2013

Spatial and Seasonal Variation in the Stable Isotopic Composition of Thirteen-lined Ground Squirrel Fecal Pellets as an Estimate of Variation in Diet

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Honors Thesis

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April 1, 2013

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ABSTRACT

We performed stable isotope analyses of fecal pellets to investigate temporal and spatial patterns in the diet of free-ranging, thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*). Samples were collected opportunistically from a population in SW Michigan from May to September 2012. We found a statistically significant increase in $\partial^{13}C$ values throughout the study period, possibly correlating to a late-season flush of C-4 plants. Also, samples collected within 25 m of a cornfield had a mean $\partial^{13}C$ value of -20.470‰, a value significantly higher than those outside the 25 m border (mean=-26.192‰). This pattern may be indicative of a C-4 rich diet probably due to increased corn consumption. Similarly, we found that the $\delta^{15}N$ values of pellets collected near the cornfield were also significantly higher (mean of 3.229‰) than those further away (mean=1.765‰). This may imply increased animal/insect consumption, or, more likely, is an artifact of artificial fertilizers.

INTRODUCTION

It is known that the omnivorous diet of *Ictidomys tridecemlineatus,* commonly known as the thirteen-lined ground squirrel, varies in the proportion of animal to plant consumption at different points in the active season. For example, a study of a population in Colorado found that approximately 44% of stomach content consisted of animal matter, predominately beetles, moth or butterfly larvae, and grasshoppers. This percentage varied considerably throughout the active season and was as high as 61.4% in late summer/early fall and as low as 36.4% in the early spring. Conversely, plant matter averaged 54.5% and, like animal matter, varied throughout the active season (Flake 1971). Furthermore, the ratio of C3 to C4 plants fluctuates seasonally as temperature varies. A study on a mixed prairie system in South Dakota showed that there was a significantly higher proportion of C3 plants in the spring and fall, but a higher proportion of C4 plants in the summer (Ode 1980). As the plant composition of a habitat changes with time, it is probable that the type of vegetation a squirrel consumes also changes seasonally.

Although several studies exist on the ecology of thirteen-lined ground squirrels (Flake 1971; Streubel 1978), none have addressed diet using the stable isotopic composition of fecal pellets, a biological component shown to have very minimal isotopic fractionation to that of foods consumed (Sponheimer 2003). This project specifically looks at seasonal variances in $\delta^{13}C$ and $\delta^{15}N$ of ground squirrel feces to estimate trends in C3 and C4 plant consumption as well as fluctuations trophic level (Sponheimer 2003).

$\delta^{13}C$ analysis determines the ratio of ^{13}C to ^{12}C in a sample, relative to a standard and expressed in parts per thousand. Fluctuations in this ratio are particularly useful in determining diet because the $\delta^{13}C$ signature of body tissue reflects that of foods consumed (DeNiro 1978). The different photosynthetic pathways of C3 and C4 plants cause these plant types to have different $\delta^{13}C$ values. Photosynthesis in C3 plants includes integration of atmospheric CO_2 with an enzyme called ribulose-1,5-bisphosphate carboxylase (RUBISCO). RUBISCO discriminates against the uptake of ^{13}C, causing C3 plants to have a $\delta^{13}C$ ranging from -35‰ to -21‰. Alternately, phosphoenolpyruvate carboxylase or PEP carboxylase assimilates atmospheric CO_2 in the C4 pathway and does not discriminate as strongly against ^{13}C. Consequently, C4 plants have a $\delta^{13}C$ between -14‰ and -10‰ (Ehleringer 1986; Kelly 2000; West 2006).

Similarly, $\delta^{15}N$ analysis determines the ratio of ^{15}N to ^{14}N and can be used to determine the trophic position of an animal (Drever 2000). The trophic level of an animal relates to its position in a food web and distinguishes between primary producers (e.g. plants), primary consumers (e.g. herbivores), secondary consumers (e.g. predators), and so on. On average, the ^{15}N content of animal tissue is enriched 3‰ from that of its diet (DeNiro 1981); this enrichment can be attributed to the preferential excretion of ^{14}N. When protein is digested and broken down by a consumer, the body uses those amino acids to build other proteins as well as carbohydrates through processes known as deamination and transamination. The nitrogenous waste created as by-products from these processes are excreted from the body in the form of either uric acid or urea; urea and uric acid have a lower $\delta^{15}N$ than that of diet. Due to this preferential elimination of ^{14}N from the body, we see a higher $\delta^{15}N$ relative to that that of food consumed (Kelly 2000). Thus, $\delta^{15}N$ values may indicate whether an animal is primarily eating vegetation or animal matter.

