Controlled Chaos: Three-Dimensional Kinematics, Fiber Histochemistry, and Muscle Contractile Dynamics of Autotomized Lizard Tails*

Timothy E. Higham[1,†]
Kathryn R. Lipsett[2]
Douglas A. Syme[2]
Anthony P. Russell[2]
[1]Department of Biology, University of California, 900 University Avenue, Riverside, California 92521; [2]Department of Biological Sciences, University of Calgary, 2500 University Drive Northwest, Calgary, Alberta T2N 1N4, Canada

Accepted 8/25/2013; Electronically Published 10/11/2013

ABSTRACT

The ability to shed an appendage occurs in both vertebrates and invertebrates, often as a tactic to avoid predation. The tails of lizards, unlike most autotomized body parts of animals, exhibit complex and vigorous movements once disconnected from the body. Despite the near ubiquity of autotomy across groups of lizards and the fact that this is an extraordinary event involving the self-severing of the spinal cord, our understanding of why and how tails move as they do following autotomy is sparse. We herein explore the histochemistry and physiology of the tail muscles of the leopard gecko (*Eublepharis macularius*), a species that exhibits vigorous and variable tail movements following autotomy. To confirm that the previously studied tail movements of this species are generally representative of geckos and therefore suitable for in-depth muscle studies, we quantified the three-dimensional kinematics of autotomized tails in three additional species. The movements of the tails of all species were generally similar and included jumps, flips, and swings. Our preliminary analyses suggest that some species of gecko exhibit short but high-frequency movements, whereas others exhibit larger-amplitude but lower-frequency movements. We then compared the ATPase and oxidative capacity of muscle fibers and contractile dynamics of isolated muscle bundles from original tails, muscle from regenerate tails, and fast fibers from an upper limb muscle (iliofibularis) of the leopard gecko. Histochemical analysis revealed that more than 90% of the fibers in original and regenerate caudal muscles had high ATPase but possessed a superficial layer of fibers with low ATPase and high oxidative capacity. We found that contraction kinetics, isometric force, work, power output, and the oscillation frequency at which maximum power was generated were lowest in the original tail, followed by the regenerate tail and then the fast fibers of the iliofibularis. Muscle from the original tail exhibited greater resistance to fatigue, followed by the regenerate tail and then the fast iliofibularis fibers. These results suggest that the relatively slow and oxidative fibers found within the tail musculature have a significant impact on contractile function, which translates into a trade-off between longevity of performance and power after autotomy.

Introduction

Sacrificing a body part in order to survive has evolved independently in several groups of invertebrates (Eisner and Camazine 1983; Mladenov 1983; Amaya et al. 2001; Ramos et al. 2004) and vertebrates (Wake and Dresner 1967; Arnold 1984; Bellairs and Bryant 1985; Dubost and Gasc 1987; Zani 1996; Maginnis 2006; Bateman and Fleming 2009). Caudal autotomy is the loss of the tail by its fracture at distinct regions of weakness (Arnold 1984) and is common among lizards and salamanders. Research focusing on lizards has revealed that post-autotomic movements of the tail can be extremely rapid, complex, and of long duration (Clark 1971; Dial and Fitzpatrick 1983; Higham and Russell 2010, 2012). Not surprisingly, tail autotomy in lizards has attracted the interest of scientists over the last 250 yr. For example, John Hunter (1861), in the mid-1700s (his findings were not published until 1861), observed tail autotomy in lizards and was captivated by the fact that the tail continues to move and writhe for some time following disconnection from the body. Research pertaining to autotomized tail function has focused on only a few species of lizard that exhibit autotomy (Longstaff 1907; Clark 1971; Dial and Fitzpatrick 1983; Daniels et al. 1986; Rumping and Jayne 1996; Pafilis et al. 2005; Higham and Russell 2010, 2012). Given that autotomy and subsequent tail movement are critical for escaping predators (Congdon et al. 1974; Dial and Fitzpatrick 1983, 1984; Vitt and Cooper 1986), it is important to understand the

* This paper is based on, and was prepared in association with, a presentation given in the symposium "Caudal Autotomy and Regeneration in Lizards: Patterns, Costs, and Benefits" at the Seventh World Congress of Herpetology, University of British Columbia, Vancouver, British Columbia, Canada, August 8–14, 2012 (http://www.worldcongressofherpetology.org).

† Corresponding author; e-mail: thigham@ucr.edu.

Physiological and Biochemical Zoology 86(6):611–630. 2013. 1522-2152/2013/8606-2180$15.00. DOI: 10.1086/673546

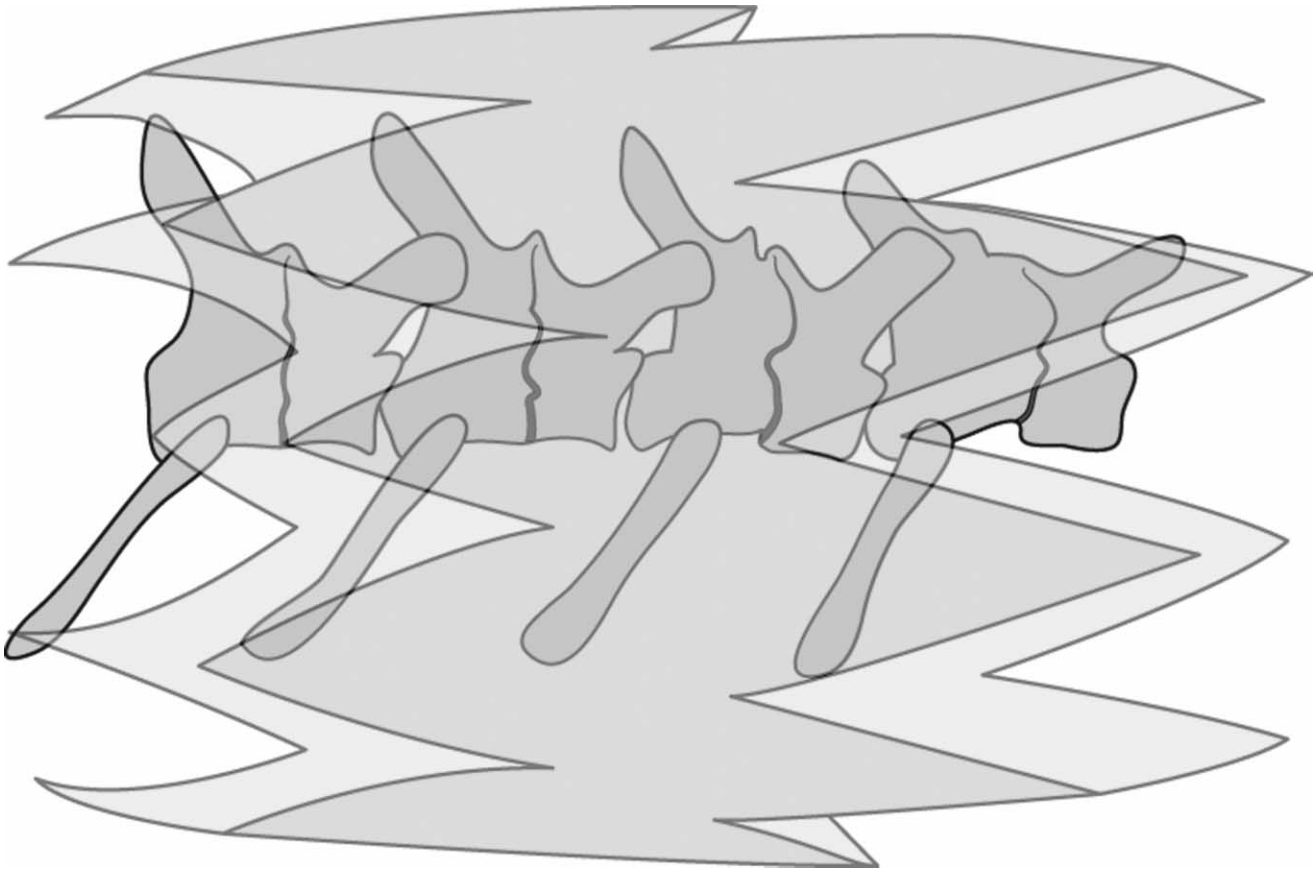

Figure 1. Lateral view of the caudal vertebrae of a skeletal preparation (provided by the University of Chicago museum) of *Eublepharis macularius*. Overlain on the caudal vertebrae is the musculature of a single caudal segment. Caudal autotomy typically occurs intravertebrally, as illustrated by the gray-filled fissures within the centra that depict fracture planes. A color version of this figure is available online.

physiological basis of contraction and the resultant movement patterns.

