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Correlating diet and digestive tract specialization: Examples from the lizard family Liolaemidae

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Abstract

A range of digestive tract specializations were compared among dietary categories in the family Liolaemidae to test the hypothesis that herbivores require greater gut complexity to process plant matter. Additionally, the hypothesis that herbivory favors the evolution of larger body size was tested. Lastly, the association between diet and hindgut nematodes was explored. Herbivorous liolaemids were larger relative to omnivorous and insectivorous congeners and consequently had larger guts. In addition, small intestine length of herbivorous liolaemids was disproportionately longer than that of congeners. Significant interaction effects between diet and body size among organ dimensions indicate that increases in organ size occur to a greater extent in herbivores than other diet categories. For species with plant matter in their guts, there was a significant positive correlation between the percentage of plant matter consumed and small intestine length. Herbivorous liolaemids examined in this study lacked the gross morphological specializations (cecum and colonic valves) found in herbivores in the families Iguanidae and Agamidae. A significantly greater percentage of herbivorous species had nematodes in their gut. Of the species with nematodes, over 95% of herbivores had nematodes only in the hindgut. Prevalence of nematodes in the hindgut of herbivores was $2 \times$ that of omnivores and $4 \times$ that of insectivores.
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Introduction

Herbivores and carnivores confront disparate dietary and digestive challenges. While animal matter is easily digested and assimilated, most of the energy in plant material is contained in fibrous components such as cellulose, for which all vertebrates lack the enzymes to digest (Stevens, 1980). Thus, most herbivores possess symbiotic microflora capable of fermenting fibrous components. The end products of fermentation are absorbed by the herbivore and significantly contribute to daily energetic requirements. The degree to which symbiotic microflora are able to ferment plant material is highly dependent on particle size and retention time of digesta, the latter of which is in turn determined by gut structure and function (reviewed in Stevens, 1980; Stevens and Hume, 1998).

Comparative studies indicate that distinct differences in gut structure and function exist between herbivores and carnivores. The alimentary tract, particularly the large intestine, of herbivores is consistently longer and

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more voluminous than that of similar sized carnivores (Schieck and Millar, 1985). Additionally, herbivores may possess gut specializations absent in carnivores such as a cecum, intestinal valves, and hindgut nematodes. These specializations facilitate the fermentation of fiber by microflora and lengthen retention time of digesta, thereby increasing digestive efficiency (Stevens and Hume, 1998). For example, a study comparing eight species of small mammals from six genera found herbivorous species had a larger and more complex cecum and hindgut than omnivorous and carnivorous species (Barry, 1976, 1977). Although many studies have demonstrated significant morphological differences between herbivores and carnivores, most comparative studies are confounded by phylogeny because they compare distantly related animals. Furthermore, early comparative studies relied on analyses of ratios (e.g., gut length/body length) to control for body size that may have artificially biased results (Schieck and Millar, 1985).

The lizard sub-family Liolaemidae is well suited for phylogenetically independent comparisons of dietary correlates because of the large number of species that represent the full range of dietary strategies: herbivory, omnivory, and insectivory. Of the ~170 identified species in Liolaemidae, at least 20 are herbivorous. Of those, all 10 species of *Phymaturus* are herbivorous; the remaining 10 species are widely distributed across the genus *Liolaemus* (Adamson, 1994; Espinoza and Tracy, 2000; Espinoza et al., 2004). The herbivorous Liolaemidae can be used to investigate the fundamental morphology for herbivory because they are reported to lack many of the more sophisticated specializations of the gut (i.e., cecum, colonic valves) present in mammalian and more specialized reptilian herbivores (Troyer, 1991).

The presence of nematodes in the hindgut of a wide variety of species of Liolaemidae presents the opportunity to investigate the association between herbivory and hindgut nematodes. Many studies investigating digestion in reptilian herbivores have noted large colonies of nematodes in the hindgut (Schad, 1964; Dubuis et al., 1971; Nagy, 1977; Iverson, 1982; Bjorndal and Bolten, 1990; Dearing, 1993), but the relationship between nematodes and feeding strategy has not been addressed. Iverson (1982) estimated the population of nematodes in the hindgut of a single herbivorous lizard to be in excess of 15,000. Hindgut nematodes may in fact be parasitic, but both their wide occurrence in reptilian herbivores and the high densities in which they occur within a single animal suggests that hindgut nematodes may aid in the digestion of plant matter by herbivorous reptiles.

