Protein Quality and Its Effect on Grasshopper Size

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Abstract

Protein affects insect in numerous ways. An increase in protein can dramatically increase in individuals overall fitness. Benefits of increased protein levels are; an increase in development time, larger body size, and less time spent foraging. The site for this study is the University of Notre Dame Environmental Research Center, which located in the upper peninsula of Michigan. In this experiment I will be manipulating nitrogen levels in the soil. I will elevate levels, by adding a commercial fertilizer. I monitored nitrogen levels on a weekly basis to maintain constant level; I also noted phosphorus and potassium levels within the soil. For the second part of my experiment I will provide individuals with two food options, and determine if there is a preference for a particular option. In this study I will be using Camnula pellucida, or the clear winged grasshopper. My hypothesis is that a preference will be shown in food option, and elevated protein levels will increase an individual's overall fitness.

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Introduction

Phytophagous insects primarily feed on plants. There are three types of phytophagous insects; monophagous, which feed on a single genus of plants, oligophagous, which feed on a number of plants in a single family, and polyphagous, which feed on a large number of plants from different families. Most polyphagous species feed only on dicotyledons, while fewer feed only on monocotyledons (Jonas & Joern, 2008). Grasshoppers primarily feed on grasses and forbs. Diet selections for phytophagous species are closely associated with plant chemistry (Behmer, 1994).

Grasses cover over 40 percent of the Earths landscape, which provide a large, but nutritionally poor resource for herbivores (Williams et al., 1968, Tscharntke & Greiler 1995). In grasses nutrient concentrations are lower when compared to forbs. One reason forbs are better quality is that they contain higher amounts of nitrogen, phosphorus, and sugar (Randolph et al., 1995). Many nutrients are retained the chloroplast and cell membranes (Carpita et al., 1968, Baron et al., 1988). Proteins and carbohydrates are often concentrated in tough bundle sheath cells (Barbehenn et al., 2004). In many cases grasshoppers can alter their feeding habits by eating more food or by actively selecting food of better quality. In general many insects consume carbohydrate and protein in proportions that are near its individual needs to increase its overall fitness (Waldbauer & Friedman., 1991, Simpson et al., 2004).

Grasshoppers are under constant predation from many spiders and birds. Thus grasshoppers are under constant pressure to increases their overall fitness in order to survive. Because of predation and competition it is important that they grow at a relatively fast pace. Feeding on high quality plants can lead to success by insect herbivores, and possibly even result in population outbreaks of the herbivore (Joern & Behmer, 1998). Plants that are higher in nitrogen and lower in carbon are considered to be high quality (Strengbun et al., 2008). When a species is consuming a plant of low quality there are many differences that are evident. Reduced body size and prolonged development are often linked to nutrient deficiencies such as lower levels of nitrogen (Bernays & Chapman., 1978; Mcneil & Southwood., 1978; Mattson, 1980; Scriber & Slansky, 1981; Dadd, 1985).

Nitrogen is the most common resource that limits production in plants (Vitousek & Howarth., 1991). Nitrogen is found naturally in soils, but is lost through leaching and plants absorption. Decomposition of plants and animal waste provide much of the nitrogen found in soils. Precipitation adds about five to ten pounds of nitrogen annually (Christy & Smith., 1993). Plants use nitrogen found in soils and convert it into proteins. Proline, a five-carbon chain, is one such protein that is used for direct energy (Behmer & Joern., 1994). In commercial fertilizers nitrogen is often in the form of ammonium nitrogen, which is rapidly converted to nitrate in the soil (Christy & Smith., 1993). Other minerals can affect the nitrogen content in soils. Having elevated levels of minerals like; phosphorus, potassium or sulfur, can often lower nitrogen levels in the soil. Terrestrial insect herbivores growth is often limited by water, nitrogen and secondary chemical content of plants (Mattson, 1980; Scriber, 1981; Strong, 1984; White, 1984; Bernays, 1990).

In this study I will be examining the Camnula pellucida, or clear winged grasshopper. Camnula pellucid can be found widely across North America. Camnula pellucida lives in a variety of habitats, ranging from grasslands, to mountain meadows (USDA, 1994). Camnula pellucida primarily feeds on grass, but consumes small amounts of forbs. It can be identified by the distinct cracking sound made during flight (Borror & Delong, 2005). During its growth it will typically undergo about five instar stages before reaching adulthood (Parker, 1924). Camnula pellucida, like many grasshoppers is active during the day and inactive at night. During peak hours of the day many Camnula pellucida can be found on the tips of grass and other vegetation to bask in the sunlight. By basking in the sun Camnula pellucida self regulates its body temperature, which is important because varying temperatures modify grasshopper behavior (Parker, 1924). When temperatures begin to decline Camnula pellucida starts to seek shelter.