There are several key aspects of tail autotomy that likely differ between species depending on ecology, morphology, physiology, and constraints imposed by locomotion when the tail is attached to the body. Tails of lizards vary considerably in length and shape, ranging from relatively short and stubby structures to longer and thinner ones. Tails with reduced mass can likely move faster when cast off, which may potentially aid in distracting a predator more effectively, but the effectiveness of distracting a predator also increases with tail size (Daniels et al. 1986). Although the tails of some lizards that are used for a specialized function have lost the ability to autotomize (e.g., those of chameleons), others have not. For example, geckos and alligator lizards (*Elgaria* and *Abronia*) readily autotomize their tails, but some also use the tail as a prehensile appendage when moving among branches (Etheridge 1967; Bauer 1998). Tail use in species that do not employ it in prehension has received very little attention (Jusufi et al. 2008, 2010, 2011; Gillis et al. 2009; Libby et al. 2012), but it is possible that the importance of the tail during locomotion may have a strong influence on its behavior once autotomized, and some data on the ecological consequences of tail autotomy (e.g., predation, energetics, survival, growth, and escape) are available for lizards (Dial and Fitzpatrick 1981; Medel et al. 1988). Although this information is beneficial in assessing tail function and in interpretation of autotomy, detailed anatomical analyses or physiological studies are rare (but see Fisher et al. 2012 and Ritzman et al. 2012).

Reports of tail movements following autotomy are often qualitative, largely because of the high speeds at which they occur. Some, however, have attempted to quantitatively document the fracture event and subsequent tail movement. The act of autotomy itself involves the contraction of caudal muscles that span a fracture plane (Bellairs and Bryant 1985; fig. 1). However, the exact mechanism beyond this depends on the species, situation, and abiotic factors such as temperature. Tails are capable of autotomizing with no external contact (Sheppard and Bellairs 1972), although an anchor point (e.g., predator's jaws) is often the trigger (Bellairs and Bryant 1985). Poulton (1895), when referring to tail loss in lizards, noted that mutilation stimulates the nervous and muscular mechanisms in the tail. In addition, he postulated that the movements of autotomized tails are an adaptation for the purpose of aiding

escape from a pursuing enemy. Although this hypothesis was tested during the second half of the twentieth century, it is unclear how distracting diverse tails are to a variety of predators. Rather than simply resulting from the maintenance of metabolic activity in the amputated tissue, Poulton (1895) suggested that the movements of autotomized tails are the result of selective pressures. This provocative idea relates to the details regarding the evolution of tail autotomy, which are still relatively unknown.

Longstaff (1907) observed the autotomized tail of a South African gecko, *Pachydactylus maculatus*, and found that it was cast off when the animal was euthanized (using chloroform) but that it took much longer to cease moving than did the body, wriggling with a spiral movement. Additional qualitative observations have been published over the last 100 yr, including those of Clark (1971), in which tail movements were observed in *Lygosoma laterale*, *Anolis carolinensis*, and *Sceloporus undulatus*. Clark stated that the movements of the tail of the skink are more rapid and intricate than those of the other species examined. However, most studies of autotomized tails mention that the movements were too fast to observe closely. An alternate approach to the quantification of movement has been to record the time it takes for the tail to stop moving after autotomy. This has been done for several species of lacertid, and these averaged 6–8 min of movement following autotomy (Pafilis et al. 2005). The advent of affordable camera technologies now permits detailed, high-speed assessments of the three-dimensional motions of autotomized tails.

Three studies have examined the neuromuscular control of autotomized tails using electromyography (EMG; Rumping and Jayne 1996; Higham and Russell 2010, 2012), all focusing on geckos. Whereas the Tokay gecko (*Gekko gecko*) exhibits a cyclic and repeating pattern of burst activity (Rumping and Jayne 1996), as measured using EMG, the leopard gecko (*E. macularius*) exhibits complex neuromuscular control (Higham and Russell 2010, 2012). The latter underpins the complexity of behavior exhibited by the tail, which includes jumps, flips, and rhythmic swings. However, it is unclear whether the leopard gecko is unusual relative to other geckos in this regard or whether it is more generally representative. Analyses of more species is thus needed. The ballistic behaviors, such as jumping and flipping, are accompanied by an increase in the intensity of muscle bursts and an increase in the variation from burst to burst. Higham and Russell (2012) suggest that the transition between a rhythmic behavior (swinging back and forth) and a ballistic behavior (flipping) is similar to gait transitions exhibited by vertebrates. For example, at a given location along the length of the tail, muscles of the left and right sides are recruited alternately during rhythmic swings (in order to bend the tail to either side), whereas they are recruited simultaneously during jumps and flips (in order to bend the tail dorsoventrally). A similar alteration of pattern of recruitment can occur in vertebrates as they transition between gaits (e.g., trot-to-gallop transition in terrestrial vertebrates; Crone et al. 2009). Whether the basis of this shift in behavior is similar between autotomized tails and locomotor movements in other vertebrates remains untested.

In autotomized tails of *E. macularius*, the EMG burst amplitude decreases and the burst duration increases with time (Higham and Russell 2012). This is likely due to fatigue, which is exacerbated given that the autotomized tail is ischemic. Tail movements after autotomy are commonly thought to be driven by anaerobic metabolism given the ischemic conditions, and elevated levels of lactic acid have been observed in autotomized tails of lacertid lizards (Pafilis et al. 2005). That tails of some species of gecko can move for 30 min following autotomy suggests that some mechanism permits high endurance in the absence of an influx of oxygen. This might include a high oxidative capacity, allowing more extensive utilization of the limited oxygen available, and/or a slow rate of energy utilization, allowing prolonged activity but with reduced power output. Although we know something about how the tail is controlled, no study has yet examined the contractile abilities of the tail musculature, which might provide clues about the mechanisms of movement and the capacities of the tail following autotomy. If a lizard benefits from having an autotomized tail that moves continually, it might not be surprising that the tail exhibits modifications for endurance capacity, especially given the fact that the tail is ischemic.

Here we employ an integrative approach to reveal diverse aspects of tail autotomy. This is accomplished by presenting and integrating the results of two independent but related investigations, both of which provide insight into the evolutionary significance of tail autotomy. By doing this, we hope to reveal potential avenues for future research that endeavors to understand the fundamental principles, diversity, and evolutionary mechanisms of tail autotomy in lizards. We initially examine the three-dimensional tail movements across four species of gecko to determine whether the movements of leopard gecko tails are unusual or more general among geckos. These preliminary results provide a glimpse into the possible axes of variation across diverse groups of lizards. To compliment this, we compare the ATPase and oxidative capacity of muscle fibers and contractile dynamics of isolated muscle bundles from original tails, muscle from regenerate tails, and the fast portion of an upper limb muscle (iliofibularis) of the leopard gecko (*E. macularius*). This sets the stage for the final section of this article, in which we provide a framework for future work.

Tail Kinematics and Contractile Muscle Dynamics

We determined the three-dimensional movements immediately following autotomy in a controlled setting for four species of gecko. We tested the hypothesis that, because they live primarily on the ground in desert habitats, where tails would remain in close proximity to the owner following autotomy, the leopard gecko would exhibit more complex (increased repertoire of behaviors) and forceful movements than would the tails of species (*Chondrodactylus bibronii*, *Tarentola mauritanica*, *Hemidactylus turcicus*) that may not remain nearby following autotomy because these species often occupy more inclined struc-

tures, such as rock cliffs (Hodar et al. 2006). The tails of the latter, therefore, will likely travel a considerable distance (by falling) from the vicinity of the original owner immediately after autotomy, potentially limiting the importance of tail movements during predatory interactions. Although we expected the magnitudes of movements to differ depending on ecology, we also hypothesized that the four species in this study might exhibit the same general movements, reflecting the shared capacity of the tail to jump, flip, and swing.

For most lizards, the time that the tail remains active following autotomy is relatively short, ranging from 50 s to 15 min (Arnold 1984; Meyer et al. 2002; Pafilis 2005), but an exceptional case is noted for the tail of the leopard gecko, which may move for up to 30 min after autotomy (Higham and Russell 2010, 2012). We employ our previous observations of behavior and muscular activity of the autotomized tail of this species as a basis for exploration of muscle fiber ATPase activity, oxidative capacity, and contractile physiology to help build the foundation for comparative studies.