METHODS

Throughout a portion of the active season fecal samples were collected from ground squirrels at the Andrews University Air Park. On a weekly basis, ground squirrels were live-trapped at random using wire mesh traps crafted by members of our research team. When a squirrel was spotted, a trap was positioned over the burrow opening and staked into the ground. These were checked every 15-30 minutes for a capture of a squirrel. When a specimen was trapped, it was transferred into mesh field bag where the sex of the animal could be determined. After recording the GPS coordinates of the capture location, the squirrel was weighed and released. We collected fecal samples opportunistically; if the squirrel provided fecal pellets, these samples were collected in a glass tube, labeled, and stored in a freezer. Andrew Brassington collected these samples from the start of the project in mid-May until mid-July. I took over at this point and continued this process until the end of September.

In preparation for isotope analysis, fecal samples were removed from the freezer and dried in a laboratory oven between 8 and 10 hours at 70°C. Each sample was then ground using a mortar and pestle and sifted through a 40-mesh sieve (sieve size = 0.422 mm). These were packaged and sent to the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah for $\delta^{13}C$ and $\delta^{15}N$ analysis. This analysis was done using a Delta Plus isotope ratio mass spectrometer (Finnigan-MAT, Bremen, Germany) interfaced with an Elemental Analyzer (model 1110, Carla Erba, Milan, Italy). The instrument precision for $\delta^{13}C$ is ± 0.15 ‰ and ± 0.2 ‰ for $\delta^{15}N$.

The mass spectrometry results were analyzed graphically and statistically with SPSS 19 for Mac. Analysis included a correlation between isotope values and time of study period, a t-test comparing isotope values between spatial clusters, and a t-test comparing isotope values between sexes within spatial clusters. Isotope values were also plotted spatially using ArcGIS 10.

RESULTS

Fecal pellet stable isotope values for $\partial^{13}C$ displayed a positive correlation with day of collection throughout the study period for samples collected within 25 m of a cornfield as well as those collected further away (Fig. 1; Near cornfield: r= 0.404, n=34, p<0.01; Away from cornfield: r= 0.315, n=38, p<0.05). There was no such correlation between $\partial^{15}N$ values and time.

Stable isotope values clustered spatially (Figs. 2 & 3); fecal pellets collected within 25 m of a cornfield showed significantly higher $\partial^{13}C$ values (mean=-20.470, *sd* =3.988, *n*=34) than those collected further from the cornfield (mean=-26.192, *sd* =4.042, *n*=38; t = 6.035, p<0.001). Likewise, samples collected near a cornfield displayed significantly higher $\partial^{15}N$ values (mean=3.229, *sd* =0.804, *n*=34) than those collected further away (mean=1.765, *sd* =1.07, *n*=38; t = 6.485, p<0.001). There is a significant correlation between $\partial^{13}C$ and $\partial^{15}N$ values (Fig. 4; r = .728, *n*=72, p<0.01)

Fecal samples collected from male specimens captured within 25 m of a cornfield had significantly higher $\partial^{15}N$ values (Fig. 5; mean=3.656, *sd*=0.727, *n*=10) than did females within the same spatial cluster (mean=3.048, *sd*=.799, *n*= 22; t=2.048, p<0.05). No difference was observed between males and females in $\partial^{15}N$ away from the cornfield, or in $\partial^{13}C$ of either spatial cluster.

DISCUSSION

The increase in $\partial^{13}C$ values throughout the study period shows there was a definite seasonal trend in vegetation consumption (Fig. 1). The diet of the squirrels is more C3 plant dependent in spring and early summer and gradually shifts towards C4 plants in mid and late summer. A higher proportion of C4 plants in diet may suggest a higher ratio of C4 plants in habitat composition. This is consistent with the findings of Ode (1980), who reported a flush of C4 plant during the warmest parts of the season.