In this experiment I will be analyzing three food options to determine if there is a preference for a particular option. The options that I will be using are: dandelion, store bought romaine lettuce, and an altered sweet white violet. Determining which plants to use was somewhat difficult, because all green plant tissue does not constitute food for a herbivore (Belovsky & Slade, 2008). In determining food options I choose dandelion which is common and grows abundantly around the study site. Romaine lettuce was chosen because it is a known food for many herbivores. I chose sweet white violet, because I noticed that it was being consumed at the study site. Sweet white violet will be altered by elevating the nitrogen content within the soil. By elevating nitrogen levels, I will determine if protein quality has an effect on grasshopper development. I hypothesis that there will be a preference in food options, and that having an elevated nitrogen content will have an impact on development.

Methods

Study Site

Grasshoppers are common at University of Notre Dame Environmental Research Center East (UNDERC), which is located on the border between Wisconsin and the Upper Peninsula of Michigan (Figure 1). I will be collecting grasshoppers at “grasshopper nation’, which is located on the western side of property at UNDERC (Figure 2). The elevation at the site is 513 meters which was recorded using a Garmin® geographic position satellite. I also recorded geographic information system (GIS) points to outline my study site. I sectioned a study site that was a rectangular shape that measured 270 feet by 100 feet. Within this study site I created a grid system with individual plots that measured ten feet by ten feet, totaling 270 individual plots (Table 3).

Soil testing

After completing a grid system of the location I randomly chose 27 plots (table 1), and tested for Nitrogen, Phosphorus, and Potassium levels. To test the levels I used a La Mottte® Soil NPK kit. The testing kit was a visual test used to test for Nitrogen, Phosphorus, and Potassium. The Soil kit measured three levels; Low, Medium, and High for each. Low nitrogen levels are indicated at 40 pounds an acre, Medium, at 160 pounds an acre, and high at 320 pounds an acre. Low Phosphorus levels are at 8 pounds an acre, medium at 20 pounds an acre, and high at 64 pounds an acre. A low potassium level was 40 pounds an acre, medium levels at 80 pounds an acre, and high levels at 160 pounds and acre. I collected two samples from each of the 27 plots. Each sample was from a depth of six inches. I took samples at six inches because most nutrients can be found within the root layer of the soil. Both samples collected within each grid were about one cup. Each of the 27 soil samples were hand sifted, to exclude twigs, rocks, stones, or large material. I then put each sample into glass dishes, and put the samples into a drying oven for 48 hours. During the drying time I stirred the samples to ensure that all moisture was evaporated. After the soil was completely dry I used a soil sieve measuring .025 mm to exclude larger material. After sifting the soil I followed the standard instructions that were included in the kit to test the soil. After testing the soils I found that a majority of the soils were relatively low in nitrogen and phosphorus levels, but medium in potassium levels. Data for the first tests are shown in Table 1.

In this experiment I manipulated the nitrogen levels within the soil and noted the phosphorus and potassium levels. I noted the other levels because recent evidence suggests that phosphorus may have an important role in regulating performance and growth (Sterner & Robinson, 1994; Schade et al., 2003; Perkins et al., 2004). For the levels I converted the low, medium, and high levels to grams per square foot. After calculating the levels I applied Miracle Grow® plant fertilizer to each of the 27 plots. I elevated nitrogen levels to 21 grams of nitrogen per square feet, which was indicated by a medium level in the soil test kit. After three days I again took samples from each of the 27 plots and followed the same procedure for testing nutrient levels. Results of the second samplings are shown in Table 2. Of the 27 plots I chose ten plots to continue fertilizing (Table 2). I chose the ten plots based on the vegetation present. I continued to fertilize until available food was unusable. Plots were unusable when it became difficult to collect plant species form. By the end of the trials I had two plots that were still being fertilized, plot number 20 and plot number ten. Throughout the experiment I continued to fertilize to keep a constant nitrogen level of medium, or 21 grams per square feet.

Collecting

All Camnula pellucida individuals were collected within the study site. After collection grasshoppers were taken to the lab and placed in containers. The containers used were a transparent plastic, which measured 30 cm by 19 cm with a height of 11.5 cm. The ends of the containers had three to five holes drilled on the end that were one cm in diameter. Holes were drilled to allow air circulation for grasshoppers. The holes were then covered by a plastic mesh, which prevented grasshoppers from exiting the containers. Following previous experiment grasshoppers were fed ad libitum on romaine lettuce for 48 hours, and I assumed that all individuals were in a nutritionally similar state before conducting any trails (Behmer & Joern, 1994).