The objective of this study was to test the hypothesis that herbivorous species in Liolaemidae have a larger and more complex gut relative to that of omnivorous and insectivorous species. Specifically, we predicted that herbivorous lizards would consume a more fibrous diet than omnivorous or insectivorous lizards and therefore should have a longer and more voluminous gut. The greatest differences in volume and length among diet strategies should be in the hindgut, the primary site of fermentation in herbivorous lizards. Furthermore, there should be a positive correlation between plant consumption and hindgut size. Additionally, we tested the hypothesis that herbivory favors the evolution of larger body size in lizards (Pough, 1973; Cooper and Vitt, 2002). We predicted that herbivorous species in Liolaemidae would be larger than both omnivores and insectivores. Finally, we investigated the association between herbivory and hindgut nematodes. If hindgut nematodes facilitate digestion of plant matter by herbivorous lizards, they should occur in herbivorous lizards more frequently than in omnivorous or insectivorous lizards.

Materials and methods

Specimen collection

Adult lizards were either noosed or caught by hand in a variety of habitats along the Andean cordillera in Argentina from January 12, 2003 to February 13, 2003 (latitudes: 26–43°S, longitudes: 66–70°W). Noosing occurred during the same time each day (1000–1400 h) at sites ranging in altitude from 600 to 3000 m. Species classifications were made based on both morphological data and mtDNA analysis (unpub. data).

Morphological measurements

Lizards were euthanized with a pericardiac injection of Tiopental Sodico (Abbot®) for tissue collection for a molecular phylogeny study (in progress). Snout vent length (SVL) was measured, to the nearest millimeter, for each animal. Directly following euthanasia, the gastrointestinal (GI) tract (mid-esophagus to anterior to the cloacal aperture) was excised and preserved in 70% EtOH. Gut measurements were recorded in the laboratory and made without knowledge of lizard species or diet type. Each GI tract was cut into sections by organ and the following data were recorded for each lizard: length of the stomach; volume of the stomach including contents; length of the small intestine; length of the large intestine; volume of the large intestine including contents. Volume measurements included gut contents; gut contents were removed prior to measurement of length. All mesenteries were cut from organs and lengths were measured using a length of string, allowing it to conform to the curvature of the organ. The string length

was then measured to the nearest millimeter. Volume (nearest 0.1 ml) was measured as water displacement in a graduated cylinder. Initial water level was recorded, the organ was placed in the graduated cylinder and the resultant water level was recorded. Organ volume was calculated as the difference between the final and initial water levels.

Diet classification

Stomach and large intestine contents were identified as either plant or arthropod using a $10\times$ dissecting scope. The volume that was plant matter was recorded for both organs. Individual lizards were classified into diet categories based on the amount of plant matter contained in the stomach: herbivores $\geqslant 85\%$, omnivores 11–84%, insectivores $\leqslant 10\%$. These categories were selected for consistency with those used in other lizard studies (Cooper and Vitt, 2002; Espinoza et al., 2004). Species were assigned diet categories based on the diet classification of individuals of that species. Species were designated herbivorous or insectivorous only if all individuals collected from that species were classified into the same diet category. If diet classifications varied among individuals of a given species, the species was classified as omnivorous.

Fiber analysis of stomach contents

To quantify fiber levels consumed by herbivorous lizards in the wild, acid detergent fiber (ADF) analysis was performed on the plant matter extracted from lizard stomachs. ADF analysis quantifies cellulose, lignin, and cutin content. Using forceps, plant matter was isolated from the stomachs of herbivorous lizards and dried. After drying, plant samples were ground using a Wiley mill, and passed through a 1 mm screen. Fiber was measured according to the methods of Goering and VanSoest, 1970 using an ANKOM fiber analyzer. To accurately determine fiber content 0.2 g replicates were run for each sample.

Nematode removal

All visible nematodes were removed from the alimentary tract. For each lizard, the location (stomach vs. hindgut) of all nematodes was noted. Nematode specimens from each organ were preserved in 70% EtOH for identification by Dr. Stephen Goldberg, Whittier College, California.

Statistical analysis

The average SVL of lizards in each diet category (herbivore, omnivore, and insectivore) were compared using an analysis of variance (ANOVA). Analyses of covariance (ANCOVA) were used to contrast organ measurements and diet category with SVL as the covariate for length measurements and the cube of SVL as the covariate for volumetric measurements. Using SVL^3 as a proxy for mass introduces more conservative bias into the analysis but measurements of mass were not available. When interaction terms were not significant they were removed from the model. For all analyses, differences between diet categories were determined with post hoc analyses (Tukey's HSD). Residuals were calculated between organ measurements and SVL and linear regression was used to examine the relationship between the residuals and percentage of plant matter in the stomach. All analyses were performed using species averages and not individual values to avoid biasing statistical tests toward over-represented species. Initially, species with sample sizes less than three individuals ($n = 4$) were removed from the statistical analyses, but doing so did not affect the results or conclusions, thus these four species were included in the final analysis. Differences in nematode presence, location, and species between diet categories were examined with a χ^2 analysis.