Unlike $\partial^{13}C$, there is no such temporal pattern in the fecal pellet $\partial^{15}N$ composition and, thus, no apparent seasonal variation in trophic position. However, by plotting data points according to geographic location we were able to observe a dramatic spatial difference in both the $\partial^{13}C$ and $\partial^{15}N$ values of samples collected within a relatively small area (Figs. 2 and 3). The higher $\partial^{13}C$ values of samples collected within 25 m of a the cornfield indicate that these squirrels consumed a larger proportion of C4 plants (most likely corn), whereas the lower $\partial^{13}C$ values from samples collected greater than 25 m away from the cornfield imply that these individuals relied more on C3 plants. Likewise, samples collected near the cornfield had higher $\partial^{15}N$ values than those collected further away. This higher fecal ^{15}N content may indicate that these squirrels consumed a higher proportion of insect/animal matter than those found further away. However, because of the direct correlation between $\partial^{13}C$ and $\partial^{15}N$ values in the context of the cornfield, it may be more likely that the fertilizer used on the cornfield had an enriched $\partial^{15}N$ value relative to the soil of the surrounding area. Together the spatial patterns in $\partial^{3}C$ and $\partial^{15}N$ values emphasize the power of using the stable isotopic composition of feces as a tool to discern between different food groups present in diet.

We also observed that those fecal samples from male squirrels within 25 m of a cornfield had significantly higher $\partial^{15}N$ values than those collected from females within the same spatial cluster (Fig. 5). This may suggest that the males here did indeed consume more animal or insect matter, but this hypothesis needs to be tested. There was no such difference found between sexes for $\partial^{13}C$ values.

These data will be incorporated into a larger study investigating stable isotopic composition of squirrel tooth enamel and its relationship to diet. Recently, a study conducted here at Andrews University looked at stable isotopes in tooth enamel towards the end of the active season and throughout hibernation and found a peak in $\delta^{13}C$ in late summer; this could be due to a shift in C4-based diet at this time in the season (Jang 2012). Data from the Jang et al. (2012) study coupled with that of diet and a current investigation of tooth enamel will be used to determine what type of information we can infer from seasonal variation in stable isotopes in rodent enamel. This information may be used to develop an analog to assist in future fine-scale paleoecological modelling studies of seasonal variation fossil rodent diet.

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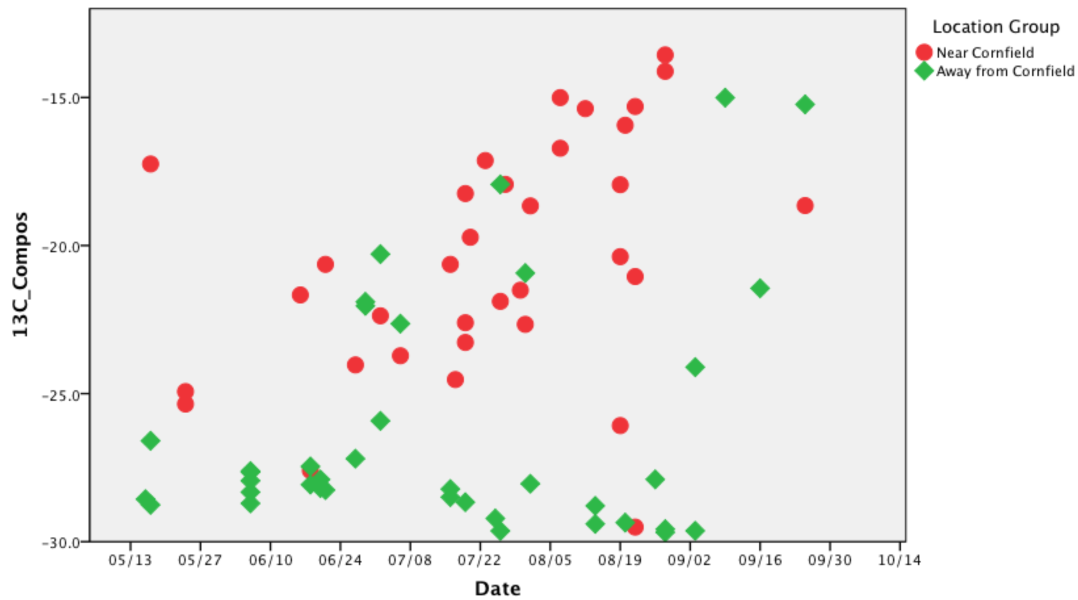

FIGURE 1 – ∂[13]C values plotted over time.

