Costs of Loss: A Comparison of Biochemistry and Morphology of Original and Regenerated Lizard Tails

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INTRODUCTION

Caudal autonomy is a common and effective method of predator evasion in many lizard species. However, because the tail contributes a multitude of beneficial functions, there are often costs associated with autonomy (Vitt and Caldwell, 2008). For example, the effects of tail autonomy on locomotion, social status, and reproductive fitness are well established (Martín and Avery, 1998; Martín and Salvador, 1993; Downes and Shine, 2001).

Furthermore, morphological differences may exist between the original and regenerated tail that could alter the biochemistry and energetics. **We tested the hypotheses** that lizard tail biochemistry or morphology or both differ after regeneration.

A decrease in caudal protein storage ability has been documented in the African house gecko, *Hemidactylus mabouia*, presumably because the cartilaginous rod which replaces ossified vertebrae in the regenerated tail reduces potential muscle attachment sites (Meyer *et al*., 2002). We predict a similar reduction of protein storage ability across several species of lizard, accompanied by a concurrent increase in lipid content. Further, we intend to examine the specific morphological changes that occur between original and regenerated tails and predict that they will support the biochemical changes.

METHODS

Study species: We examined four lizard species: 3 geckos and 1 skink (Figure 1). We housed all lizards individually and provided a mixed diet and water *ad libitum* throughout the study.

(A) *Hemitheconyx caudicinctus* (B) *Gekko ulikovskii* (C) *Hemidactylus turcicus* (D) *Eumeces fasciatus*

Figure 1. Lizard species used in study: (A) African Fat-Tail Gecko, (B) Golden Gecko, (C) House Gecko, and (D) Five-Lined Skink.

Tail collection: We induced caudal autonomy twice in all lizards at the same break point distal to the vent (Figure 2). We removed tails at the beginning of the study and again after 15 weeks of regeneration. After each round of tail removal, we sectioned each tail into proximal, medial, and distal portions. We then sectioned each of these portions in two for biochemical and morphological analyses.

Figure 2. Manual tail removal

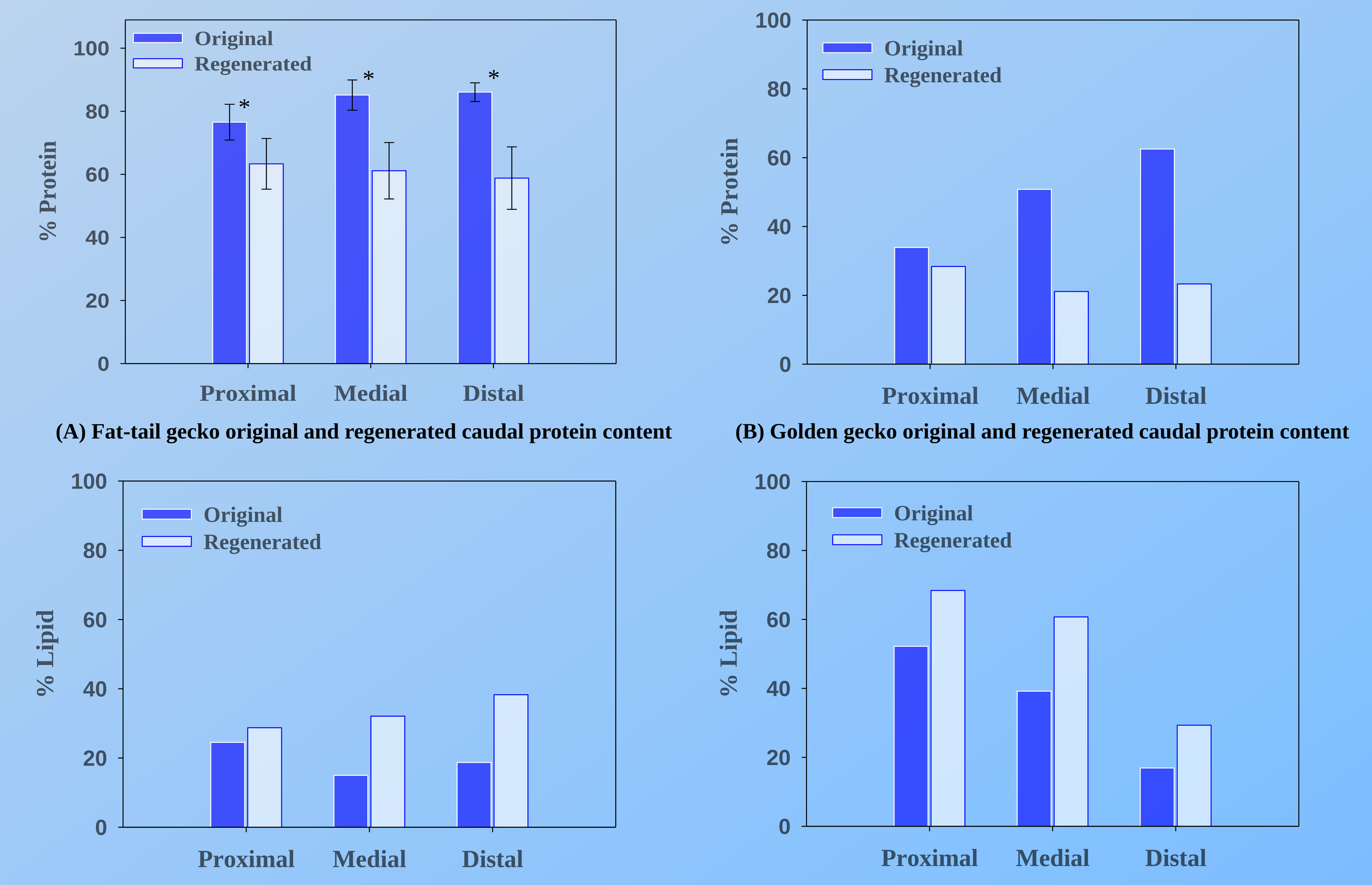
Biochemical analysis: Assays were conducted in an external lab to assess caudal protein, lipid, and wet matter content for proximal, medial, and distal tail segments.