Results

Diet classification

The digestive tracts and gut contents of 22 Liolaemidae species (204 individuals) were examined (Table 1). Based on stomach content analyses, seven species were classified as insectivores, eight species as omnivores, and seven species as herbivores.

Fiber analysis of stomach contents

ADF analysis was performed on plant matter isolated from the stomachs of herbivorous lizards ($n = 12$). Representatives of all herbivorous species were included in this analysis. In the samples examined, the mean percentage of plant matter consumed by the lizards was 97.8% ($\pm 1.8\%$); this included leafy material and fruit parts such as seeds, stems, and skin. The mean ADF content of the samples was 43.9% ($\pm 2.3\%$).

Morphological measurements

Herbivorous species were larger ($F_{2,18} = 16.3$, $P < 0.0001$) than omnivorous or insectivorous species. Herbivores were 33% larger than omnivores, while omnivores were 18% larger than insectivores (Table 2).

Herbivorous lizards had larger guts, in part because of their larger body size (Table 2). There was a

Table 1. Species examined and diet classification based on percentage of plant matter in the stomach

	Sample size	Percent plant matter
Insectivores		
L. austromendocinus	16	6
L. darwinii	28	4
L. grosseorum	3	0
L. ornatus	1	0
L. gracilis	2	0
L. koslowskyi	10	0
Omnivores		
L. bibroni	14	61
L. boulengeri	24	72
L. fitzingeri	13	69
L. olongasta	2	48
L. petrophilus	6	17
L. donosobarrosi	8	38
L. elongatus	8	25
L. umbrifer	11	30
Herbivores		
L. rothi	4	89
L. dorbigny	3	97
L. buergeri	10	86
L. kriegi	7	100
P. sp.	3	100
P. zapalensis	5	97
P. palluma	2	100

significant effect of SVL on all of the organ dimensions examined: stomach length ($F_{1,15} = 43.2$; $P < 0.0001$), stomach volume ($F_{1,13} = 12.3$; $P = 0.004$), small intestine length ($F_{1,16} = 18.6$; $P = 0.0005$), large intestine length ($F_{1,14} = 18.4$; $P = 0.0008$), large intestine volume ($F_{1,14} = 6.7$; $P = 0.02$). A main effect of diet was not consistent across all organs because of the deviations in slopes among diet categories (Fig. 1). There was a significant effect of diet on small intestine length ($F_{1,16} = 4.8$; $P = 0.02$), with herbivores having a small intestine $1.7\times$ the length of omnivores and $2.5\times$ the length of insectivores.

There were significant interactions between diet and body size due to large differences in slopes among diet categories (Fig. 1). Interaction terms were signifi-

cant in analyses of large intestine length ($F_{2,14} = 6.9$; $P = 0.008$), stomach volume ($F_{2,13} = 5.9$; $P = 0.02$), and large intestine volume ($F_{2,14} = 6.7$; $P = 0.03$). In each case, the slope of the line was steeper for organ dimensions in herbivorous lizards than omnivores or insectivores. Thus, the increase in organ size over a similar size range was much greater in herbivores than in either insectivores or omnivores. For example, over a 2-cm range in SVL, the increase in large intestine length was $10\times$ greater in herbivores than in omnivores and insectivores.

For all lizard species with plant matter in their guts, there was a significant correlation between the percentage of plant matter in the stomach and small intestine length ($r = 0.48$, $F_{1,12} = 11.02$; $P = 0.006$; Fig. 2a), and a marginally significant relationship between the percentage of plant matter in the stomach and large intestine length ($F_{1,12} = 3.99$; $P = 0.07$; Fig. 2b; all other variables: $P > 0.05$).

Nematodes

Nematode type and location were highly correlated with diet category in Liolaemidae (Fig. 3). A significantly greater percentage of herbivores had nematodes in their digestive tract than either omnivores or insectivores ($\chi^2 = 8.1$, df = 2, $P < 0.03$; Fig. 3a). Among the lizards with nematodes, there was a significant difference in the location of nematodes in the GI tract among diet categories ($\chi^2 = 24.8$, df = 4, $P < 0.01$; Fig. 3b). Herbivorous species had a greater proportion of nematodes in the hindgut than in the stomach or both organs ($\chi^2 = 10.6$, df = 2, $P < 0.01$). More than 95% of the herbivorous lizards had nematodes only in the hindgut. This was almost $2\times$ that of omnivores and almost $4\times$ that of insectivores. In omnivorous species, the number of nematodes in the stomach and the hindgut did not significantly differ, whereas insectivorous species had nematodes in the stomach significantly more often than in the hindgut or both organs ($\chi^2 = 13.7$, df = 2, $P < 0.01$).