Trials

For my experiments I conducted two types of trials. For one trial I observed if protein quality had an impact on grasshopper growth and development. For this trial I had three food types; dandelion, romaine lettuce, sweet white violet. For each of the food options I had five individuals that were feeding exclusively on one food type. I chose to only include individuals that were in their third or fourth instars. Adults were excluded from this study, because they have already undergone much their development. Protein does little for adults except for females, which is needed for reproduction. During this study I gave each grasshopper fresh food every day. I also removed the old plant material, and cleaned out any frass that was present in the cages. In the beginning of the experiment I took measurements of each grasshopper. Measurements were taken on the hind femur. Hind femur length was chosen because there is significant differences in the length from a third instar measurements of 3.4-3.8mm, to adults 10.5-11.8mm (USDA, 1994). Final measurements were taken at the end of the experiment as well as the weight of each individual (Figure 3).

In the food preferences tests, I compared dandelion, romaine lettuce, and sweet white violet. In this test I conducted a paired test to include all possible combinations. For each test I had three groups of grasshoppers that I would rotate, one group was undergoing trials, one group which was being fed ad libitum on romaine lettuce after each trial, the last group all food was taken away. I took food away from grasshoppers 20 hours before conducting a trail to insure that individuals would consume food during trials. To determine the amount of food each grasshopper was consuming I weighed out five grams of each options and dried all in an oven for 48 hours. I replicated this five times for each food option. I dried the food until no moisture was present. I then averaged the weight of the five replicates, and I assumed this average was my control weight to determine the total amount of food that had been consumed. All food used in food preference trials was weighed out to five grams. After weighing out food I placed each food option in Aquapic's®, waterpics that were modified. The waterpic's original measurements were 124mm long, with an opening of 14mm. I modified each waterpic by using a hand saw to remove 60mm from the end. I modified each waterpic to allow each grasshopper the freedom of feeding on the stem, or leaf of each plant. Trials were started at 2300 each night. Individuals were placed in containers with the two food options to avoid interrupting normal feeding times, and to allow time to acclimate to a new environment (Behmer & Joern, 1993). Each trial lasted 24 hours before the next trial was started. At the end of each trial all vegetation was removed, then put in a oven to obtain a dry weight. The dry weight was then subtracted from the control weight. This procedure was completed for each food option, and throughout the duration of the experiment.

Results

To obtain statistical data for both studies, I used Systat® 13 and Excel®. To calculate my values I used a modified type two selection test that is normally used to calculate predation (Manly, 1974). I used this formula, because I had two dependent variables, and I needed one independent value. My two dependent variables were the two food options that were presented. From the modified predation results, I transformed the values, using Hotelling's (1947) formula the proportion of food eaten was subtracted from the control amount. A proportional value was then obtained, and Hotelling's T^2 test was then applied to obtain a Hotellings Value of 182,231.282. . Having high Hotellings number indicates there was a significant difference in the food options that were presented. When I calculated dandelion and sweet white violet using Hotellings formula and received a value of 527,121.340632. When romaine and sweet white violet were calculated with Hotellings formula I had a value of 460,071.678393 for all three tests I obtained a P-value less than 0.0001. Using SYSTAT 13 I ran an ANOVA

(P-value ≥ .001) which indicated that there was a preference for food options. I also created a chart (Figure 2) that illustrates the preferences.

To analyze protein quality I ran an ANOVA test to calculate differences in food options. I also tested for normality using Shapiro Wilkinson's test. When running an ANOVA (P-value 0.056, DF 0.592, and Fvalue of .592), which indicated that there was not a significant difference in protein quality and the final lengths of the individual grasshoppers.

Discussion

In my experiment that I conducted for food preference, there was a clear preference for dandelion and sweet white violet (Figure 5). Sweet white violet also had a dramatic effect on survival rates of the individual grasshoppers. At the end of the protein quality trials, there were still two grasshoppers alive that had been feeding on sweet white violet. Having two individuals that were still alive on the modified protein option indicates that a higher nitrogen level has an impact on grasshopper survival rates. At the end of my trials there was a clear difference in individual weights (Figure 4), the grasshoppers that had been feeding on sweet white violet had a larger body size, and weighed more than the other food options. In the other two food options all grasshoppers had died. In the protein quality tests there was one major flaw in my experimental design. The number of grasshoppers used was relatively low. For future experiments many more grasshoppers should be used in trials to adjust for grasshoppers that die in trials. Using a calorimeter or other instrument that could calculate the exact values of nitrogen and protein should be used in future trials.