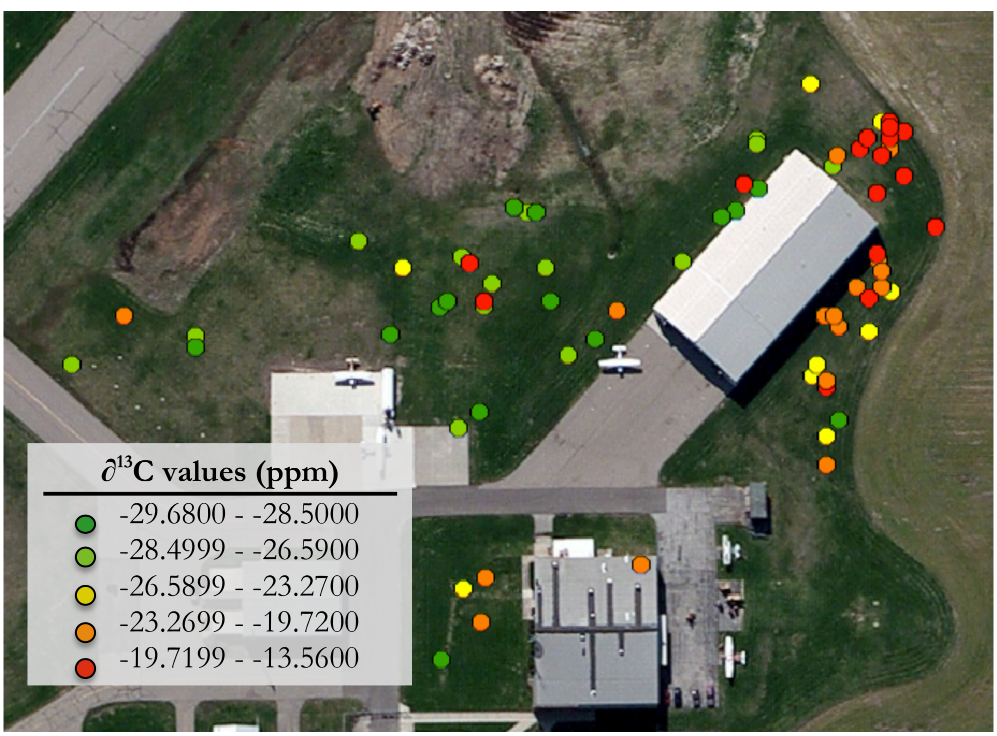

FIGURE 2 – ∂ [13]C values plotted by location on a map of the Andrews University Airpark. A cornfield is located along the ride side of the image. North is to the right.

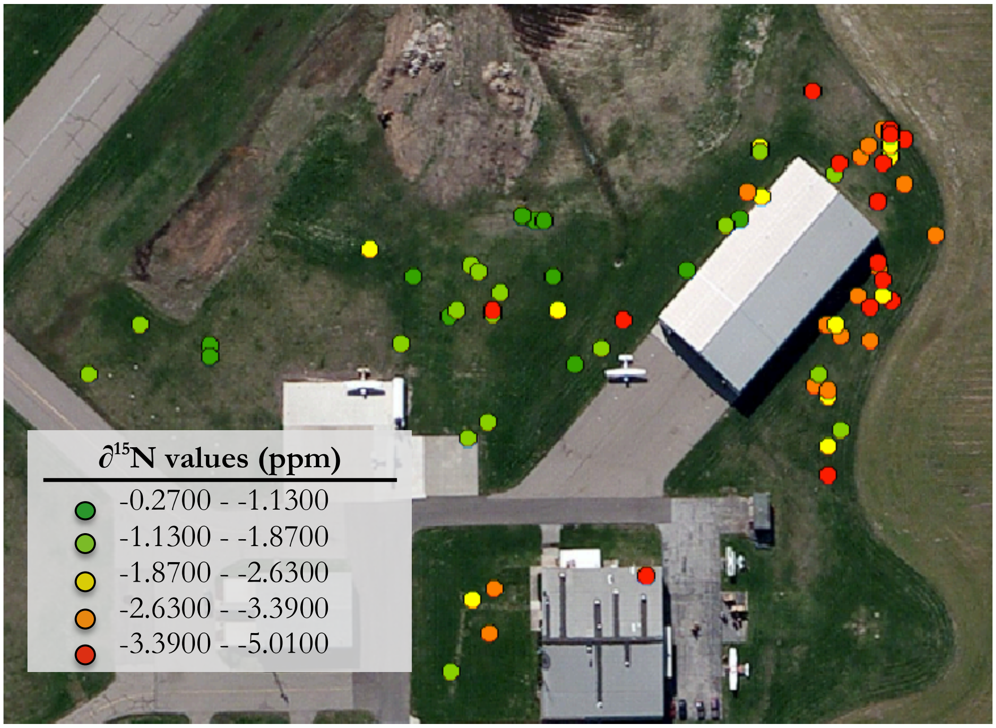

FIGURE 3 – $\partial\ ^{15}N$ values plotted by location on a map of the Andrews University Airpark. A cornfield is located along the ride side of the image. North is to the right.