Reptilian skeletal locomotor muscles are often composed primarily of fast glycolytic (FG) fibers (Gleeson 1983; Bonine et al. 2001; Meyer et al. 2002), which have a high capacity for generating rapid movement, but fatigue quickly. Therefore, such muscles might not be expected to sustain movement following autotomy for the lengths of time observed for the tail of *Eublepharis macularius* (Meyer et al. 2002). A smaller proportion of fast oxidative glycolytic (FOG) fibers and an even smaller proportion of slow oxidative (SO) fibers (Gleeson 1983), both of which are associated with higher resistance to fatigue, are found in many reptilian muscles. The presence of such slow and oxidative fibers in the tail musculature may promote sustained activity following autotomy. Yet patterns of fiber distribution in limb skeletal muscles of reptiles appear highly conserved, including those of *Gekko gecko* and *Dipsosaurus dorsalis* (Putnam et al. 1980; Mirwald and Perry 1991), and others have suggested a high degree of conservation in the profiles of metabolites in tail muscle following contractions after autotomy (Pafilis et al. 2005). These observations suggest that the potential duration of tail movement across species may not be highly variable, leading to the question of which properties of the tails of leopard geckos confer the ability for such extended periods of movement after autotomy. Histochemical muscle staining in other reptiles has demonstrated primarily FG fibers in the tail, with FOG fibers located superficially and along the septa and a crescentic FG region around a circular FOG region located close to the femur in the iliofibularis, corresponding, respectively, to the white and red regions visible in the bisected muscle (Putnam et al. 1980; Mirwald and Perry 1991). Thus, we anticipated that muscles in the leopard gecko would have similar distributions and that contractile mechanics would reflect a predominantly FG phenotype for all muscles, but that resistance to fatigue of the tail muscles would be unusually high on the basis of the prolonged activity of the autotomized tail in this species.

To investigate these properties in the leopard gecko, histological identification of muscle fibers and contractile mechanics were studied in muscle segments of the original tail and compared with the characteristics of muscle in the regenerate tail and the fast fibers of the iliofibularis muscle as a representative fast limb skeletal muscle. Histochemical staining was used to detect succinate dehydrogenase (SDH) activity (and therefore oxidative capacity) and to detect actomyosin ATPase activity, which correlates with several measures reflecting muscle speed. These measurements were used to profile the oxidative and contractile capacity of the fibers resident in the muscles and to document their distribution. The contractile mechanics of these three muscle preparations were also tested, including kinetics of contraction and relaxation, work and power output, and fatigue resistance using the work loop technique (Josephson 1985), which allows determination of the work and power output of muscle when moving and activated in a manner similar to that which occurs in cyclically active muscle in an animal. Fatigue was measured as the failure to sustain work output during repeated contractions (Fitts 1994) and was used to assess the performance of tail muscles compared with fast limb fibers.

Material and Methods

Three-Dimensional Kinematics across Four Species of Gecko

All animal care, handling, and experimentation followed procedures approved by the University of California, Riverside, Animal Care Committee, following Institutional Animal Care and Use Committee guidelines. The species examined were *Chondrodactylus bibronii*, *Tarentola mauritanica*, *Hemidactylus turcicus*, and *Eublepharis macularius*. For each, three to five individuals were obtained from the pet trade and transported to the University of California, Riverside, for experimentation. Animals were housed individually in 10-gal aquariums and were kept on a regular light cycle (12 h of light per day) and given food and water ad lib. Lizards were transported to the filming arena (25 cm × 25 cm), which had a base of grid paper and walls of Plexiglas. The base of the tail was gently pinched using forceps to initiate autotomy. Tail movements were recorded using two high-speed cameras (Photron APX-RS) operating at 1,000 fps. One camera obtained a lateral view, while the other obtained a dorsal view via a mirror mounted at 45° above the arena. Up to 12 s of movement was recorded for each tail.

Videos were digitized in Matlab using DLT DV5 (Hedrick 2008) to obtain x, y, and z coordinates of the base and tip of each tail. After importing the three-dimensional coordinates into Microsoft Excel (ver. 14.0.0 for Mac), displacement of the tip and base of the tail, amplitude of bending, frequency of bending, and velocity (first derivative of displacement) were quantified. Data were smoothed using a quintic spline, and the instantaneous values of each variable were determined. For amplitude measurements, values were scaled to the length of the tail. For velocity measurements, values were presented as tail lengths per second. The effective tail length was determined by quantifying the straight-line distance between the tip and base of the tail.

To determine the differences between species, a two-way

ANOVA was performed with species as a fixed factor and individual as a random factor (nested within species). The dependent variables were maximum tail velocity and average excursion of the tail.

Contractile Dynamics

All animal care, handling, and experimentation followed procedures approved by the University of Calgary Animal Care Committee, following Canadian Council on Animal Care guidelines. Leopard geckos were obtained from a commercial supplier. Fourteen tails (seven original and seven regenerate) and nine iliofibularis muscles were used for the study of muscle mechanics, and 15 tails (10 original and five regenerate) and five iliofibularis muscles were used for histochemical observation. The animals were mature, of both sexes, of 39–52-g body mass, and of 8.9–12.0-cm snout-to-vent length. They were maintained individually in plastic cages (28 cm × 15 cm × 10 cm) in an environmental chamber at 28°C with a 12L : 12D photoperiod using fluorescent lights and fed 15 meal worms twice a week (supplemented with calcium and Fluker's Repta-Vitamin powder with beta-carotene). Fresh water was supplied twice a week in a dish, and cages were kept humid by spraying the walls and saturating a sponge in each cage.

Histochemistry

Ten original tails (i.e., not regrown following autotomy) were examined. Before autotomy, the animals were cooled to 4°C to reduce postautotomic movement of the tail. The tails were marked on the ventral surface with a felt-tip pen, dividing them into five sections of equal length. The posterior aspect of each section was demarcated by a dot. The sections were labeled 1 through 5, proximal to distal, respectively. Autotomy of the tails was then induced by applying pressure with the fingertips at its base. Autotomized tails were immediately cut transversely into five sections using the marks as a guide, and each section was cut in half sagittally. One sagittal half from each of the five sections was frozen in liquid nitrogen and stored at −80°C for subsequent histochemical investigations. Five regenerate tails were examined. Animals were cooled as indicated above and euthanized with T-61 (Intervet Canada, Kirkland, Quebec); tails were then severed, marked, and sectioned, and portions were frozen as for the original tails. Five iliofibularis muscles were obtained from the specimens that provided the regenerate tails by removing and freezing a hind limb in the same manner as the tails.

Original tail sections, regenerate tail sections, and hind limb samples containing the iliofibularis muscle were sectioned (14 μm) with a cryostat at −30°C and attached to microscope slides. Samples were taken from the anterior surface of each tail section and from the middle of the upper hind limb. Four slides were prepared from each tail segment and each hind limb, each carrying four replicate samples (for a total of 16 sections per sample). Within 48 h of cryosectioning, half of the slides were subjected to NADH diaphorase–SDH staining and half to actomyosin ATPase staining, following procedures described by Gleeson (1983), Higham et al. (2011), and O'Connor et al. (2011). Fibers staining darkly for ATPase indicate high ATPase enzyme activity, and those staining darkly for SDH indicate a high oxidative capacity (Gleeson et al. 1980; Gleeson 1983; Bonine et al. 2001).

NADH diaphorase–SDH staining entailed submersion of slides in a fixative (5% formalin, 0.2 M Na cacodylate, 68 mM $CaCl_2$, 0.24 M sucrose, pH 7.6) for 30 s. Slides were then immersed in Tris buffer (0.2 M, pH 8.0) for two bouts of 45 s each of fresh solutions and the incubation medium (1 mM NADH, 16 mM Na succinate, 0.6 mM nitro-BT in Tris buffer) for 105 min. This was followed by rinsing for 3 min in running tap water, 3 min in 75% EtOH, two bouts of 3 min in 95% EtOH, two bouts of 3 min in 100% EtOH, and three bouts of 3 min in CitriSolv.

Acid/mildly alkaline–stable-ATPase staining entailed submersion of slides in 5% formalin for 30 s, rinse solution (18 mM $CaCl_2$ in 100 mM Tris, pH 7.8) for two bouts of 45 s, and incubation medium (60 mM NaCl, 60 mM glycine, 25 mM $CaCl_2$, 3 mM ATP, 22.5 mM NaOH, 1 drop saturated KOH in alkaline distilled water at 35°C) for 15 min. This was followed by three 1-min bouts of wash solution (1% $CaCl_2$), 1 min in alkaline distilled water, 3 min in cobalt solution (2% weight/volume cobalt [II] chloride in alkaline distilled water), 1 min in alkaline distilled water, and 3 min in ammonium sulfide solution (1% volume/volume ammonium sulfide light in alkaline distilled water). The slides were then rinsed for 90 s in running tap water; dehydrated by immersing them for 3 min in 75% EtOH, two bouts of 3 min in 95% EtOH, and two bouts of 3 min in 100% EtOH; and finally cleared with three bouts of 3 min in CitriSolv.