Based on the data collected there was a clear preference for Dandelion. Although during duration of the study the total amount eaten for sweet white violet was 64.3 grams. The total amount consumed for the two other options were; 43 grams of dandelion, and only 16.2 grams of romaine was eaten. There are many reasons that grasshoppers preferred dandelion. One theory is that grasshoppers may have had exposure to the plant before trials were run, which may have altered the study. This could be explained by the results; both sweet white violet and dandelion were preferred over romaine lettuce. Both sweet white violet and dandelion grow abundantly around the study site. Romaine lettuce may have been unfamiliar to the grasshopper. Thus romaine lettuce may not have been preferred, because it does not grow naturally at the study site. For the amount eaten it was unknown the actual protein content for each of the food options. For instance romaine lettuce may have had higher protein content then the other options. If this were the case then the grasshopper would only need to consume a small amount of food to meet its nutrient needs. This would explain why little was consumed by each individual. If this were true, and sweet white violet had a lower protein content, then more sweet white violet would have to be consumed to meet its individual nutrient needs. In the future a trial on two food options between sweet white violet and dandelion should be conducted to determine the preference between the two options.

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APPENDIX

Table 1

Table one shows data from the first 27 soil samples. Each plot size was 10 feet by ten feet. The plot numbers are on the left column of the table and the nutrient

SOIL Levels		First Testing	
Plot	Notrogen	Phosphorus	Potassium
3	LOW	MEDIUM	MEDIUM
7	LOW	MEDIUM	HIGH
8	LOW	MEDIUM	LOW
10	LOW	MEDIUM	HIGH
20	LOW	LOW	HIGH
27	LOW	LOW	MEDIUM
33	LOW	LOW	MEDIUM
40	LOW	LOW	HIGH
69	LOW	MEDIUM	HIGH
71	LOW	LOW	HIGH
79	LOW	LOW	HIGH
89	LOW	LOW	MEDIUM
109	LOW	LOW	MEDIUM
111	LOW	LOW	HIGH
127	LOW	LOW	HIGH
133	LOW	LOW	MEDIUM
136	LOW	LOW	HIGH
148	MEDIUM	LOW	MEDIUM
153	LOW	MEDIUM	HIGH
162	LOW	LOW	MEDIUM
182	LOW	MEDIUM	MEDIUM
188	LOW	LOW	MEDIUM
196	LOW	LOW	MEDIUM
240	LOW	LOW	MEDIUM
254	LOW	LOW	HIGH
264	LOW	MEDIUM	MEDIUM
265	LOW	LOW	MEDIUM

Table 2

Table two shows the data from ten plots that were used in my trials. All plots sizes were ten feet by ten feet. Plot numbers are listed on the left columns, and adjacent rows are nutrient levels.

SOIL Levels		SET of TEN	
Plot	Nitrogen	Phosphorus	Potassium
3	LOW	MEDIUM	HIGH
11	MEDIUM	LOW	HIGH
20	MEDIUM	MEDIUM	HIGH
40	MEDIUM	LOW	MEDIUM
69	MEDIUM	LOW	MEDIUM
79	MEDIUM	MEDIUM	HIGH
89	LOW	LOW	HIGH
136	MEDIUM	LOW	MEDIUM
148	LOW	MEDIUM	MEDIUM
253	LOW	LOW	MEDIUM

Table 3

Table 3 shows the plot arrangement. All plots measured ten feet by ten feet. The lighter colors indicate the 27 original plots that were sampled. Darker colors indicate the ten samples that were used in the later part of the study. The ten plots that were used in the later part of the study were continuously fertilized to maintain a medium nitrogen level.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135
136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162
163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189
190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216
217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243
244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270

