Testing the cost of diet switching as a mechanism

for the evolution of strict herbivory in lizards

MS Thesis Proposal

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Abstract

Herbivory is rare in reptiles generally, and particularly so in lizards. My study seeks to explain why strict herbivory should evolve in lizards. Captive herbivorous reptiles raised on a high protein diet grow faster and reach sexual maturity more quickly than those fed a low protein diet. Given several disadvantages associated with eating plants, and the apparent advantages to herbivores that supplement their diets with animals, why should strict herbivory evolve in nature? I will test the hypothesis that switching from an herbivorous to a carnivorous diet, and back again to herbivory, is energetically costly for herbivorous lizards. This cost may be manifested as a decrease in digestive efficiency resulting from a concomitant disturbance of the gut endosymbiont community, which is responsible for fermenting plant fiber. If this hypothesis is supported, then the energy lost from the predicted decrease in digestive efficiency should be greater than the energy that would be gained from supplementing the diet with animals. My study imposes diet-switching treatments on desert iguanas (*Dipsosaurus dorsalis*) and compares their digestive efficiency and the community structure of their gut microbes before and after diet switches. I will determine whether significant changes in digestibility and endosymbiont community structure are associated with diet switches.

Background

What an organism eats and the way it forages is a fundamental determinant of its physiology, ecology, and behavior (King 1996). Herbivory is a dietary specialization that has broad-reaching biological implications, yet there have been few studies that address the question of how diets evolve (Cooper and Vitt 2002). Herbivory is rare in lizards and in reptiles generally, and for more than 40 years investigators have attempted to explain its paucity (Szarski 1962; Ostrum 1963; Sokol 1967; Pough 1973; Iverson 1982; Cooper and Vitt 2002; Espinoza 2002). However, no study has addressed *why* strict herbivory should evolve given the many challenges associated with acquiring, digesting, and subsisting on a diet of plants. These challenges include digesting and assimilating a relatively lower quality food, excreting the high concentrations of salts sequestered in plant tissues, increasing the processing time of food in the gut, and perhaps, higher energetic costs for digestion (Iverson 1982; Zimmerman and Tracy 1989; Bjorndal and Bolten 1990; Espinoza 2002).

Disadvantages of Herbivory

Plants are a lower quality food source than animals because most plant tissues (particularly leaves) contain less digestible energy and nutrients on a gram-per-gram basis. A diet of plant material has relatively high potassium and sodium content requiring herbivores to have a means of excreting the excess salts (King 1996). Moreover, vertebrates do not have endogenous enzymes to digest plant fiber (cellulose, hemicellulose, and lignin), so folivores require a symbiotic relationship with gut microorganisms to ferment energy-rich volatile fatty acids from plant fiber (Troyer 1991; Stevens and Hume 1995; Bjorndal 1997). In addition, the process of

fermentation necessitates longer retention of digesta, thereby limiting the amount of food that the animal can ingest at a given time (Stevens and Hume 1995).

Herbivores that Cheat Reap Rewards

High protein diets (comparable to those eaten by carnivores) provide clear advantages to herbivorous reptiles. Growing herbivorous and omnivorous reptiles fed higher proportions of animal matter grow faster, achieve a larger body size, and have larger clutches of eggs than do those that consume diets less rich in animal matter (Vogt and Guzman 1988; Avery et al. 1993). Anecdotal reports also indicate that captive herbivorous *Sauromalus ater* raised on a high-protein diet (equivalent to carnivorous diets) grow faster and reach sexual maturity more quickly than do those fed a low protein diet (Mayhew 1963). Moreover, herbivorous lizards, which have evolved from omnivorous or carnivorous ancestors, apparently retain the ancestral ability to digest animal matter (Espinoza 2002). When fed dandelion flowers, carnivorous lizards (*Crotaphytus bicinctores*) had lower digestive efficiencies than herbivorous lizards (*S. ater*) on the same diet. Herbivorous lizards (*S. ater*), not surprisingly, digested the flowers efficiently, but also digested the insects as well as did the carnivores (Ruppert 1980). Apparently, herbivores can digest animal matter because their capacity to do so has not been evolutionarily lost. Carnivorous lizards may not be able to digest plant tissues because they lack the morphological and physiological specializations required for herbivory (see *Specializations for Herbivory*, below). Collectively, these studies beg the question, why don't herbivores switch entirely or primarily to a diet of insects when these prey items become seasonally available in their natural environment (Espinoza 2002)?

