors are visible at different angles (Mason, 1927). Multilayer refractors are the most common of the three types and this allows you to see two or three different hues. Photonic crystals are usually reserved for weevils that have iridescent miniature scales and require microscopic lenses to see its full effect. Diffraction gratings are present when you can see the full spectrum of white light – red, orange, yellow, green, blue, violet. Iridescence is the only phenotype I observed using a dissecting scope.

RESULTS

A total of 69 different species in 15 families were collected: 38 diurnal, 28 nocturnal, and three species seen in both times of the day (Fig 1). Because the sample size found to be both diurnal and nocturnal was so small, I decided to remove this group from data analysis. Majority of the diurnal species belonged to the leaf beetle family, *Chrysomelidae* (n=18). I found the most nocturnal species of the scarab beetle family, *Scarabaeidae* (n=8). Families that were strictly diurnal were *Coccinellidae, Lycidae,* and *Tenebrionidae*, while *Carabidae, Cleridae, Lampyridae,* and *Ptilodactylidae* only held nocturnal species.

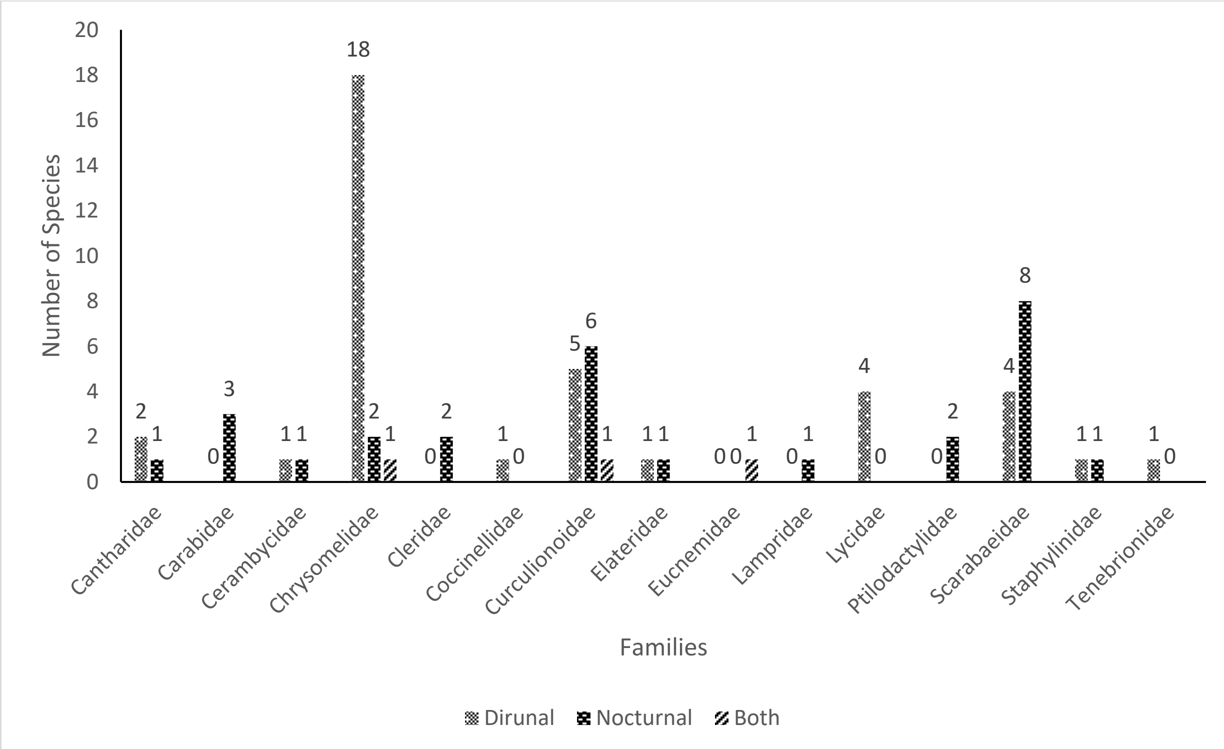

Figure 1: Number of species collected per family of Coleoptera and their time of day activity

Although the average sizes between diurnal and nocturnal beetles were similar (with diurnal beetles averaging slightly larger) the standard deviation shows that sizes between the species varied greatly (Fig 2). Further t-test analysis showed that size is insignificant – independent of diurnal or nocturnal activity ($t = 0.11$, $df = 64$, $p = 0.91$).