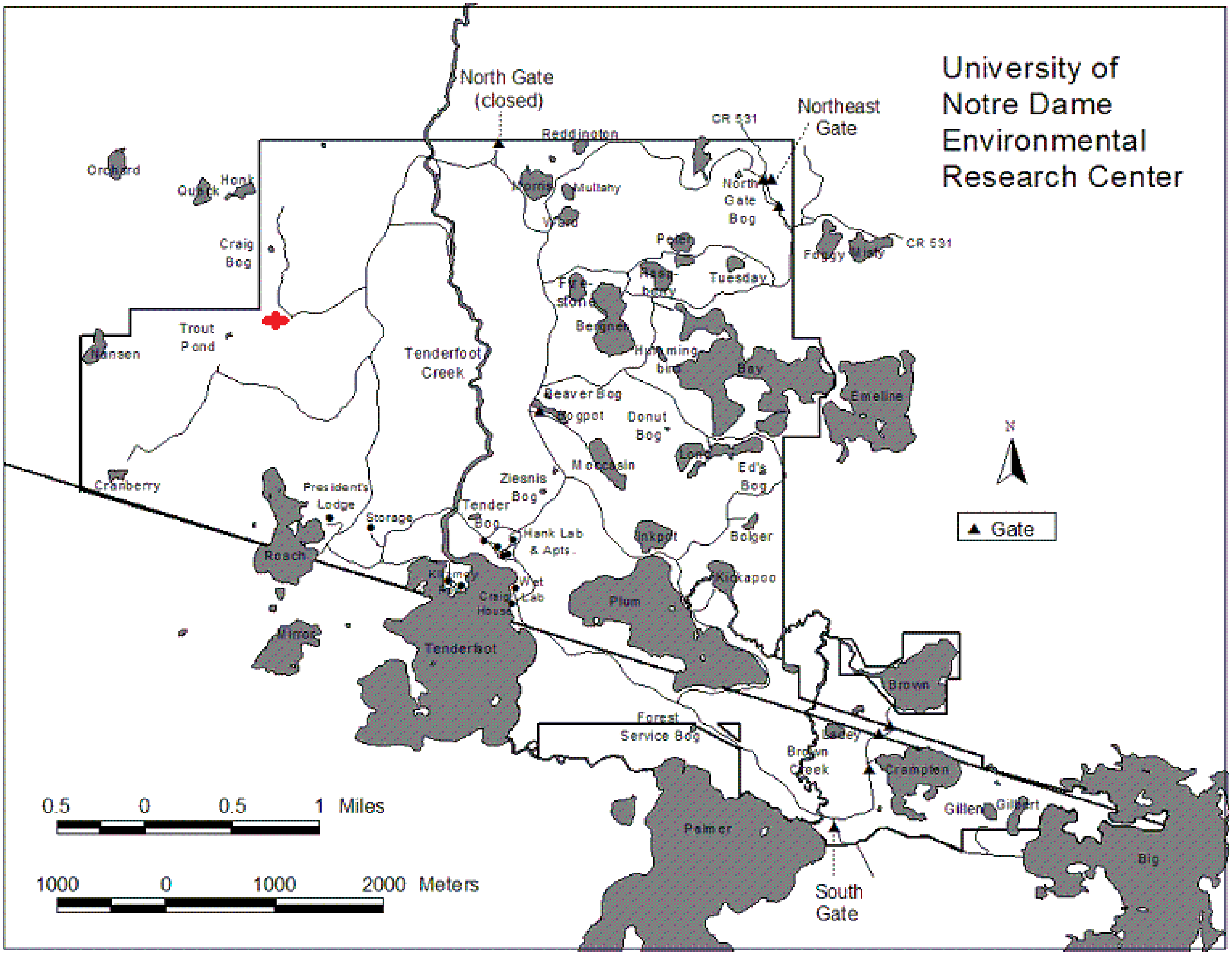

Figure 1

Figure one shows a map of UNDERC property. University of Notre Dame Environmental Research Center East (UNDERC) is located on the border between Wisconsin and the Upper Peninsula of Michigan. The red star shows the location of grasshopper nation.