After staining, the slides were mounted with DPX and examined under a microscope, and patterns of staining were observed and recorded. Five original and five regenerate tails at sections 1 and 3 as well as four iliofibularis samples were selected as being representative. These were photographed, and the images were analyzed using ImageJ (Abramoff et al. 2004). Images were converted into 8-bit gray scale, and dark was defined as a RGB hue below 120, with light being above 120. For tail samples, the number of fibers within an area approximately 0.5 mm^2 (adjusted to be 0.6% of the area for each section examined) were counted and classified as dark or light staining for both ATP and SDH stains. As the iliofibularis sections were considerably smaller than the tail samples, the number of fibers within a fixed area of 0.5 mm^{-2} was counted, half of this coming from the red region and half from the white to obtain relative intensities of SDH and ATPase staining in red and white fibers to compare with results from the tail muscle. The areas analyzed were selected to be near the center of the red and white regions of the iliofibularis and toward the periphery of the tail sections, so that the slower fibers near the periphery were accounted for in the analysis (see "Results"). This approach tended to bias the counts of slow fibers upward in the tail sections, but it ensured that the slow fibers were captured in the analysis, and

it is noted that the majority of the tail section deep to the periphery appeared to be composed of FG fibers.

Values are reported as mean ± SEM. Statistical significance was set at $P < 0.05$. To determine whether proportions of light- to dark-staining fibers were the same along the length of the tails, for both original and regenerate tails and for both ATPase and SDH stains, paired-sample *t*-tests were run comparing sections 1 and 3. To compare proportions of light to dark staining between the original tail, regenerate tail, and iliofibularis muscle, a one-way ANOVA or a Kruskal-Wallis test was performed. When significance was found, pairwise comparisons were made using a Tukey test or Dunn's method.

Muscle Contractile Properties

Seven original tails and seven regenerate tails were examined. Regenerate tails were obtained from animals whose original tails were autotomized at least 14 wk earlier, providing adequate time for the regenerate tail to regrow to mature proportions (Lynn et al. 2013). Animals were cooled to 4°C in a refrigerator for 10 min to reduce activity, then autotomy of original and regenerate tails was induced as described above for the histochemistry of original tails. The tails were immediately placed in ice-cooled physiological saline (115 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 20 mM $NaHCO_3$, 3 mM NaH_2PO_4, 5 mM glucose, pH 7.8) until movement ceased (normally 2–4 min). Tails were then placed in saline on the chilled stage of a dissecting microscope, the skin was removed from the dorsal/mediolateral/proximal third of the tail, and a small segment of muscle spanning two or three myomeres was removed from the superficial surface of the tail in a proximal, dorsomedial location. This segment was further reduced to a single myomere in length, including the superficial surface down to a depth of 0.5–1 mm and with myosepta extending from each end. Short segments of 6-0 silk suture were tied to the myosepta on either end of the preparation and used to secure the muscle into the recording apparatus (see below).

Nine iliofibularis muscles were studied. Animals were anesthetized with 5% isoflurane and then euthanized via cardiac bleed. The left hind limb was removed and placed in saline on the chilled stage of a dissection microscope. The iliofibularis muscle was removed with a portion of the ilium attached at one end and the distal tendon at the other. The slower red region of the muscle was removed, leaving the fast white region for study, allowing comparison of the biomechanics of the tail muscles with a known FG fiber type. Silk sutures were then tied onto the ilial bone fragment and tendon and used to secure the muscle into the recording apparatus.

Tail and iliofibularis samples were placed into a chamber containing physiological saline, with one end tied by the silk sutures to a servomotor (model 350 dual-mode servo; Cambridge Technology, Cambridge, MA) and the other to a force transducer (model 402A; Aurora Scientific, Aurora, Ontario; fig. 2). The saline in the chamber was pumped into a reservoir, where it was bubbled with either pure oxygen or room air and then flowed back into the chamber. Pure oxygen was used during measures of force and power output to ensure adequate oxygen delivery to the muscles. However, room air was used during measures of muscle fatigue to more closely mimic the reduced oxygen available to muscles when ischemic following autotomy. Temperature in the chamber was maintained at 28°C.

Muscles were activated with a stimulator (Grass S44; Grass Technologies, West Warwick, RI) that gated a battery-driven current source connected to platinum-plate stimulating electrodes positioned on either side of the muscle preparation. The stimulator was, in turn, controlled by a computer using a custom software program written using LabView (National Instruments, Austin, TX) and a 12-bit digital/analog converter card (PCI MIO 16E 4; National Instruments). The computer also controlled the servomotor and collected force, position, and stimulus signals (500 Hz). Muscles were activated with a train of pulses (1-ms pulse duration) at a frequency of 100 Hz. The stimulus voltage was increased until the threshold for maximal twitch force was determined, and this voltage was then increased by 50% to ensure that the preparations were maximally activated. Muscle length was adjusted to that producing maximal isometric twitch force.

A brief (20-ms) train of pulses (i.e., three pulses) was used to elicit brief isometric contractions, as this gave a much more robust and consistent force response than single shocks while being short enough in duration to not cause a sustained tetanus. The time for force to rise from 10% to 90% of maximal and for force to fall from 90% to 10% were measured from the contractions as one gauge of muscle speed. Maximal isometric tetanic force was then measured, with the duration of tetanic stimulation (100–600 ms) required for force to attain a plateau being determined separately for each preparation.

Muscle power output was assessed using the work loop technique (Josephson 1985). Muscle length was oscillated in a sinusoidal trajectory by the servomotor. The amplitude of length change (strain) was fixed at 14% peak to peak, as determined in preliminary experiments to result in near maximal work output for both tail and limb muscle. Work was measured over a range of cycle frequencies, with the stimulus phase and duration adjusted at each frequency to maximize net work output. Power was calculated as the net work done per cycle multiplied by the cycle frequency. To further refine identification of the cycle frequency that resulted in maximal power output, a quadratic equation was fit to the power-cycle frequency data for each preparation, solved for the first derivative, set equal to zero, and then solved for cycle frequency. The statistical significance of each regression was not assessed due to there being only a few data points (five typically) composing each curve as a result of attempts to minimize exertion of the preparations before measuring resistance to fatigue (see below). However, it was confirmed that the peaks so calculated were highly representative of the data used to fit the curves (see "Results" and fig. 9). Three of 21 preparations did not yield a concave-down quadratic fit and were excluded from the curve-fitting analysis.

To investigate resistance to fatigue, muscles were made to perform 100 consecutive cycles of work, and the fall in work output was assessed. During the fatigue experiment, muscles