The nematode species found were also highly related to diet category. Nematode species in the digestive tract of liolaemid lizards included *Physaloptera retusa*,

Table 2. Mean morphological measurements for each diet category. Length was measured in mm and volume in ml. One standard error is given in parentheses

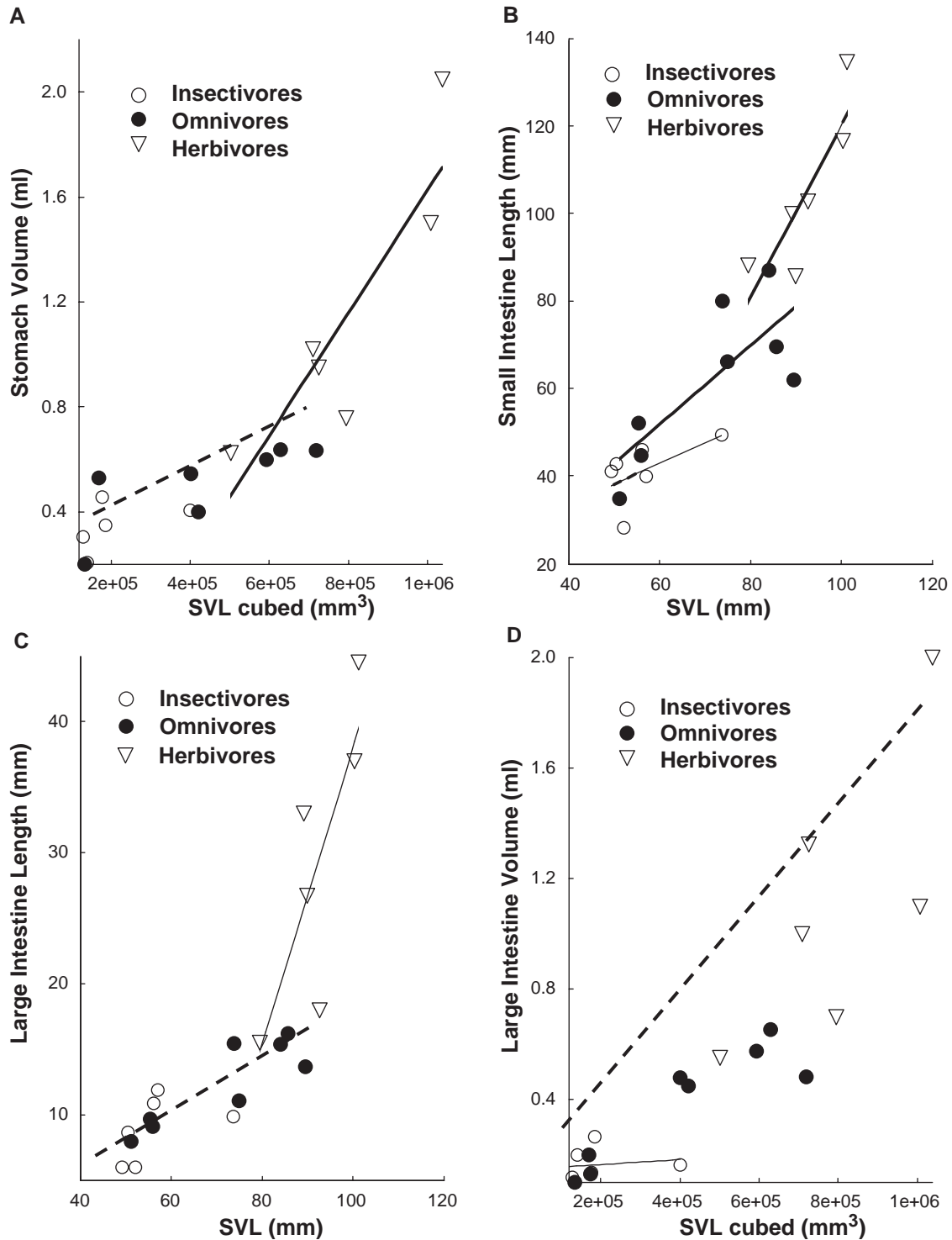| Diet category	Body size	Stomach		Small intestine	Large intestine	
	SVL	Length	Volume	Length	Length	Volume
Herbivores	94.2 (1.2)	42 (2.2)	1.2 (0.2)	117 (10.6)	29.1 (3.2)	1.06 (0.1)
Omnivores	70.8 (5.0)	27.5 (2.3)	0.45 (0.1)	62.4 (5.6)	12.2 (1.0)	0.36 (0.1)
Insectivores	60.0 (3.8)	23.5 (1.4)	0.41 (0.1)	48.7 (5.1)	10.0 (0.9)	0.22 (0.03)

Fig. 1. Organ dimensions versus body size: stomach volume (A), small intestine length (B), large intestine length (C), and large intestine volume (D). Dashed lines represent lines for which the slopes are not significantly different among diet categories (e.g. (A) – insectivores and omnivores).