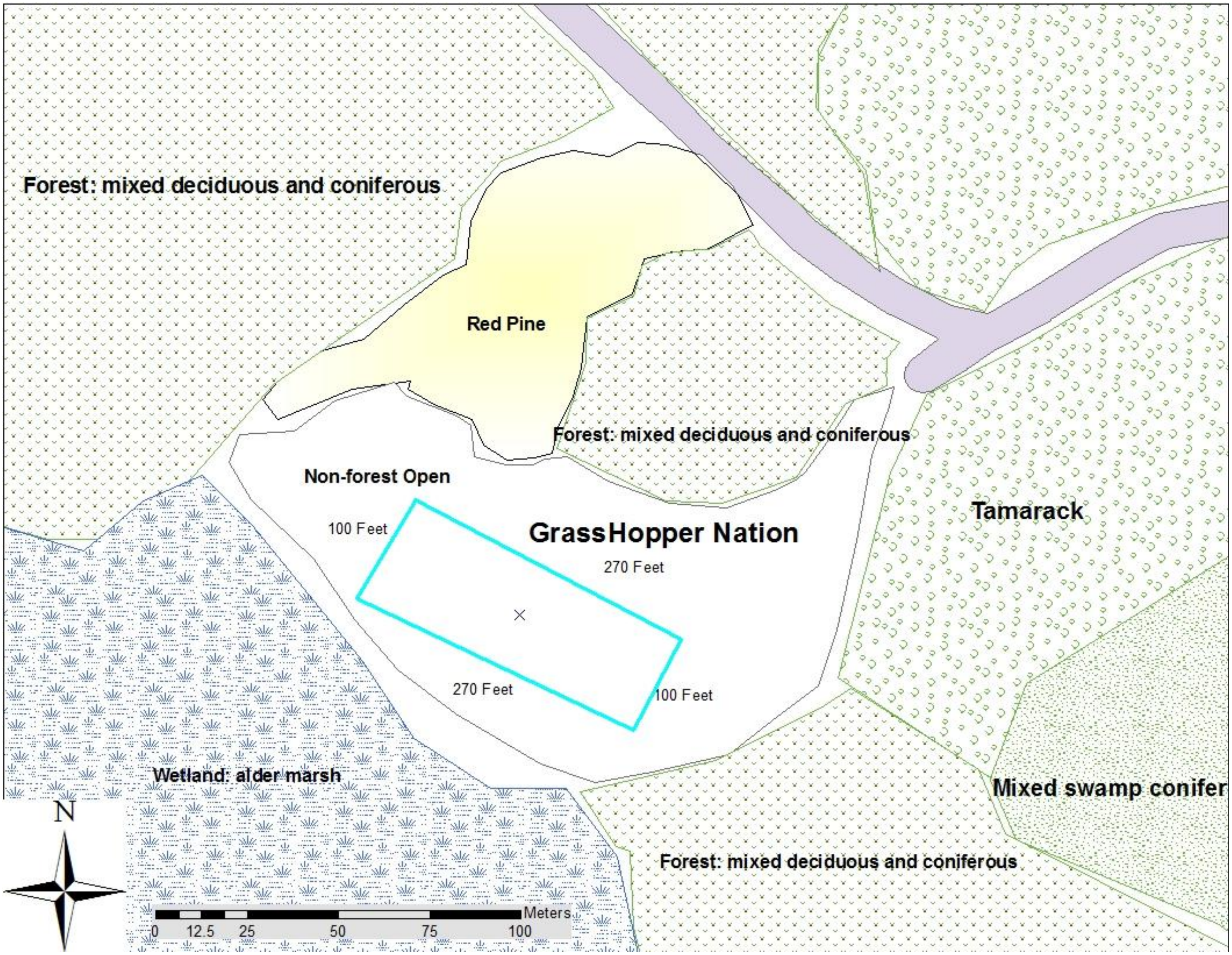

Figure 2

Figure two shows a map of grasshopper nation. Grasshopper nation is located on the west side of property at UNDERC. The average elevation is 513 meters. The length of my study site was 270 feet by 100 feet. The study site is primarily an open grassy area surrounded by mixed forests, tamarack trees, red pines, mixed conifers, and a marshy area.