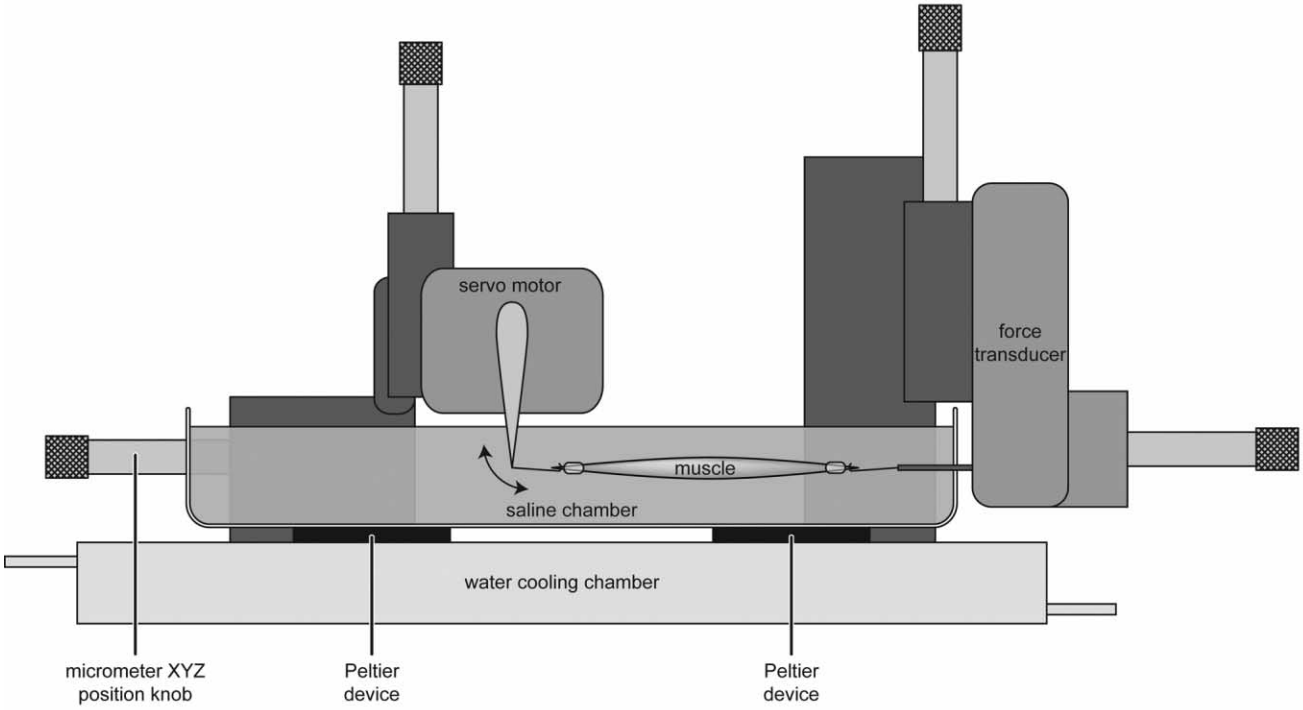

Figure 2. Experimental apparatus used to measure mechanical performance of isolated segments of tail and limb muscles. The muscle was immersed in a chamber filled with physiological saline. One end of the muscle was attached to the arm of a servomotor that was used to oscillate the length of the muscle in a sinusoidal fashion during measures of work and power. The other end of the muscle was attached to a force transducer that measured force produced by the muscle. The muscle was activated via platinum stimulation plates placed alongside the muscle (not shown). The saline in the chamber was circulated to a reservoir (not shown), where it was aerated and then flowed back into the saline chamber. Muscle length was adjusted using micrometer positioning stages attached to the servomotor and transducer. The temperature of the saline was maintained at 28°C with two Peltier devices that transferred heat between the saline chamber and a water-cooling chamber. A color version of this figure is available online.

were made to work at a cycle frequency near that resulting in maximal power output, as determined above (3 Hz for tail muscles and 8 Hz for fast iliofibularis fibers; see "Results"). This allowed an assessment of the ability of the muscles to sustain contractions when working at rates near their inherent maxima. The saline was bubbled with atmospheric air rather than pure oxygen during these measures to more closely mimic ischemic conditions. After the fatigue trials were complete, the muscles were monitored for recovery of work output by allowing them to rest and measuring work output only once every 5–10 min. Although all muscles recovered to some extent, recovery tended to be much less than complete (see "Results"), suggesting that subjecting the muscles to 100 consecutive cycles may have exceeded their ability to recover.

At the end of each experiment, the muscle samples were trimmed of obvious dead and connective tissue, blotted on filter paper to remove surface moisture, and weighed on an analytical balance (CP124S; Sartorius, Goettingen, Germany). Measurements of work and power were expressed either per mass or relative to the maximum that occurred within each preparation to facilitate comparison of changes with cycle frequency. Measurements of force were expressed relative to the cross-sectional area of the preparation.

Isometric force was monitored routinely over the course of each experiment after measures of power at each cycle frequency, and where there was a decline this force was used, along with the force produced at the start of each experiment, to adjust values of power to what would be expected if there had not been a decline. Preparations showing a decline in force greater than 20% (except during fatigue trials) were not included in the analysis.

For statistics, measurements of force rise and fall times, frequency of maximal power output, maximal force, work and power output, and work output at cycle 10, 50, and 100 of the fatigue trials were compared between the three muscle types using a one-way ANOVA or a Kruskal-Wallis one-way ANOVA. When significance was found, pairwise comparisons were made using a Tukey test or Dunn's method. Statistical tests were performed using SigmaStat statistical software (ver. 3.1.1; Systat Software, Chicago, IL).

Results

Three-Dimensional Kinematics

All gecko tails exhibited complex and dynamic motions, including jumps, flips, and swings. Qualitatively, it appeared that

the tail of the leopard gecko exhibited the most dramatic movements, often performing more than three consecutive flips in a single midair flight immediately following autotomy.

The extent to which the effective length of the tails changed during rhythmic swings differed significantly between species (fig. 3) and was negatively related to the frequency of tail bending. Both frequency and maximum bending were significantly different between species (ANOVA, $P < 0.05$; fig. 3), with *Hemidactylus turcicus* exhibiting the highest frequency and lowest value of bending (lowest excursion; fig. 3). Peak tail velocity was significantly different between the species (ANOVA, $P < 0.05$; fig. 4), with *Tarentola mauritanica* exhibiting the highest peak velocity (fig. 4), reaching around 100 tail lengths s^{-1}, whereas other species attained velocities between 60 and 80 tail lengths s^{-1}. The tip of the tail moved considerably faster than the base of the tail for each species, frequently reaching a velocity twice that of the base.

Histochemical Staining Proportions

Proportions of dark staining for ATPase between the selected superficial regions of the original and regenerate tails and a portion of the iliofibularis consisting of about 50 : 50 red-to-white fibers were not significantly different (Kruskal-Wallis test, $H = 4.061$, df $= 2$, $P > 0.131$; table 1). Proportions of dark staining for SDH were significantly greater for the iliofibularis sample compared with both regenerate and original tail samples but were not different between the original and regenerate tails (ANOVA, $F = 16.261$, df $= 2, 21$, $P < 0.05$; table 1).

Histochemical Staining Patterns

Original Tails. In all five cross sections of original tails from all 10 specimens examined, staining for ATPase was mostly dark (fig. 5). Staining was more intense along the borders of muscle bundles from adjacent tail segments and along the vertical and horizontal septa. However, some light-staining fibers were present along the peripheral surface of the tail, and these were most apparent in the more posterior tail sections (fig. 5). Cells staining light for ATPase appeared smaller in cross section than did those staining darkly.

Original tail sections revealed primarily light staining for SDH, with dark-staining fibers along the superficial subcutaneous border and adjacent to the horizontal and vertical septa (fig. 6). Dark-staining fibers were often found in a cluster along the vertical septa, at the midpoint between the vertebrae and the skin. Dark staining was also found along the borders between interdigitating segmental muscle blocks (fig. 6). Dark-staining cells situated superficially and adjacent to the vertical septum were often smaller in cross section than were surrounding lightly stained fibers.

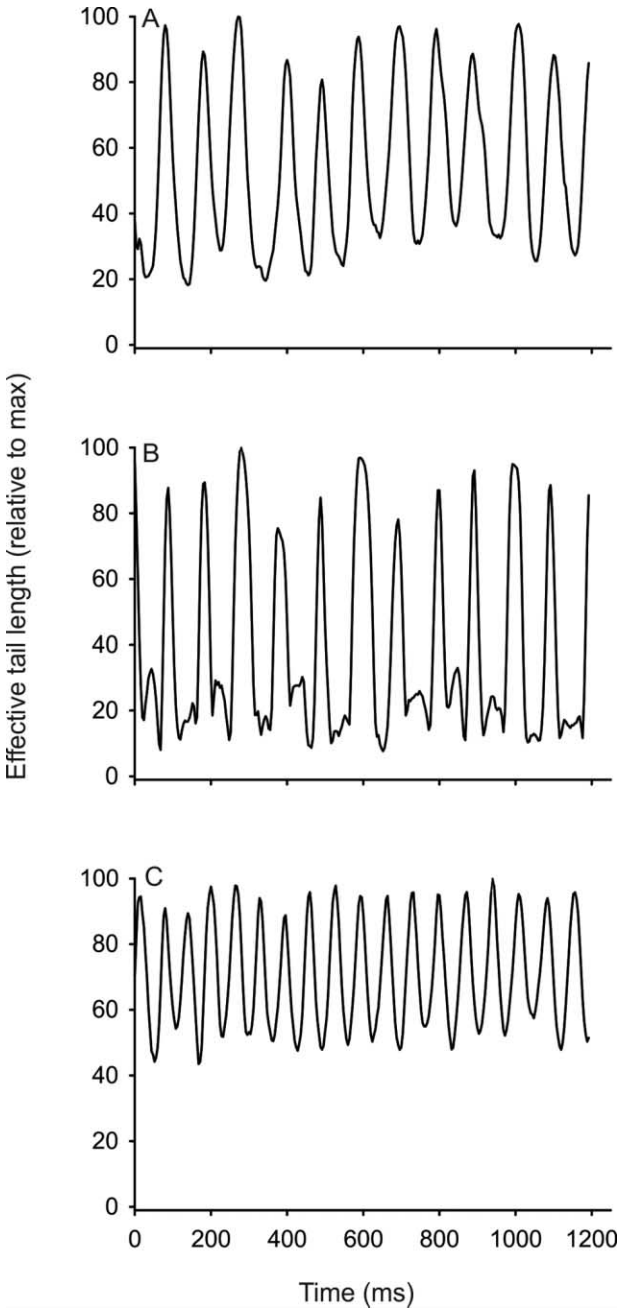

Figure 3. Representative plots of the effective tail length (distance from tail base to tip relative to the length of the tail) for *Chondrodactylus bibroni* (*A*), *Tarentola mauritanica* (*B*), and *Hemidactylus turcicus* (*C*). A value of 100 indicates that the tail is completely straight, representing the length of the tail. Values less than 100 indicate that the tail is curved and that the tip and the base of the tail are closer together. The minimum value occurs at the moment when the tip and base of the tail are close or in contact. For *T. mauritanica*, the small positive bump following each larger curve indicates that the tip and base of the tail cross paths.