Specializations for Herbivory

Herbivorous lizards, like other herbivorous vertebrates, exhibit a number of specializations that are considered to be adaptations for a diet of fibrous plant tissues. Adaptations that are relevant to this study include larger body size (generally >100 g; Pough 1973; Iverson 1982; Cooper and Vitt 2002; Espinoza 2002) and a larger gut relative to carnivorous lizards (Iverson 1982; Espinoza 2002), microbial hindgut endosymbionts that ferment plant fiber (Troyer 1991; Bjorndal 1997), and the presence of one or more transverse valves or evaginations in the colon of most strictly herbivorous lizards (Iverson 1982).

Herbivorous lizards tend to have a larger body size than carnivorous lizards. Larger lizards have lower mass-specific metabolic rates, and thus lower energy requirements, but higher gross energy needs relative to smaller lizards (Pough 1973). Chasing small, elusive energy-rich insects is not thought to be an efficient way for large lizards to forage because large lizards are less agile and spend more energy to move their bodies than small lizards (Pough 1973). Consequently, large reptiles should rely on food resources that are plentiful and require less energy to obtain, such as plants (Pough 1973).

Herbivorous lizards have larger relative gut sizes than carnivorous lizards. The large gut of herbivorous lizards is accounted for by a longer midgut and a more voluminous hindgut when compared to omnivorous and carnivorous species (Iverson 1982; Espinoza 2002). The large gut permits a higher volume of plant material to be processed, which is necessary because of the lower extractable energy and nutrients per gram of plant material compared to animal matter, and because of the longer gut retention time needed to extract that energy from plant tissues (Iverson 1982, Troyer 1991, Bjorndal 1997).

Vertebrates do not possess endogenous enzymes for digesting plant fiber, therefore they rely on fermentation by microbial endosymbionts to obtain a substantial portion of the energy bound in plant fiber (Iverson 1982; Troyer 1991; Bjorndal 1997). These endosymbionts include bacteria and protozoa that ferment plant fiber, producing short-chain fatty acids (Bjorndal 1997). In reptiles, fermentation occurs in the small intestine (some freshwater turtles) or large intestine (tortoises and lizards) (Bjorndal 1997).

Transverse and semilunar valves may serve several functions in the digestion of plant material. First, they presumably slow the passage of digesta, which should allow more energy and nutrients to be extracted from a food bolus. Second, these partitions increase the surface area of the gut, which should enhance absorption. Third, the valves may help retain the endosymbionts that are responsible for fermenting plant fiber (bacteria and protozoa) or mixing the food bolus (nematodes) (Iverson 1982; Troyer 1991; Bjorndal 1997; Espinoza 2002).

Under What Conditions Does Herbivory Evolve?

The evolution of plant eating (i.e., omnivory) in reptiles is correlated with seasonally hot, arid environments (Cooper and Vitt 2002; Espinoza 2002). Recent herbivorous reptiles tend to occur in seasonal environments where food quality and quantity fluctuate (Cooper and Vitt 2002; Espinoza 2002). Most herbivorous reptiles occurring outside of the tropics and subtropics are found in warm deserts (Zimmerman and Tracy 1989; Espinoza and Tracy 1997; Espinoza 2002).

How the Cost of Diet Switching Could Preclude Carnivory?

Changes in diet have long been recognized to disrupt digestion in herbivores. When domestic cows and other ungulate herbivores are moved from a dry pasture to a green pasture they

experience a disruption in digestion colloquially termed "sour" stomach (Allen 1868). Among reptiles, hatchling desert tortoises experience a substantial reduction in digestive efficiency following a diet switch from forbs to grasses, and vice versa, and the recovery period takes ~3 wk (C. R. Tracy et al. unpubl. data). Changes in diet also affect the community structure of gut endosymbionts, although few studies have attempted to quantify these changes (e.g., Santo Domingo et al. 1998; Simpson et al. 1999). Given that changes in the species of plants eaten can change digestive efficiency, a dramatic change in energy assimilation is expected when an herbivore switches from an carnivorous to an herbivorous diet. If the concomitant decline in energy assimilation is greater than the energy that is gained when an herbivore supplements its diet with insects, then herbivorous lizards should not switch to eating insects when the latter become seasonally available (Espinoza 2002). Conversely, approximately 10% of all "carnivorous" lizards supplement their diet with plant material (Cooper and Vitt 2002; Espinoza 2002). Facultative herbivory is not expected to pose an energetic challenge for carnivorous lizards because they tend to eat high-energy, easily digestible plant material, such as fruits and flowers (reviewed by Cooper and Vitt 2002; Espinoza 2002). Moreover, carnivores are not known to depend on endosymbionts for nutrient extraction, so disturbance of the microbial community following a diet switch is unlikely to pose an energetic challenge.