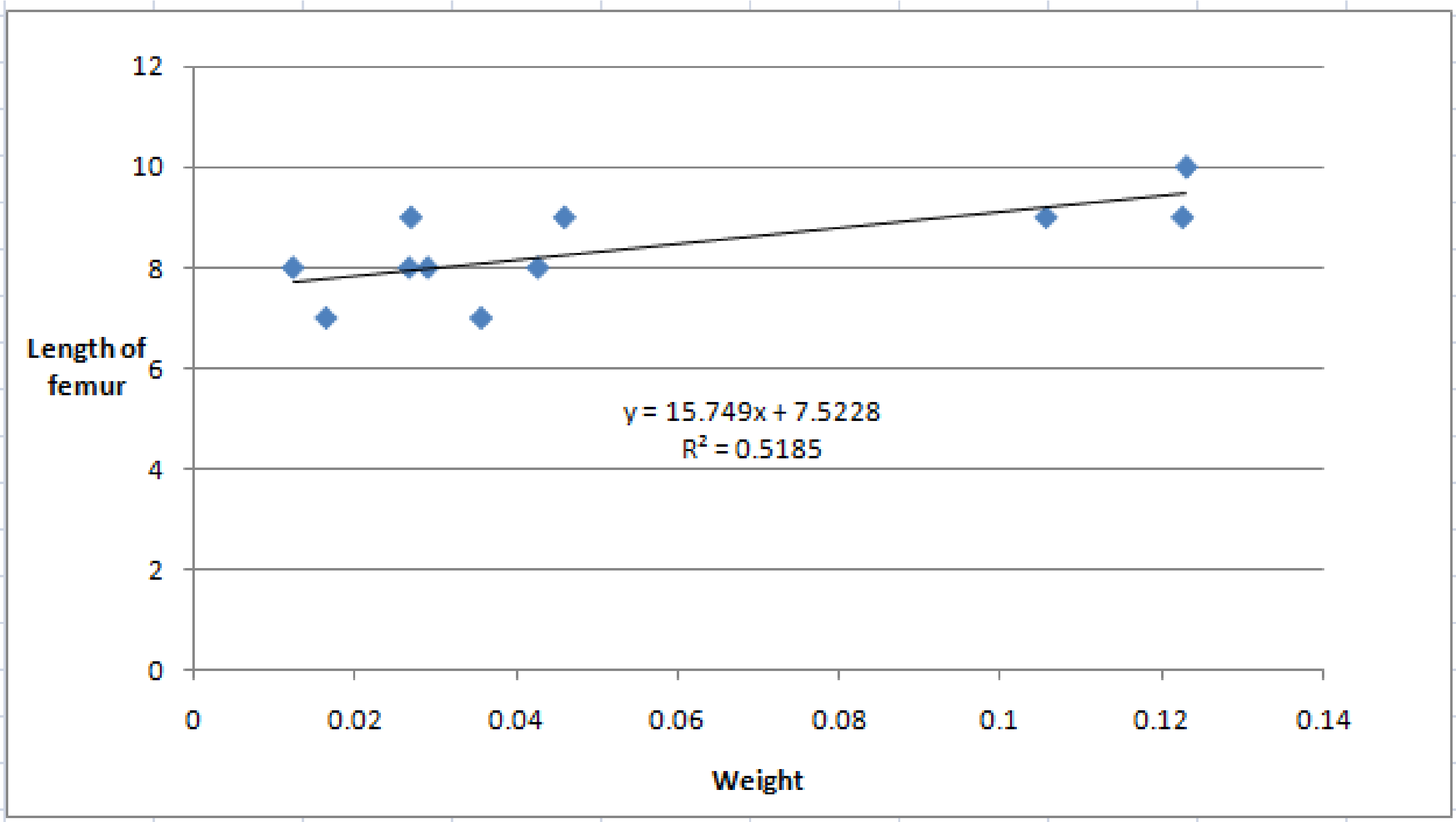

Figure 3

Figure three illustrates the final growth of individuals in the feeding trials. The three points that are on the higher weights are all from the Altered Sweet White Violet.

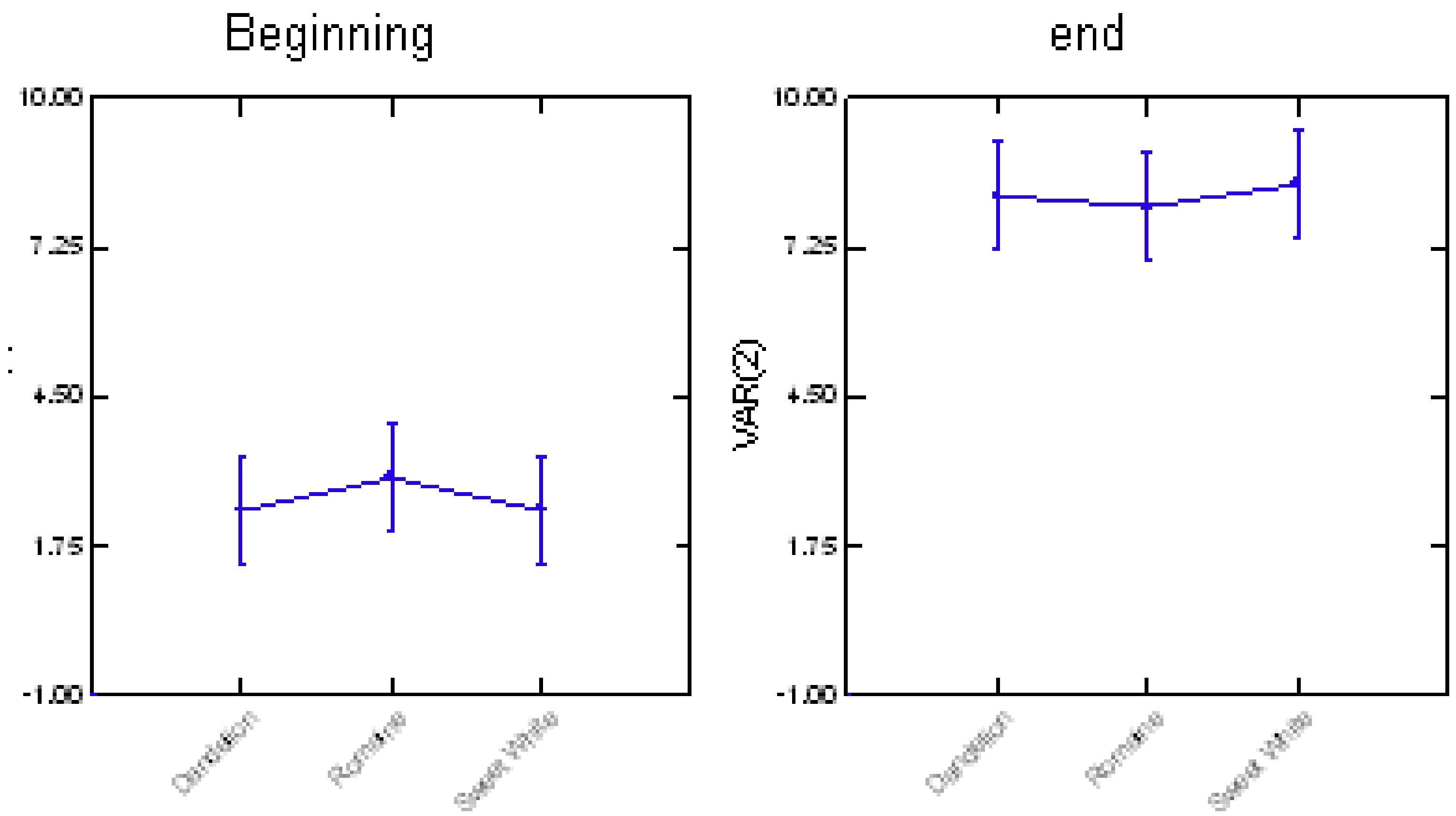

Figure 4

Figure four illustrates the beginning average weights of individual grasshoppers that were used in the study. figure on the left side shows the beginning weights, Dandelion is listed first, then Romaine lettuce, followed by Sweet white violet.