Regenerate Tails. The regenerate tail at all five cross-sectional locations for all five specimens examined revealed primarily dark staining for ATPase, with a band of lightly staining fibers at the peripheral surface of the muscle (fig. 5). This lightly staining band was more prominent in regenerate than in original tails. Lightly staining fibers were visibly smaller in cross section than were darker-staining fibers. SDH staining of the regenerate tails demonstrated mostly lightly staining fibers,

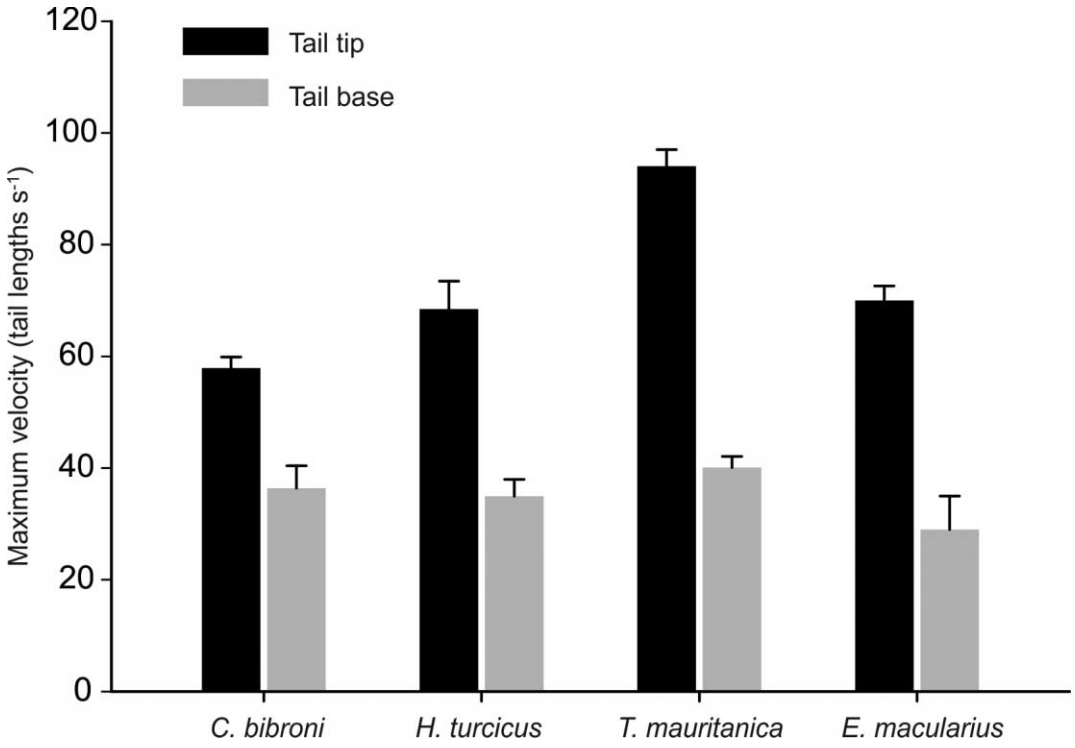

Figure 4. A plot of mean (±SEM) maximum tail velocity (tail lengths s^{-1}) for all four species included in this study. The velocities are divided into those for tail tip and tail base and represent the maximum instantaneous value observed within the initial 12 s following autotomy.

with a band of smaller, darkly staining fibers along the peripheral surface of the caudal muscles (fig. 6). Darker-staining superficial fibers for SDH corresponded with the position of the lightly staining superficial fibers, as revealed by ATPase staining.

Iliofibularis. The iliofibularis fibers stained dark for ATPase for all five specimens examined, in both the red and the white regions (fig. 5). A portion of the muscle located close to the femur had a concentration of smaller, more lightly staining fibers but was still classified as dark on the basis of the defining criteria (fig. 5). These were visibly smaller in cross section than were fibers located elsewhere (fig. 5). When stained for SDH, fibers of the iliofibularis were mostly light, but with a large, darkly staining bundle of fibers located near the femur (fig. 6). These were smaller in cross section than were the light-staining fibers elsewhere in the muscle and corresponded with the region staining more lightly for ATPase (figs. 5, 6).

Contraction Kinetics

Time for force to rise from 10% to 90% of maximal during a brief (20-ms) contraction was significantly different between the fast iliofibularis fibers and the muscle segments from the original and regenerate tails, but it was not significantly different between the regenerate and original tail muscles (Kruskal-Wallis test, $H = 16.350$, df $= 2$, $P < 0.05$). Force rose fastest for the iliofibularis (20.7 ± 0.37 ms), more slowly for the regenerate tail (25.3 ± 0.52 ms), and slowest for the original tail (26.7 ± 2.6 ms; fig. 7). Time for force to fall from 90% to 10% of maximal was significantly different between the original tail muscle and the fast iliofibularis fibers and regenerate tail muscle but was not significantly different between the regenerate tail muscle and the iliofibularis (ANOVA, $F = 23.622$, df $= 2, 18$, $P < 0.05$). Force fell fastest for the iliofibularis (25.5 ± 1.4 ms), slightly more slowly for the regenerate tail (28.4 ± 1.6 ms), and slowest for the original tail (48 ± 4.5 ms).

Table 1: Proportions of darkly staining fibers in muscle segments from original tails, muscle from regenerate tails, and approximately 50 : 50 red-to-white portions of iliofibularis muscles

	Muscle		
Stain	Original tail	Regenerate tail	Iliofibularis
ATPase	.934 ± .025	.952 ± .022	1.00 ± .00
SDH	.334 ± .521	.451 ± .067	.932 ± .042

Note. See "Material and Methods" for specific locations from which the samples were selected. For tail muscle, measures were made from sections 1 and 3 (see "Material and Methods"), and the results were combined. Dark staining for ATPase indicates fast myosin ATPase; dark staining for succinate dehydrogenase (SDH) indicates a high oxidative capacity. $n = 5$ for original and regenerate tails, and $n = 4$ for iliofibularis. Values are mean ± SEM.