If switching to eating insects causes a reduction in endosymbiont diversity as a result of changes in the gut environment, then the host should experience a reduction in digestive efficiency when it switches back to eating plant material. This decrease in digestive efficiency could cause a dramatic reduction in the amount of energy that the host obtains. Because of this, herbivores may not switch their diet to one of primarily insects when such food items become seasonally available in their environment (Espinoza 2002).

Study Species

My study will assess the energetic cost of diet switching for the desert iguana (*Dipsosaurus dorsalis*), a small (40–90 g) herbivorous iguanid (Mautz and Nagy 1987). *Dipsosaurus* is one of the smallest species of strictly herbivorous lizards (Iverson 1982; Mautz and Nagy 1987; Espinoza 2002). This species is distributed in the Mojave and Colorado deserts—highly seasonal places—and is dormant for approximately five months of the year, October–March (Norris 1953; Muth 1980). Most diurnal insects are available for a short period of time (following the rainy season) within the activity period of *D. dorsalis* (Cloudsley-Thompson 1975). A two-year study of *D. dorsalis* that included both hatchling and adults found that <1% of the diet was insects (Mautz and Nagy 1987). *Dipsosaurus* have the specializations described above to aid in the digestion of plant material, including fermentative endosymbionts (Zimmerman and Tracy 1989), enlarged mid- and hindguts, and one transverse valve in the colon (Iverson 1982).

Hypotheses and Predictions

I am testing the hypothesis that switching from an herbivorous diet to a carnivorous diet and back again to an herbivory will be energetically costly to desert iguanas because the resulting disturbance to the endosymbiont community will cause reduced digestive efficiency.

Diet Treatments and Predictions

Three groups of desert iguanas (N = 10 per group) were fed one of three diet treatments. One group was fed an herbivorous diet, switched to a carnivorous diet, and then switched back to the herbivorous diet (H ⚡ C ⚡ H). I predict that after the switch to carnivory, there should be an

increase in digestive efficiency because animal matter is a higher quality food than plants. There should also be a change in structure of the endosymbiont community as a result of substantial changes to the environment of the gut (e.g., an increase in gut pH is expected as a result of the absence of short-chain fatty acid products of fermentation; Stevens and Hume 1995). The diet switch should make the gut milieu less suitable for some microbial species. However, digestive efficiency should not diminish because endosymbionts are not required for the digestion of animal matter. After the switch back to herbivory, a sudden and significant reduction in digestive efficiency should be observed, followed by a gradual return to the original level of efficiency. This is because the endosymbiont community is expected to no longer be dominated by fiber-fermenting species during the carnivorous diet phase, but this component of the community should increase in proportion over 1–2 weeks after the switch back to an herbivorous diet (Van Soest 1982).

Because a wide variety of primarily carnivorous lizards take advantage of high-quality plant foods (e.g., flowers and fruits) when they become seasonally available (Cooper and Vitt 2002; Espinoza 2002), a second group of desert iguanas will be fed the carnivorous diet, switched to the herbivorous diet, and then switched back to the carnivorous diet (C $\rightleftharpoons$ H $\rightleftharpoons$ C). Thus, this treatment simulates the natural diet switches noted for many otherwise carnivorous or omnivorous lizards. The digestive efficiency should decline after the switch from the initial carnivorous diet to the herbivorous diet because the diet of animal matter is expected to create an environment in the gut that is not optimal for fermentative endosymbionts (Espinoza 2002). The endosymbiont community is expected to gradually recover following the diet switch from carnivory to herbivory and digestive efficiency should also gradually return to initial levels. Digestive efficiency should increase following the switch back to carnivory, but the

endosymbiont community should change substantially after this switch because of the aforementioned changes to their environment.