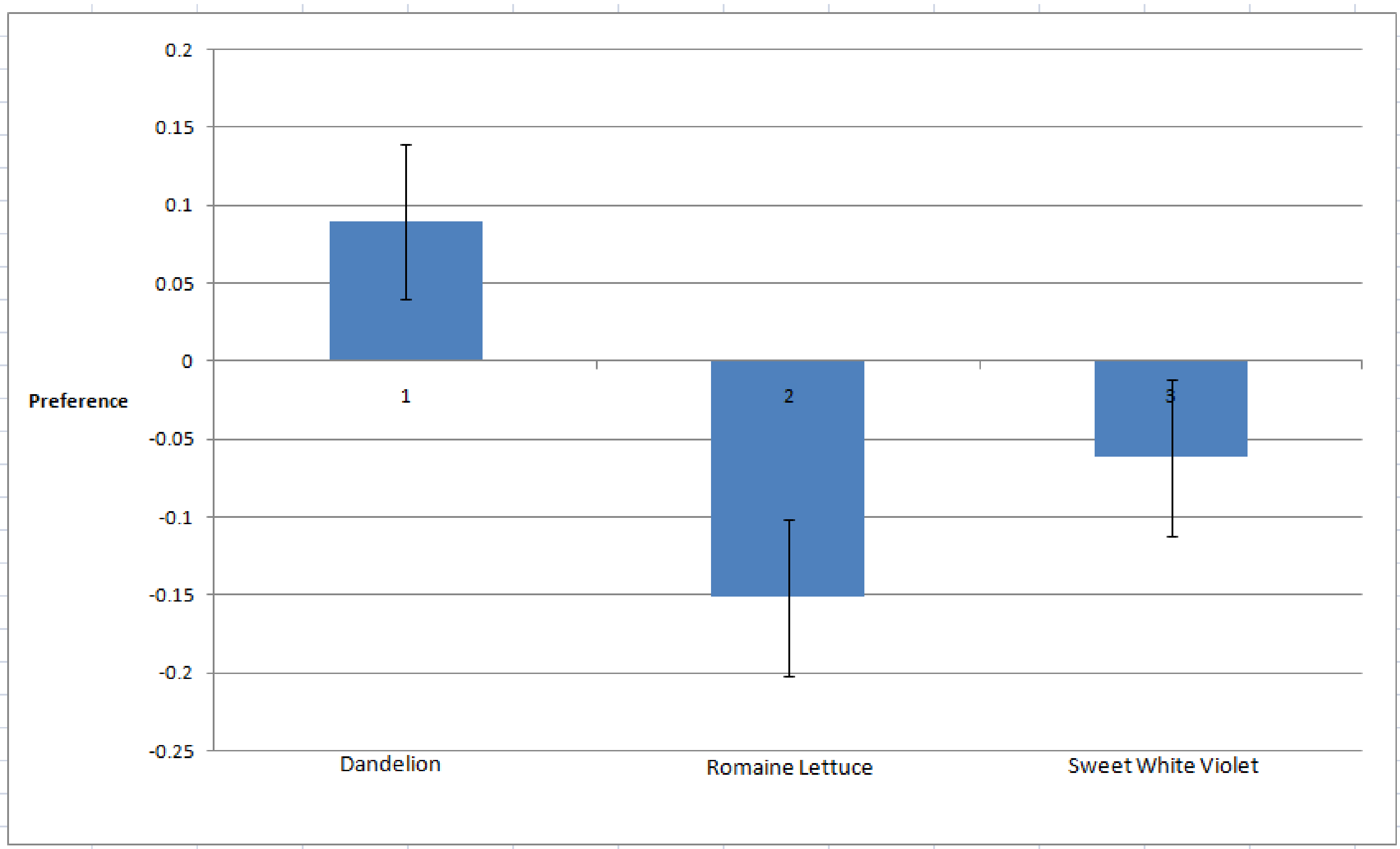

Figure 5

Figure five illustrates food preference between the food options. In this figure Dandelion is shown first, when given the option of feeding on dandelion and romaine lettuce, individuals generally chose to consume dandelion. Data for this chart was calculated by taking the mean values from a hotelling T^2 test.