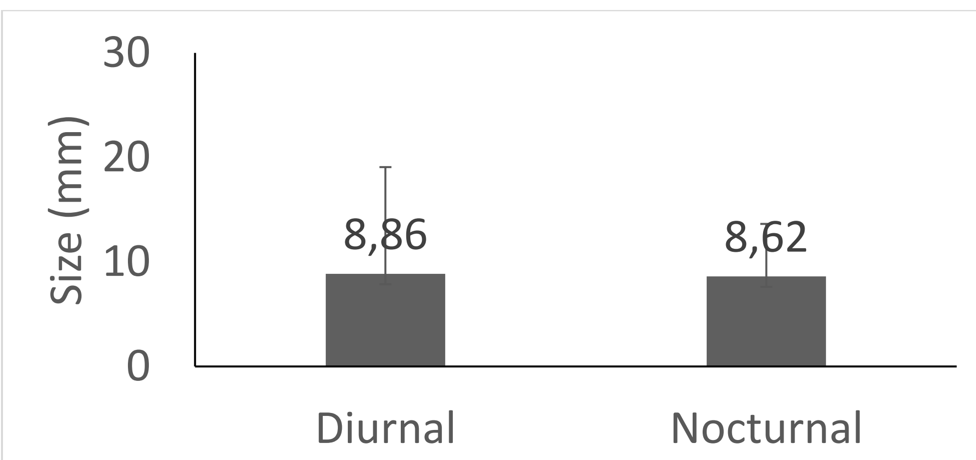

Figure 2: Average sizes of diurnal and nocturnal beetles. In some cases, I found multiple individuals of a species so I calculated the species average size before calculating diurnal and nocturnal averages.

Nocturnal beetles had a higher frequency of single colored species while diurnal species had a higher frequency of having two colors (Fig 3). Chi squared analysis proved number of colors visible on beetles to be unrelated to diurnal or nocturnal activity ($X^2 = 0.93$, df = 65, p = 0.63).

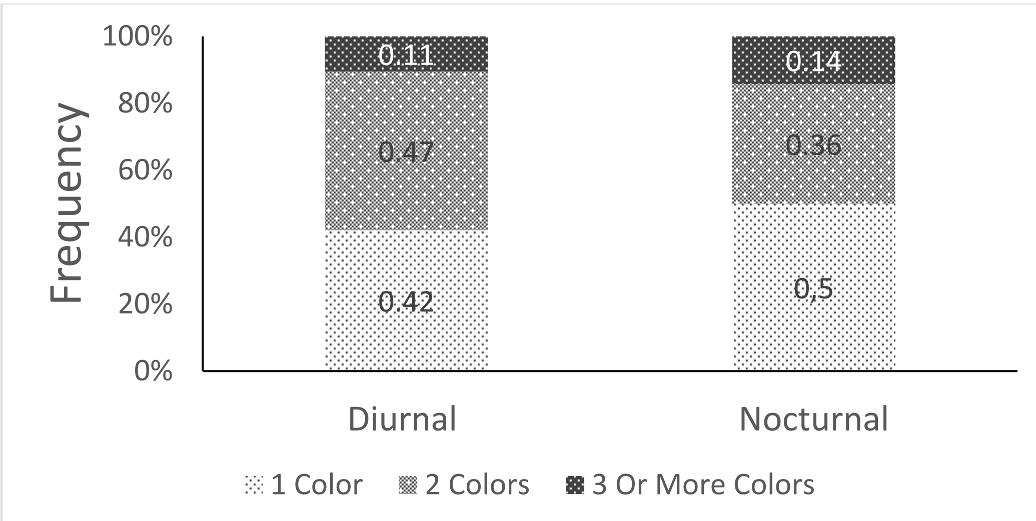

Figure 3: Frequency of total number of colors visible on diurnal and nocturnal beetles.