Parapharyngodon riojensis, Spauligodon maytacacai, and *Spauligodon loboi. Parapharyngodon riojensis* occurred more frequently in herbivores than in omnivores or insectivores ($\chi^2 = 23$, df = 2, $P < 0.001$). Each of the

above four nematode species were found in omnivorous lizards, but significantly more omnivores contained *S. maytacacai* and *S. loboi* than herbivores or insectivores ($\chi^2 = 7.2, 7.3$, df = 2, $P < 0.05$). Finally, *Physaloptera*
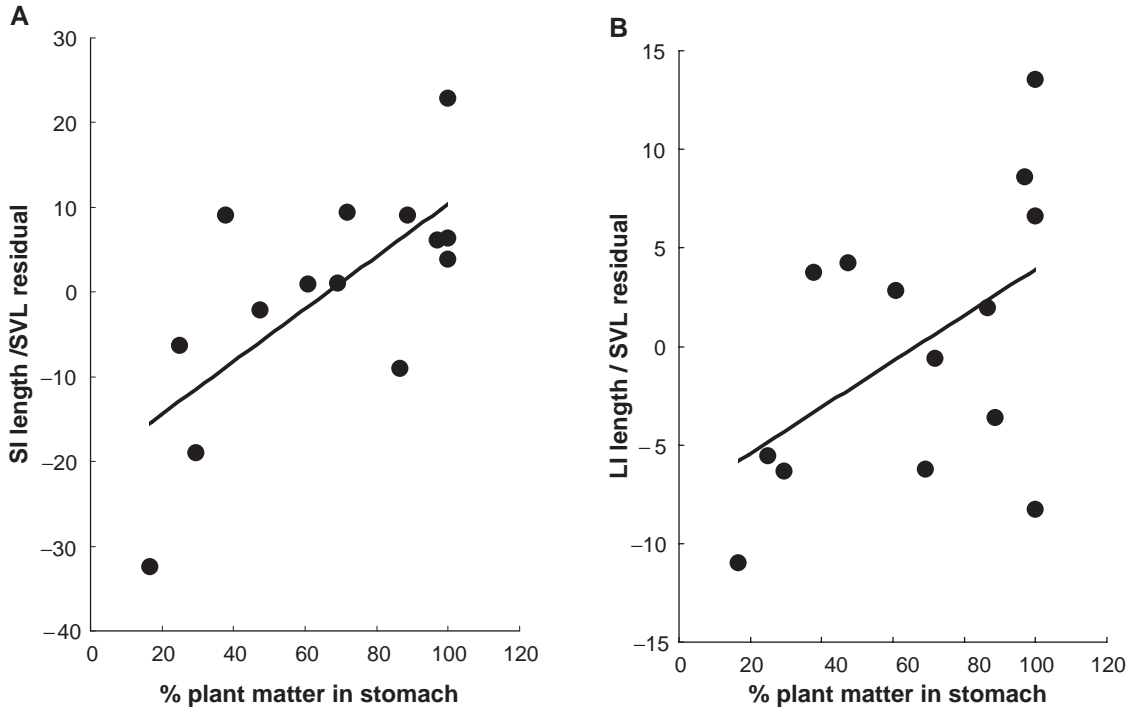
Fig. 2. Residuals of organ dimensions and body size were plotted against the percentage of plant matter in the stomach. Small intestine length (A) was significantly correlated with plant matter in the diet and the correlation between large intestine length (B) and plant matter in the diet was marginally significant.
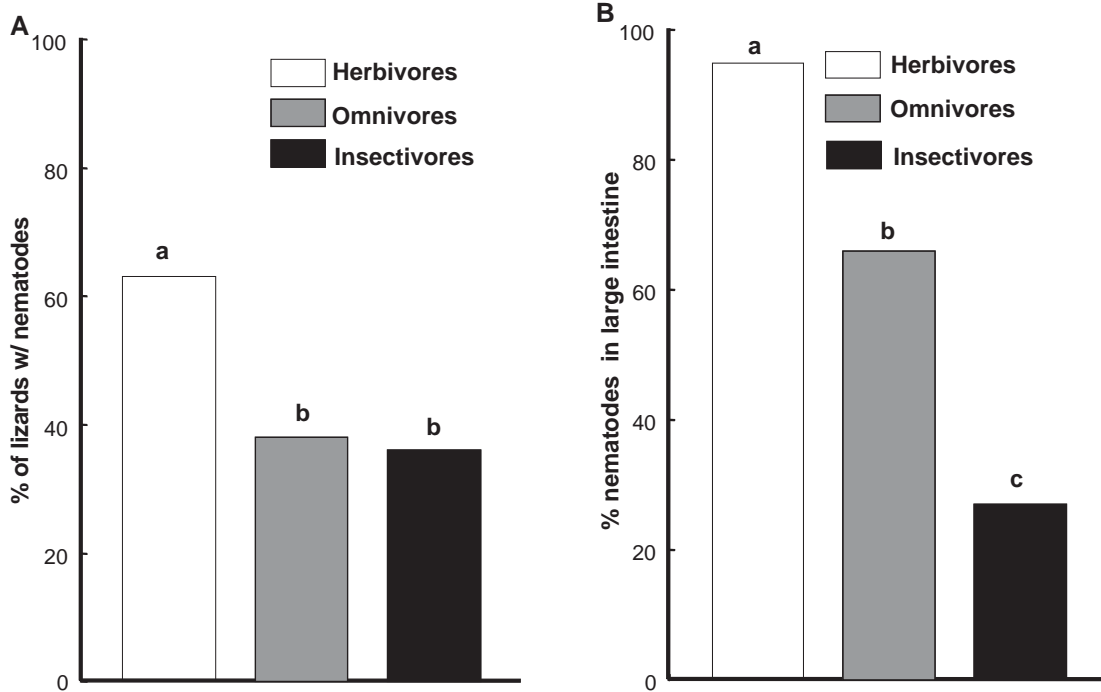