A third group of lizards will be fed an omnivorous diet throughout the experiment, with no diet switching. This treatment will serve as a control: if changes in endosymbiont community or digestive efficiency occur in this group, they can be attributed to factors other than a change in diet. In addition, the omnivore treatment will provide data on how digestive efficiency and the endosymbiont community are affected by a more diverse diet. I predict that the digestive efficiency of this group should be high and stable because of the associative effects of multiple diet items, which results in a higher digestive efficiency than would be predicted based on digestive efficiencies of single diet items (Bjorndal 1991). The endosymbiont community should also be stable, although different from communities in the guts of lizards on the herbivorous, carnivorous, or recently switched diets because of differing conditions in the gut.

Materials and Methods

Experimental Animals and Husbandry

Thirty desert iguanas were collected in Palm Springs, Riverside County, California (33.80654° N, 116.51076° W) under permit from the California Department of Fish and Game (803054–04). Adult and subadult animals of both sexes were collected. Following capture, lizards were oiled to remove external parasites (Espinoza et al. 1998), toe clipped for identification, and their snout-vent length and body mass were recorded.

Animals were housed individually in ventilated plastic containers (30.0 x 16.5 x 9.0 cm) lined with plastic grating. Containers were kept in environmentally controlled chambers (CMP

4030 Equipped Chamber, Conviron Winnipeg, Manitoba, Canada) with a 14 L:10 D photoperiod temperature programmed for 30 °C during scotophase and 35 °C during photophase (± 1 °C). The containers were randomly assigned to a new position in one of the two chambers each day to mitigate chamber effects.

Experimental Diets

Ten lizards were randomly assigned to each of three diet treatments: (1) herbivorous ⚊ carnivorous ⚊ herbivorous, in which individuals were fed rabbit chow for 5 wk, switched to a diet of crickets for 5 wk, and then switched back to the herbivorous diet for 5 wk; (2) carnivorous ⚊ herbivorous ⚊ carnivorous, in which individuals were started on the carnivorous diet for 5 wk, switched to the herbivorous diet for 5 wk, and then switched back to the carnivorous diet for 5 wk; (3) omnivorous, in which individuals are fed a 50/50 mix of rabbit chow and crickets for the duration of the experiment (15 wk). The carnivore diet consists of adult crickets (*Acheta domesticus*), which were killed by freezing (ca. –17 °C) and dried in a drying oven (105–110 °C) to constant mass. Dried crickets were ground in a coffee mill and passed through a 1 x 1-mm screen. The herbivore diet consists of ground rabbit chow (Diamond Pacific Products, Perris, CA), which according to the manufacturer contains at least 16% protein, 3% fat, and no more than 21% crude fiber. The omnivore diet consists of a 50:50 (by mass) combination of the carnivore and herbivore diets. The ground foods were prepared in sufficient quantity to feed the lizards throughout the experiment. Dried, ground foods were hydrated with distilled water (1:3 by mass) immediately before feeding. Thus, as in nature, lizards obtained their water through the hydrated food (Minnich and Shoemaker 1970): 75% water by mass. Diets were lightly dusted with a 50/50 mix of multivitamin and calcium powder (Rep-Cal® Phosphorus-free

Calcium with Vitamin D and Rep-Cal[®] Herptivite, Los Gatos, CA) weekly to ensure that lizards were receiving proper nutrition.

Lizards were force-fed daily from 0800–1100 and given sufficient food to maintain body mass (± 10%) during the digestive efficiency experiments. The amount of food ingested was determined by weighing lizards before and after feeding. Three lizards will be excluded from analyses because their body mass decreased by more than 10% during the feeding trials.

Digestive Efficiency

During the feeding trials feces were collected daily and dry mass was obtained as described above. From these data, apparent digestive efficiency was estimated using the equation:

DE = (dry mass of food ingested – dry mass of feces)/(dry mass of food ingested).

This estimates digestive efficiency because some gut tissue (e.g., sloughed cells from the gut lining) may be eliminated with the feces, resulting in an underestimation of true digestive efficiency (Van Soest 1982).

I will use repeated measures ANOVA ($\alpha = 0.05$) to determine whether there are significant differences in digestive efficiency associated with diet switching among treatment groups.

Analysis of the Gut Endosymbiont Community

Fresh feces (<10 min following defecation) were collected for 1 wk before and 2 wk after the diet switches. This was accomplished by checking the lizards every 10 min from 0800–1230 daily (the time over which most individuals defecated). Individual feces were placed in cryovials using sterile techniques and stored at –80 °C until the molecular analyses were conducted.