I found that while the diurnal species were about evenly split between light and dark coloration, nocturnal species held more dark tones than light tones (Fig 4). Out of 28 nocturnal species, 22 of them had darker colors (Fig 4). Statistical analysis showed light versus dark coloration to be significant to diurnal and nocturnal activity ($X^2 = 4.69$, df = 65, p = 0.030).

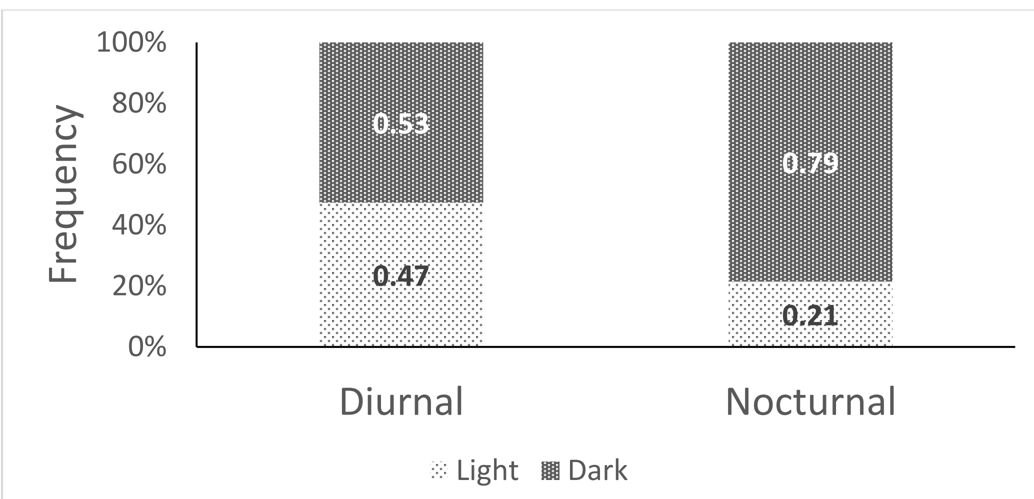

Figure 4: Frequency of light coloration and dark coloration in diurnal and nocturnal beetles.

While diurnal beetles had a higher frequency of species displaying patterns, nocturnal beetles were about 50/50 between species with patterns and species without patterns (Fig 5). Of the nocturnal species that did hold patterns, the most frequent pattern was speckles (18%). The type of pattern that occurred most on diurnal beetles was either the stripes or blocks. (18%). A Chi squared test proved presence of a pattern to be insignificant to diurnal or nocturnal activity ($X^2 = 0.85$, df = 65, p = 0.36).

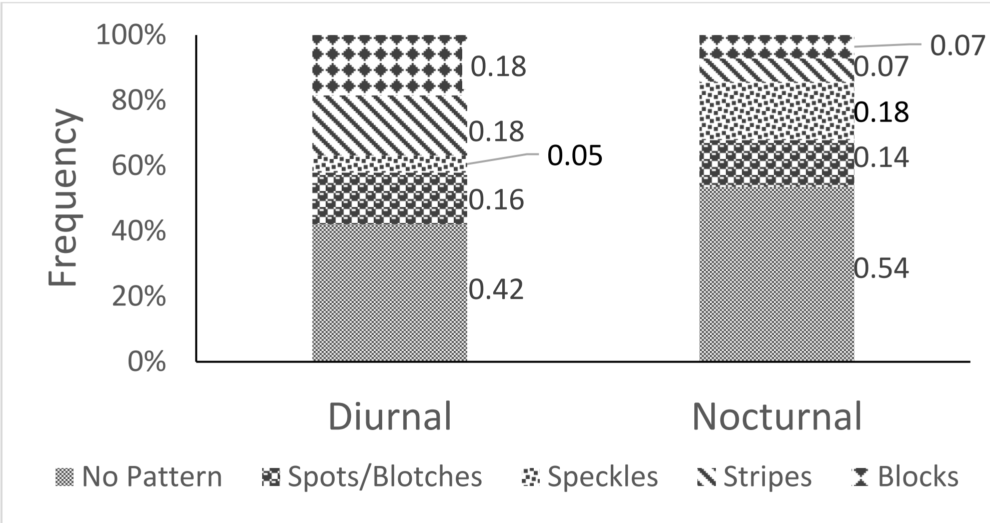

Figure 5: Frequency of different patterns on diurnal and nocturnal beetles.