Morphological analysis: We fixed proximal, medial, and distal sections in paraformaldehyde for 24 hours and then decalcified the tissue in CalEx® for 24 hours. We stored all segments in 70% ethanol until tissues were embedded in paraffin and sectioned to a thickness of 10µm. We will stain cross- and longitudinal-sections of each sample using a modified Meyer's trichrome stain so that lipid, protein, and bone / cartilage will be visually distinguishable (Figures 4 & 5). We will analyze each sample using an Olympus BX40 light microscope and capture images with an Infinity-1 digital camera. We will quantify lipid and protein composition of original and regenerated tails using Adobe Photoshop CS4.

RESULTS

Table 1. Comparison of caudal protein and lipid content in the proximal section of original and regenerated tails across four lizard species. The proximal section provided the most complete biochemical results for all species due to larger mass of this tail section. Low tail mass limited our ability to assess caudal lipid content in the house geckos.

	Fat-Tail Gecko (n=10)		Golden Gecko (n=12)		House Gecko (n=4)		Five-Lined Skink (n=3)	
	Original	Regenerated	Original	Regenerated	Original	Regenerated	Original	Regenerated
% Caudal Protein	72.2	63.3	33.9	28.4	60.0	18.8	59.0	74.6
% Caudal Lipid	24.5	28.7	52.2	68.4	Not Enough Sample	71.2	19.5	16.7



(A) Fat-tail gecko original and regenerated caudal protein content

(B) Golden gecko original and regenerated caudal protein content

(C) Fat-tail original and regenerated caudal lipid content

(D) Golden gecko original and regenerated caudal lipid content

Figure 3. Comparison of original and regenerated caudal (A & B) protein and (C & D) lipid content in fat-tail and golden geckos. * indicates significant differences between original and regenerated tail sections (p<0.05).

- **Fat-tail geckos possessed a significantly greater percentage of caudal protein in the original tail relative to the regenerated tail.**
  - A similar trend was observed in caudal protein content of all other species tested except for five-lined skinks.
- **There is an observable increase in regenerated caudal lipid content relative to original content in all four species tested.**