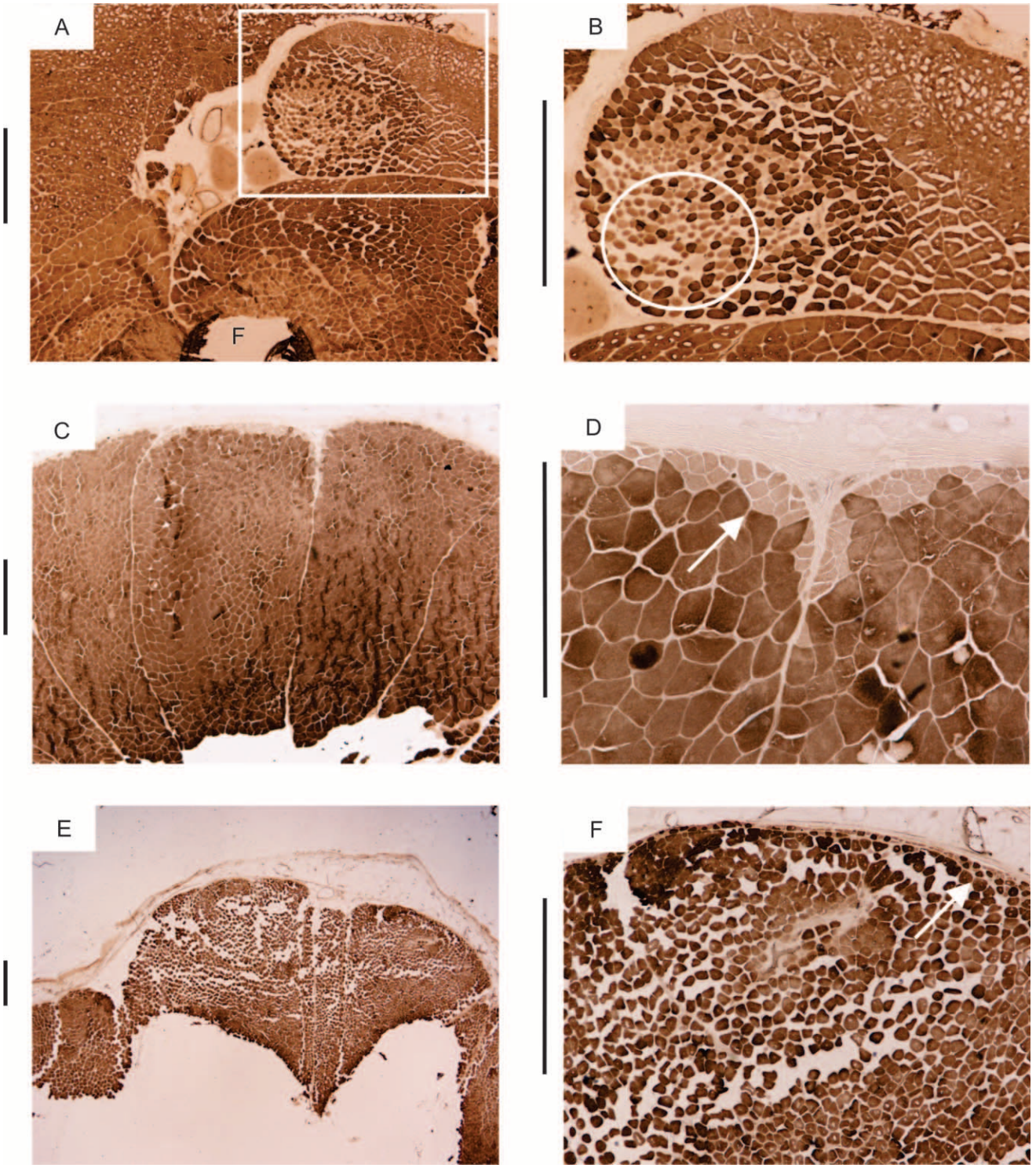

Figure 5. Histochemical sections with staining for ATPase, including low-magnification (*A*, *C*, *E*) and high-magnification (*B*, *D*, *F*) images of the iliofibularis (*A*, *B*), regenerate tail (*C*, *D*), and original tail (*E*, *F*). The scale bar to the left of each image is 1 mm. The femur (F) is indicated in *A*. The square and circle in *A* and *B* indicate the lighter-staining region within the muscle near the femur. The arrows in *D* and *F* indicate the lightly staining superficial fibers in the tail.

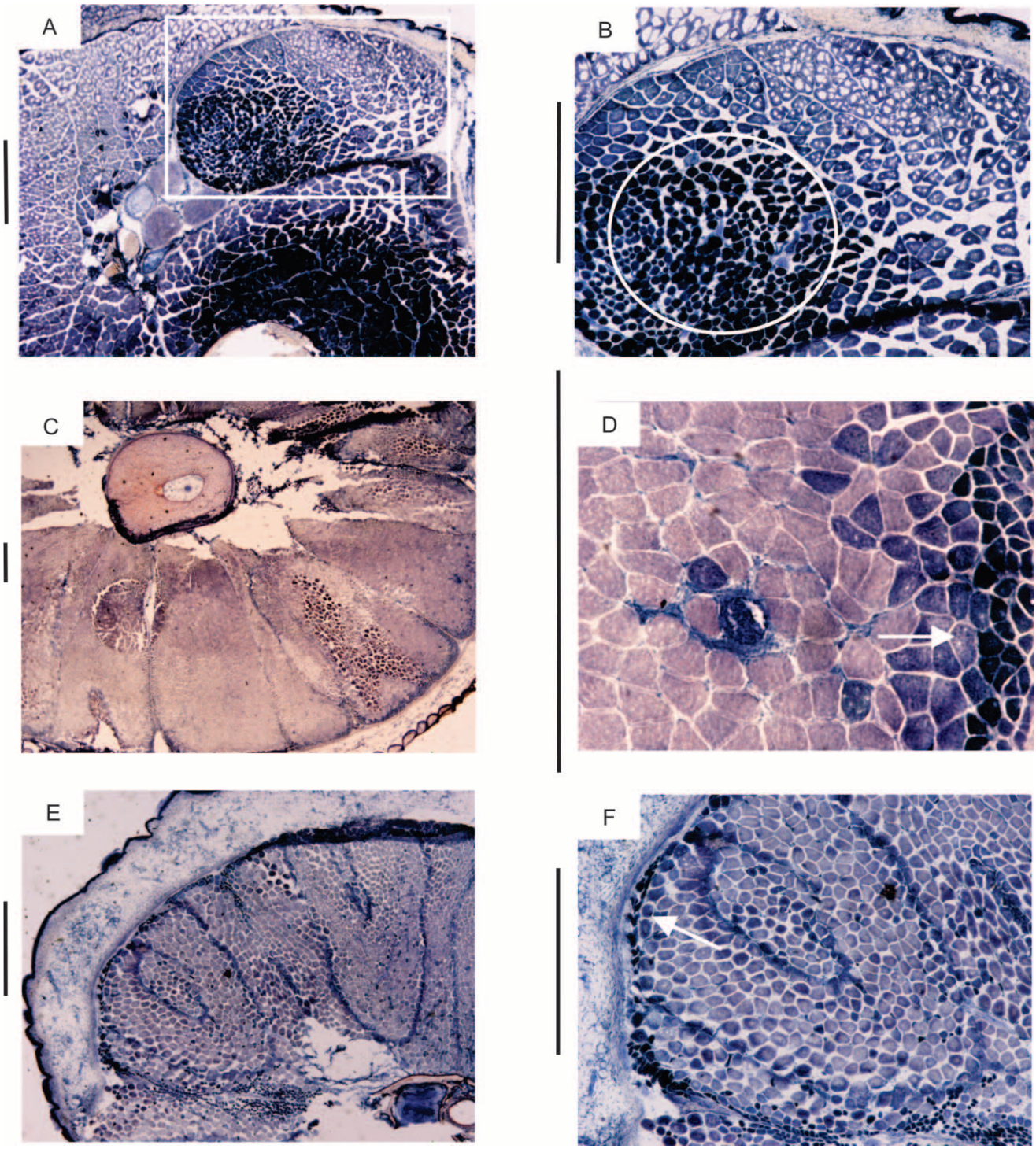

Figure 6. Histochemical sections with staining for succinate dehydrogenase, including low-magnification (*A*, *C*, *E*) and high-magnification (*B*, *D*, *F*) images of the iliofibularis (*A*, *B*), regenerate tail (*C*, *D*), and original tail (*E*, *F*). The scale bar to the left of each image is 1 mm. The square and circle in *A* and *B* indicate the darker-staining region within the muscle near the femur. The arrows in *D* and *F* indicate the darkly stained superficial fibers in the tail.