Fig. 3. (A) Percentage of lizards with nematodes by diet category. (B) Nematodes were found in the stomach and/or colon of lizards. Of the lizards with nematodes, 95% of herbivores, 66% of omnivores, and 27% of insectivores had nematodes only in their colon. Letters indicate statistically distinguishable diet categories, using post hoc analyses at $P < 0.05$.

retusa occurred more frequently in insectivores than in omnivores or herbivores ($\chi^2 = 24.7$, df = 2, $P < 0.001$).

Discussion

Although plants are an abundant food source, high fiber content presents a digestive challenge and has led to morphological specializations of the digestive tract exclusive to herbivorous animals (Stevens, 1980; Hume, 1989). The majority of herbivorous lizards exhibit extensive morphological specializations, similar to those found in mammalian herbivores, that facilitate the digestion of fiber (Iverson, 1980; Zimmerman and Tracy, 1989; van Marken Lichtenbelt, 1992). Herbivores in Liolaemidae are unique in that beyond a generally enlarged digestive tract, the gut morphology of these lizards closely resembles that of insectivorous congeners. This study system permits examination of the basic specializations necessary for herbivory and additional investigation of characteristics exclusive to reptilian herbivores.

Herbivory in lizards is rare (Pough, 1973). A number of characteristics have been hypothesized to be necessary for a lizard to be a herbivore, including distribution in a warm and stable climate, large body size, specialized dentition, specialized gut morphology, and colonic nematodes. The main objectives of this research were to: (1) determine if herbivorous species in Liolaemidae are larger in body size than omnivorous and insectivorous congeners; (2) determine if herbivorous species have a larger and more voluminous gut relative to that of insectivorous and omnivorous species, and (3) determine if hindgut nematodes occur in herbivorous species more frequently than in omnivorous or insectivorous species. The following sub-sections discuss each of these aspects.

Body size

Pough (1973) proposed that large body size (> 100 g) in lizards both requires and permits herbivory. The high mass-specific metabolic rates associated with small size may preclude smaller lizard species from consuming a plant diet low in digestible energy. In addition to higher energy requirements, smaller species have limited gut capacity because gut capacity increases in direct proportion to body size, while metabolic requirements scale as a fractional power of body size (Parra, 1978; Demment, 1985). Differential scaling between body size and gut capacity and body size and metabolic requirements forces small herbivores to process food faster and consequently causes digestion and fermentation of plant matter to be less efficient. Despite these constraints, small herbivorous lizards (30–80 g), like Liolaemidae species, exist and thrive on a strict plant diet. Although

Liolaemidae species fall below the size threshold proposed by Pough (1973), there is a diet–body size gradient within the family. We found that herbivorous lizards in Liolaemidae are significantly larger than omnivores and insectivores, supporting the hypothesis that larger body size may be selected for in herbivorous lizards. In addition to energetic savings resulting from lower mass specific metabolic rates, the thermal inertia gained through a larger body size may serve as an additional benefit to herbivorous liolaemids (Espinoza et al., 2004).