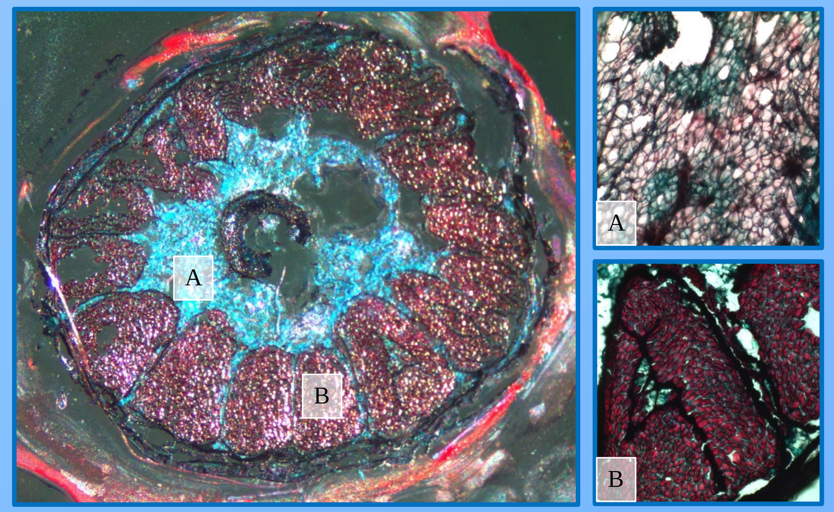


Figure 4. Digital micrograph showing (A) lipid and (B) protein tissue in cross section of lizard tail (100X magnification).

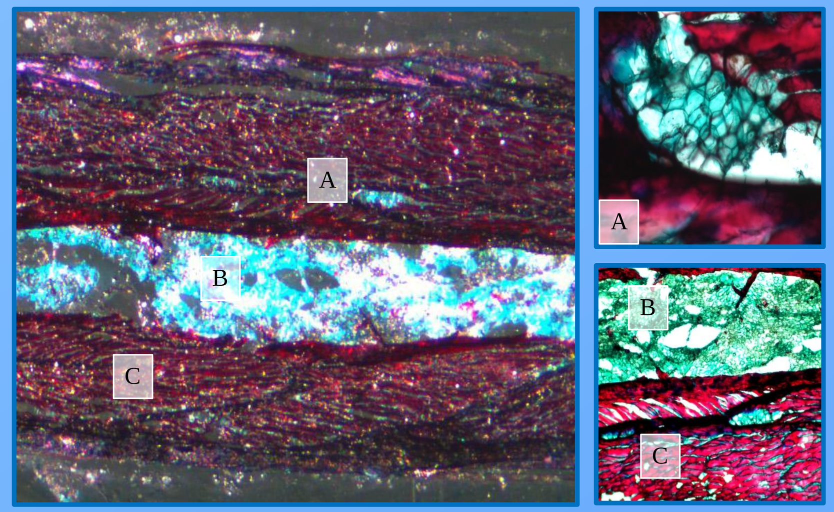


Figure 5. Digital micrograph showing (A) lipid (200x) (B) cartilaginous rod (100x), and (C) protein (100x) tissue in cross section of regenerated lizard tail.

Morphological analysis will continue through fall 2010

DISCUSSION

- **Decrease in protein content observed in regenerated tails of all 3 gecko species (Table 1)**
  - Data supports results of Meyer and colleagues (2002), by reflecting consistency across multiple species
  - Possible result of decreased muscle attachment sites in regenerated tail
  - Increase in protein content seen in regenerated five-lined skink tails could be due to small sample size or differences between gecko and skink tail anatomy
- **Increase in lipid content observed in regenerated gecko tails (Table 1)**
  - House gecko original caudal lipid content may be presumed to be <40% (because of 60% protein content), and therefore the 71.2% lipid content observed in the regenerated tails reflects a relative increase
  - Cellular structure of lipid is simpler compared with protein / muscle, therefore it may be replaced more rapidly
  - Differences between gecko and skink caudal uses may explain contrasting results
- **Proximal, medial, and distal sections contain differing percentages of lipid and protein (Figure 3)**
  - Generally, more lipid stored in proximal section and more protein stored in distal section
  - Batch processing limited our ability to perform statistical analysis for remaining data

FUTURE DIRECTIONS

- **Complete morphological analysis to quantify relative amounts of caudal protein and lipid content (Figures 4 & 5)**
  - Relate differences in morphology to biochemical changes between original and regenerated tail
- **Expand gecko species comparisons in current study**
- **Increase sample size of existing species so that statistical analysis of biochemical results may be performed**
- **Evaluate how diet influences caudal regeneration**
- **Extend EPAC lab's parallel study evaluating the behavioral effects of caudal autonomy**

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