For the two switching treatments, at least twelve fecal samples for each of eight lizards (four each treatment, >96 samples total) were analyzed for microbial diversity. Four lizards in the omnivore treatment were also analyzed, but fewer samples were used because the microbial community was expected to change less. Changes, if any, in the composition of "species" in the endosymbiont community were characterized using denaturing gradient gel electrophoresis (DGGE) followed by sequencing of PCR-amplified 16S ribosomal subunit DNA (Mackie 2002). Cells were lysed and DNA extracted and amplified using the Qiagen UltraClean Soil DNA kit. DGGE allows separation of DNA fragments differing by as little as a single base pair, which is not possible with standard gel electrophoresis (Fischer and Lerman 1983). Gel electrophoresis separates DNA fragments based on the size and charge of the fragments. DGGE is a similar technique, but the gel contains an increasing concentration of denaturant (urea/formamide). The denaturant causes folded fragments to abruptly unfold at specific positions in the gel, strongly curtailing the fragment's ability to move through the gel (Fischer and Lerman 1983). This method separates fragments according to their melting properties (Sheffield et al. 1989) (based on base-pair sequences) rather than length. Consequently, more stable fragments occupy deeper positions in the gel than do less stable fragments.

Dissimilar DGGE bands correspond to distinct microbial taxa, so the number of bands can be used to estimate species diversity (Mackie 2002). From these data, changes in the structure of the gut endosymbiont community following changes in the host's diet can be estimated. The molecular analyses were conducted at the University of Illinois, Urbana–Champaign in the laboratory of Dr. Roderick I. Mackie.

Endosymbiont species diversity will be calculated using the Simpson diversity index:

$$D = 1/\Sigma\, P_i^{\,2},$$

which emphasizes the most abundant species (Ricklefs 1997). This index was selected because the endosymbionts that are important for fermentation should be the most abundant, whereas rare species are expected to be unimportant to digestion and more likely to vary randomly with respect to treatment. Species evenness was assessed using the equation:

$$E = H/H_{\max},$$

where H is the Shannon diversity index and $H_{\max}$ is the natural log of species richness.

Diversity Database is being used to define bands and banding patterns. This information will be used to determine which species are associated with which diet for each individual lizard using Spearman's rank correlation. A multivariate synthetic variable for all of the individuals together will be generated and patterns of changes in each individual can then be plotted.

Discussion

If diet switching is associated with a reduction in digestive efficiency and a concomitant change in the community structure of gut endosymbionts, then the energetic cost of switching between different diets may preclude herbivorous lizards from eating insects in their natural environment. Consequently, strict herbivory would likely coincide with the evolution of adaptations for digesting leafy plant matter (as described above). If I find no change in digestive efficiency and/or endosymbiont community structure, then the presumed cost of diet switching can not explain why herbivorous lizards do not switch to eating insects when these protein-rich foods become seasonally available and alternatives should be investigated. The possibility of energetic constraints associated with capturing mobile prey is an alternative explanation for the evolution of strict herbivory. Herbivorous lizards are large relative to nonherbivores (Pough 1973; Iverson

1982; Cooper and Vitt 2002; Espinoza 2002). Large-bodied lizards may be forced into herbivory because they are less able to capture insect prey and/or because it takes more energy to move their large mass, causing them to expend more energy during prey capture than they would obtain from the food item if captured (Pough 1973). Another alternative explanation for the evolution of strict herbivory is that the activity patterns of lizards with high temperature preferences do not overlap with insects (Pough 1973). Insect adaptations to arid environments include avoidance of light, heat, and low humidity. Crepuscular or nocturnal activity, burrowing, and climbing vegetation to escape high surface temperatures are common behaviors exhibited by arid-adapted insects (Cloudsley-Thompson 1975) that may minimize opportunities for desert-dwelling lizards to prey upon them. However, neither of these hypotheses has been tested.

Proposed Timeline

January–June 2004: Conduct digestive efficiency investigation, gather and store fecal samples

Summer 2004: Conduct molecular analyses of endosymbiont community

Fall 2004: Analyze data, complete first draft of thesis

Winter 2004: Complete first draft of thesis

Spring 2005: Thesis defense

Progress to Date

The feeding trials and microbial analyses have been completed. Data analysis will be completed during fall 2004. The first draft of the thesis will be completed before the end of the 2004–05 winter break and the defense is planned for spring 2005.

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