Evolution of body size may be affected by a number of factors, including phylogeny, sexual selection, fecundity selection, competition, and predation (Cooper and Vitt, 2002). Although any of these factors or a combination of them could select for larger size, the ubiquity of the size–diet trend across lizard families (Pough, 1973; Van Damme, 1999; Cooper and Vitt, 2002) suggests that large body size either facilitates the processing and assimilation of plant matter or offers substantial energetic savings. Comparative digestion trials between large and small species on similar diets could address the role of body size in food processing and energetic savings.

Gut morphology

The greatest differences in GI tract morphology among herbivorous, omnivorous and insectivorous lizards were found in the small intestine and not the hindgut as initially predicted. The small intestines of herbivorous liolaemids are significantly longer than those of omnivorous and insectivorous liolaemids (Table 2, Fig. 1b). This pattern has also been found in the genus *Cnemidophorus* (Dearing, 1993) and in omnivorous lacertid species (Herrel et al., 2004). It is possible that a lengthy small intestine is caused by or facilitates digestion of a high fiber diet. Consistent with this idea, small intestine length was highly correlated with the percentage of plant matter in the stomachs of omnivorous and herbivorous liolaemids (Fig. 2a). Furthermore, fiber analysis (ADF) of stomach contents reported here revealed that herbivorous liolaemids consume diets high in fiber.

Given that herbivorous liolaemids were consuming a diet high in fiber, why was the hindgut, as the main site of fermentation, not enlarged relative to insectivorous congeners? It is possible that the type of fiber ingested by liolaemids is not suitable for extensive fermentation. The stomachs of herbivorous liolaemids contained both fruit and leaf material; the relative amounts of each plant tissue were not quantified. Both leaf and fruit can contain high levels of fiber, but may differ in suitability for fermentation (Stevens and Hume, 1998). Much of the fiber in leaf material can be fermented. In contrast,

fruits, although high in easily digestible carbohydrates, can also contain significant quantities of fiber in the seed coat and fruit skin that are not readily fermented. Folivores obtain a large portion of their energy via hindgut fermentation whereas more frugivorous animals meet their energetic demands via carbohydrate absorption in the small intestine. It is possible that liolaemids rely more on fruit to satisfy energetic demands than on fermentation of leaf material.

Changes in the quality and availability of food as well as altered energetic needs (i.e. reproduction, hibernation) can elicit up- or down-regulation of the GI tract (reviewed in Starck, 2003). Phenotypic plasticity in the GI tract is common in species consuming high fiber diets. Both avian and mammalian species given fibrous diets consume larger amounts of food and the increase in food intake due to the decrease in food quality causes lengthening of the small intestine (reviewed in Foley and Cork, 1992). Could phenotypic plasticity account for the $2.5\times$ difference in small intestine length between herbivorous and insectivorous liolaemid species? Studies investigating the effects of food quality on digestive tract length in continuously feeding herbivores have found differences in small intestine length averaging from 20% to 50% (Hansson, 1985; Green and Millar, 1987; Hansson and Jaarola, 1989; Lee and Houston, 1993; Owl and Batzli, 1998), to a maximum of 130% in Japanese quail (Starck and Rahmaan, 2003). Increases in small intestine length similar to those in this study have been documented in Burmese pythons (Secor et al., 1994; Starck and Beese, 2001) when transitioning from a fasted state to ingestion of a large meal (200% increase in small intestine length). However, the feeding regime of pythons, i.e., several days of fasting followed by a large meal, may differ significantly from that of the lizards in this study, which are likely more comparable to the continuous feeders. Given the range of gut plasticity documented in continuous feeders, it is unlikely that the difference in small intestine length between liolaemids (250%) is solely a phenotypic response to food quality and/or availability. Rather, this difference in small intestine length between herbivorous and insectivorous liolaemids seems to be the result of dietary specialization within this family.

The goal of our study was a broad-scale comparison of morphology within the Liolaemidae. A potential shortcoming of such a large survey on wild species is the lack of control in parameters such as food availability, foraging activity, and diet quality. We took several measures to reduce the variation. First, all individuals were collected within the same time range to attempt to control for variation in feeding bouts. Second, all collecting was done within a 4-week period of time to control for changes in food availability. We could not control for geographical variation in the quality of food and it is possible that animals consuming a low-quality diet could have an enlarged gut due to differences in quality and not in digestive strategy. However, differences in diet quality and its effects on the gut are restricted to herbivorous species and should not affect conclusions regarding different dietary strategies. Thus, we suggest that the differences in gut morphology are governed by differences in dietary strategy. While a laboratory study could control for variation in diet quality and quantity, it would be a monumental task to conduct such a study on 22 species simultaneously and with adequate replication.