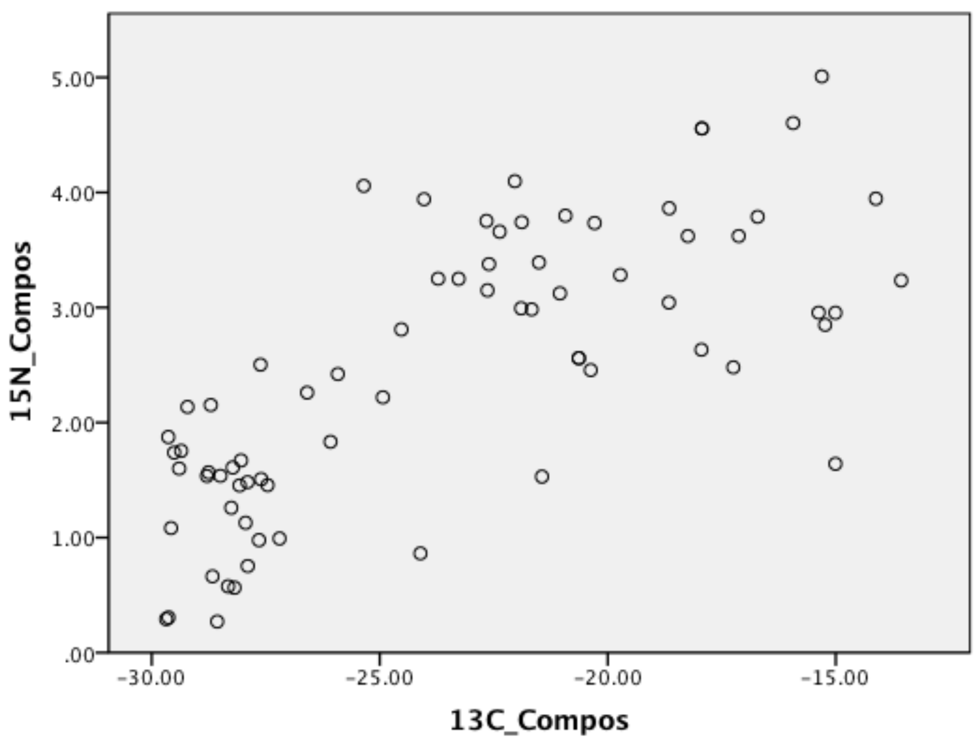

FIGURE 4 – Correlation between $\partial\ ^{15}N$ and $\partial\ ^{13}C$ values.

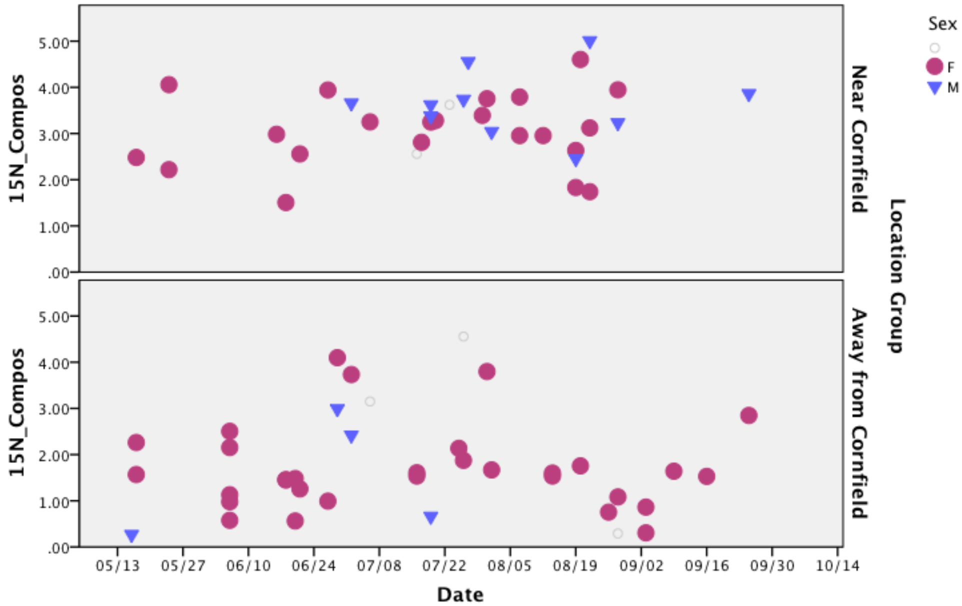

FIGURE 5 – ∂^{15}N values spatial clusters plotted over time with regards to sex.