Majority of species had a glossy texture as opposed to smooth or rough (Fig 6). Nocturnal beetles had proportionally less reflective texture than diurnal beetles. Statistical analysis proved texture of beetles to be insignificant to diurnal or nocturnal activity ($X^2 = 3.44$, df = 65, p = 0.18).

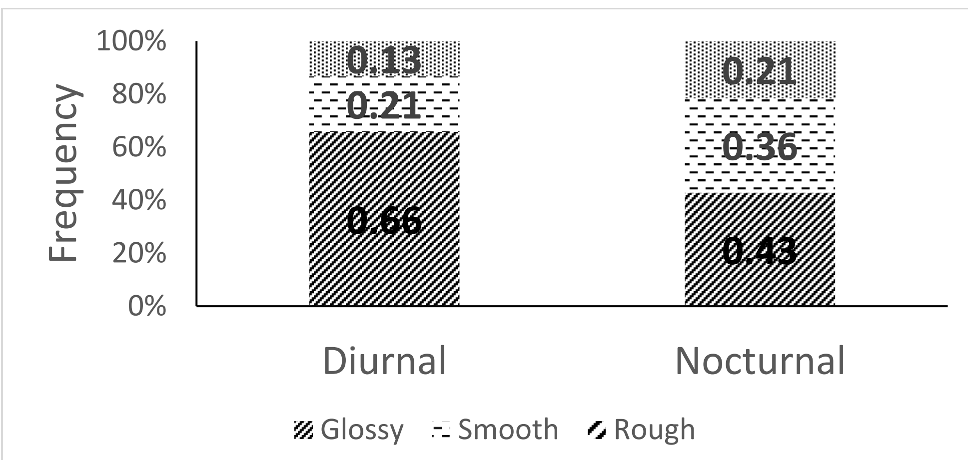

Figure 6: Frequency of different textures visible on diurnal and nocturnal beetles.

Most of the specimens did not display any iridescence (Fig 6). Of those that did, the most frequent form of iridescence was multilayer reflectors. Diffraction gratings were seen on only one species in *Scarabaeidae* which belonged to the nocturnal group. Statistical analysis showed iridescence to be independent of diurnal or nocturnal behavior ($X^2 = 0.076$, df = 65, p =0.78).