Hindgut nematodes

An intriguing correlation exists between diet and nematode presence in Liolaemidae. The percentage of lizards with nematodes, the location, and the type of nematodes within the gut were significantly different between dietary groupings (Fig. 3). Herbivores had nematodes significantly more often in their hindgut than in other sections of the gut, whereas in insectivores the majority of nematodes occurred in the stomach and in omnivores they were evenly spread throughout the gut.

There has been much speculation as to the role hindgut nematodes play in the digestion of plant material by herbivorous species of ectotherms (Nagy, 1977; Iverson, 1982; Pryor, 2003). Of the nematode species found in the gut, presence of *Parapharyngodon riojensis* (Pharyngodonidae) was significantly greater in herbivores than omnivores and very few of these worms occurred in insectivores. *Parapharyngodon riojensis* is in the family Oxyuridae, commonly referred to as "pinworms". Pinworms typically have direct life cycles, requiring no intermediate host, and are common in mammals, birds, reptiles, and amphibians. It has been suggested that pinworms are not parasitic because they are free-living, inhabiting the large intestine and apparently feeding on bacteria and other intestinal contents (Schmidt and Roberts, 1989).

Given that the hindgut is the major site of fermentation for these herbivores, it is possible that hindgut nematodes have either a direct or indirect role in the digestion of plant matter. Three possible roles have been suggested: mechanical reduction of digesta, production of usable waste products such as volatile fatty acids, and/or the regulation of microbial populations (Iverson, 1982). In some animals hindgut nematodes occupy a large proportion of the gut. These dense nematode masses may facilitate digesta retention by serving as an obstacle around which the gut contents must pass. In a previous study, roughly 40% of the total cellulase activity in the gut of herbivorous lizards was attributed to hindgut nematodes, but it was unclear if the cellulase activity found was attributable to the nematodes or associated bacteria (Nagy, 1977). Given that hindgut

nematodes occur in high densities and primarily feed on bacteria (Pryor, 2003; Pryor and Bjorndal, 2003) it is likely that their removal would have a considerable effect on both microbial communities and the host. For example, bullfrog tadpoles with hindgut nematodes exhibited higher levels of volatile fatty acids in the hindgut and greater rates of fermentation, consequently leading to accelerated development and metamorphosis (Pryor, 2003; Pryor and Bjorndal, 2003). Although the presence of nematodes appears to be beneficial for host animals, it has yet to be established how hindgut nematodes increase fermentative activity in lizards. Further research needs to be done to understand the role of hindgut nematodes (Oxyurata) in digestion by herbivorous animals.

All the nematode species found in liolaemids in this study were present in omnivores. The occurrence of *Spauligodon maytacacai,* and *Spauligodon loboi* was significantly greater in omnivores than in herbivores or insectivores. Nematodes in the genus *Spauligodon* are members of the Oxyurata and, like *Parapharyngodon riojensis*, may potentially play a role in the digestion of plant matter. The greater presence of *Spauligodon* nematodes in omnivorous lizards may be the result of a more varied diet.

Presence of *Physaloptera retusa* was significantly higher in insectivores than omnivores and almost non-existent in herbivores. *Physaloptera retusa* (order Spirurata) are parasitic worms that occur in the stomach or intestines of all classes of vertebrates. Spirurids utilize arthropods as intermediate hosts in their development, which could explain their abundance in the insectivorous species of Liolaemidae. Infestation by such parasitic nematodes may be a cost of an insectivorous diet.

Conclusion

Plant defenses to digestion, both chemical and structural, have led to specialization of the digestive tract exclusive to herbivorous animals. While gut specializations can be quite complex, as in the case of ruminants, this study demonstrates that larger body size and enlargement of the gut may be the fundamental specializations for herbivory in lizards. The enlarged small intestine in herbivorous liolaemids may be a trademark of herbivores consuming a diet high in fruit. Additionally, this study tested many of the traits posited necessary for herbivory in lizards. Although large body size may afford a degree of energetic savings in herbivores relative to insectivores, optimal size may be dependent on climatic stability in the habitat. Finally, this study showed a strong correlation between diet and gut nematodes and suggests that nematodes in the

hindgut play a role in the digestion and assimilation of plant matter.

Acknowledgements

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