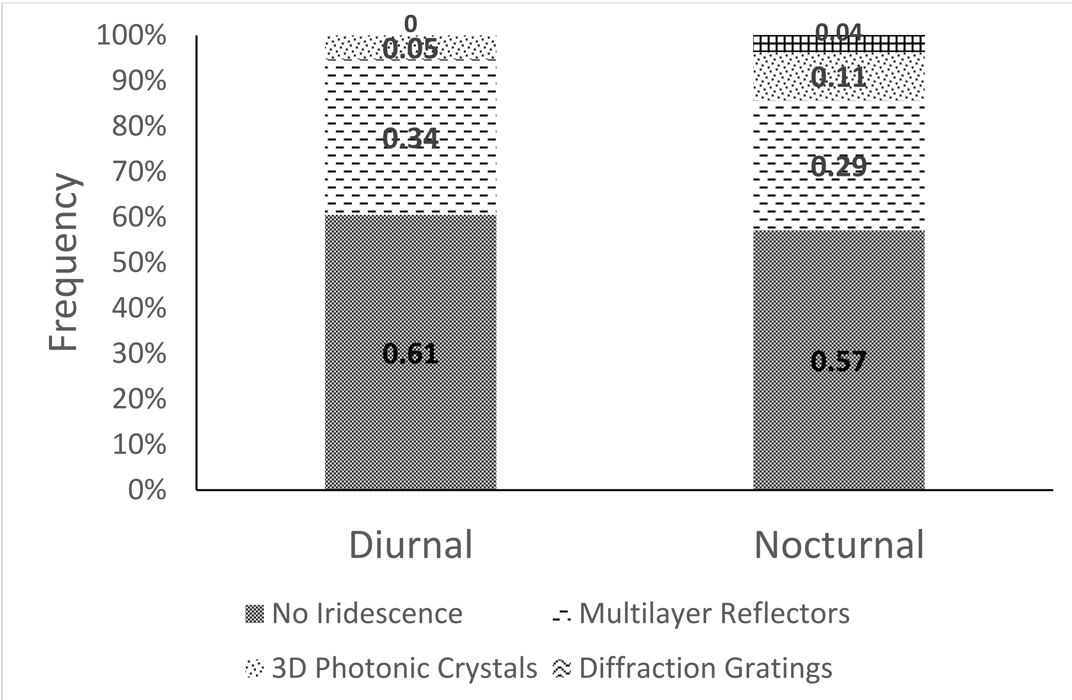

Figure 7: Frequency of different iridescence in diurnal and nocturnal beetles.

DISCUSSION

The largest sample size came from the diurnal leaf beetles, Chrysomelidae, coming to the conclusion that diurnal beetles have the highest species richness in the family of leaf beetles. On the other hand, nocturnal beetles may have their highest richness among scarab beetles, Scarabaeidae, because nocturnal scarab beetles were the second largest group collected. This information and the discovery of families that were exclusively diurnal or exclusively nocturnal also led me to believe that taxonomic category may have more correlation to time activity of beetles than their phenotypic characteristics, meaning that species in certain families evolved together to be diurnal or nocturnal.

The t-test of average size between diurnal and nocturnal beetles revealed that there is no correlation between beetle size and diurnal versus nocturnal activity. When the t-test result is removed from the analysis, the average size distribution between the diurnal and nocturnal beetles shows that they are about similar in size. However, the large standard deviation of the diurnal beetles indicates that this average is flawed due to a *large* outlier – a diurnal beetle in the longhorn family (Cerambycidae). Huffaker and Gutierrez (1999) stated that nocturnal insects tend to be larger than diurnal insects due to the lack of sunlight during their time of activity. Diurnal insects rely on the sun to regulate the temperature of their bodies as they forage in the day. Nocturnal insects on the other hand must be well adapted for thermoregulation if active during colder nocturnal temperatures. Therefore, my t-test analysis of the average sizes of diurnal and nocturnal beetles may have inaccurately determined size to be independent of diurnal or nocturnal activity due to the small sample size of 69 species.

After performing Chi squared tests on the remaining phenotypes, the only phenotype that showed significance was the light versus dark coloration. The results of the test revealed that darker coloration occurs more often than lighter coloration in nocturnal beetles as opposed to diurnal species which are split about 50% in each coloration. I hypothesize that dark coloration is an evolutionary adaptation for nocturnal beetles to stay hidden from predators against the darkness of the night. Additionally, dark coloration can also be an adaptive characteristic of beetles that are themselves predators and need to be unnoticeable when they hunt. Beetles that are diurnal hunters tend to be more metallically colored, while nocturnal ones are usually black, reinstating that they are better adapted to be darker colored (Crowson 2013).

If diurnal and nocturnal activity is independent of phenotypes such as quantity of colors, patterns, textures, and iridescence, other defense mechanisms may be the purpose of such diversity. For example, aposematism, or warning colorations, that certain insects display allow them to avoid being eaten because vibrant colors and unique patterns tell predators that they are poisonous or distasteful (Jones 1932). Better crypsis in the forest is another defense property that may influence the phenotypic differences amongst beetles. Iridescence for example give beetles crypsis against the green foliage because green is the most abundant color seen in iridescence (Parker 1998).

Sexual selection is another determinant of different phenotypes among beetles. Bezzerides et al (2007) discovered that the presence of red coloration, or less black coloration, on Asian lady beetles, *Harmonia axyridis,* is linked to higher alkaloid levels which are chemical defenses against predators. His discovery, along with prior studies stating that Harmonia axyridis with more red coloration or less black coloration have higher mating probabilities, proved that red coloration is preferred during sexual selection due to its indication of higher predator defense.

For further research into differences among diurnal and nocturnal beetles, I would increase my sample size and repeat the statistical analysis. Additionally, in my data analysis and research, I was most fascinated by the concept of iridescence than any other phenotype among beetles. As a future project, I would be interested in studying the functions of the different forms of iridescence in beetles indicated in Seago's report (2008).

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