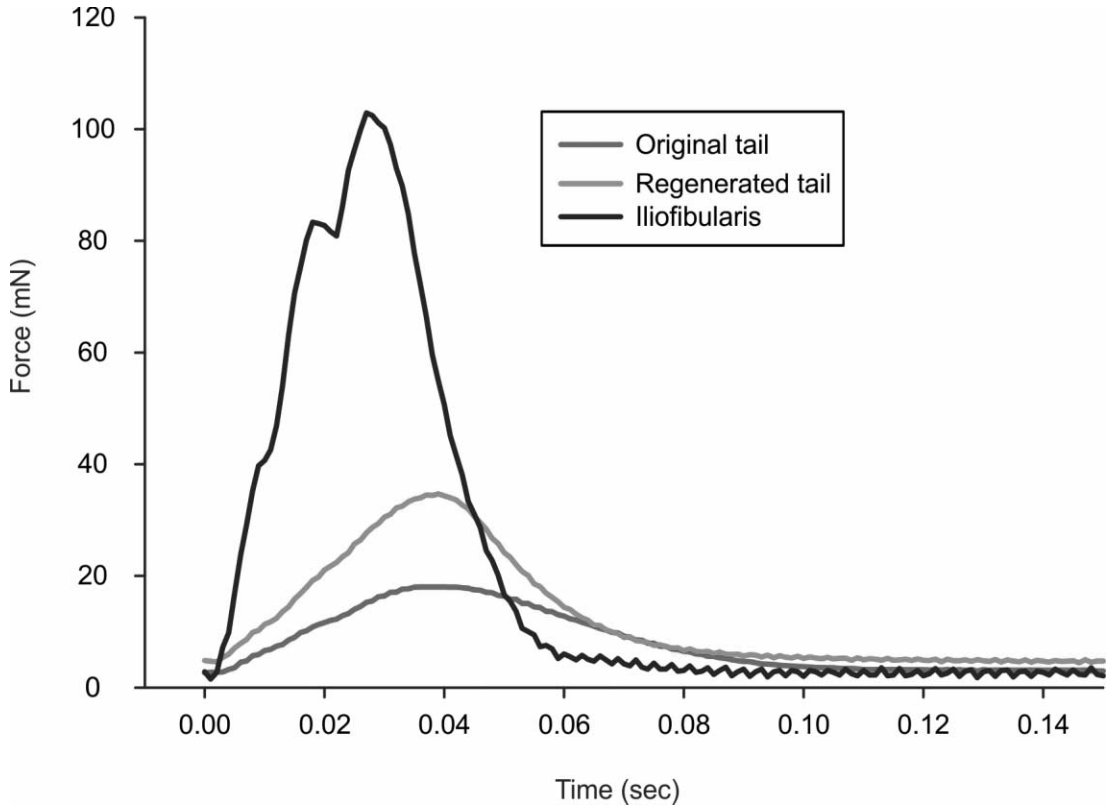

Figure 7. Representative traces showing force produced by isolated muscle segments during a brief (20 ms) isometric contraction from an original tail, regenerate tail, and fast iliofibularis fibers. See "Material and Methods" for specific locations from which the muscle segments were isolated. A color version of this figure is available online.

Force, Work, and Power Output

Maximum force per cross-sectional area during a prolonged tetanus was significantly different between all muscles examined (ANOVA, $F = 71.388$, df $= 2,21$, $P < 0.05$). The fast iliofibularis fibers produced the highest force, followed by the regenerate tail and the original tail (fig. 8*A*). Maximum net work per unit mass across all frequencies studied was significantly greater for the iliofibularis compared with the original tail muscle but was not significantly different for any other pairwise comparison (Kruskal-Wallis test, $H = 13.272$, df $= 2$, $P < 0.05$; fig. 8*B*). Maximum net power output per unit mass was significantly greater for the fast iliofibularis fibers compared with the original tail but was not significantly different for any of the other pairwise comparisons (Kruskal-Wallis test, $H = 14.500$, df $= 2$, $P < 0.05$; fig. 8*C*).

Frequency for Maximal Power Production

The cycle frequency at which power was maximal (fig. 9) was significantly different between the fast iliofibularis and both tail muscles but was not significantly different between the tail muscles (ANOVA, $F = 20.46$, df $= 2,14$, $P < 0.001$). The frequency for maximum power output was highest for the fast iliofibularis fibers (8.5 ± 1.1 Hz), lower for the regenerate tail muscle (4.6 ± 0.16 Hz), and lowest for the original tail muscle (3.1 ± 0.47 Hz).

Fatigue

The progressive decline in net work per cycle during fatigue was quantified every tenth cycle in the 100-cycle series (fig. 10*A*). Work output declined most rapidly in the fast iliofibularis fibers, with net work output reaching and remaining below 0 by about the fiftieth cycle, whereas work output of the original tail muscle remained proportionately highest for any given cycle in comparison to the other muscles examined. At the tenth cycle, relatively early in the series of contractions, none of the pairwise comparisons between muscles were statistically significantly different (Kruskal-Wallis test, $H = 6.692$, df $= 2$, $P < .05$). By the fiftieth cycle, work output was significantly different between the iliofibularis and both the original and the regenerate tail muscles but was not significantly different between the original and the regenerate tail muscles (ANOVA, $F = 20.183$, df $= 2,19$, $P < 0.05$). By the one hundredth cycle, the work output was significantly different between the original tail and iliofibularis muscles but was not significantly different for any of the other comparisons (ANOVA, $F = 6.471$, df $= 2,17$, $P < 0.05$). Work output recovered after the end of the

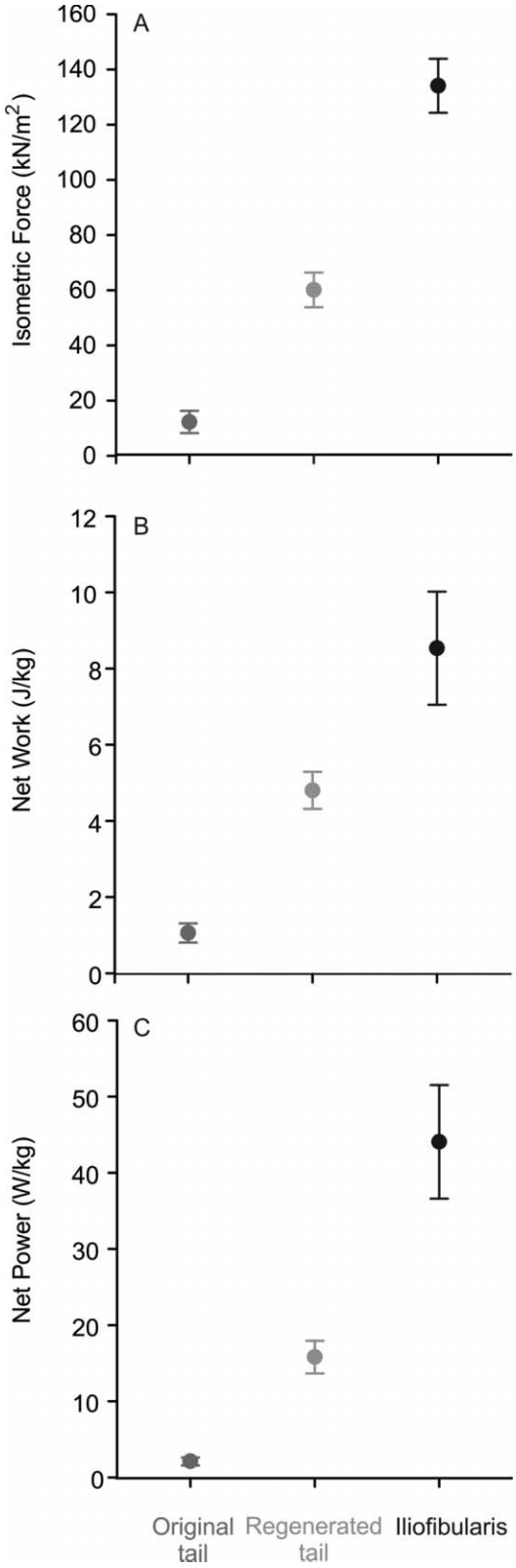

Figure 8. Measures of mechanical performance of isolated segments from original tail, regenerate tail, and fast iliofibularis muscle. See "Material and Methods" for specific locations from which the muscle segments were isolated. *A*, Area-specific, maximal, isometric, tetanic force. *B*, Mass-specific net work measured over a complete cycle of lengthening and shortening. The largest value of work recorded over a range of cycle frequencies was selected for each preparation, typically from the slowest cycle frequency tested. *C*, Mass-specific net power output over a complete cycle of lengthening and shortening. The largest value of power recorded over a range of cycle frequencies was selected for each preparation, typically in the middle of the range of cycle frequencies tested. $n = 8$, 7, and 9 for original tail muscle, regenerate tail muscle, and iliofibularis muscles, respectively. Values are mean ± SEM. A color version of this figure is available online.

fatigue trial to 48.8% ± 5.6% of initial for the original tail muscle, 46.9% ± 3.4% for the regenerate tail muscle, and 48.5% ± 7.2% for the iliofibularis muscle.

In addition to assessing the net work, an examination of the work loops revealed a progressive decline in the ability to generate force during the fatigue trial (fig. 10*B*–10*D*). As noted for net work, the decline in the ability to produce and sustain force production appeared greatest for the fast iliofibularis fibers and least for the original tail muscle. None of the muscles showed a pronounced increase in force and work during lengthening, such that the decline in net work output with fatigue was due predominantly to a failure of the ability to produce force during shortening.

Discussion

Three-Dimensional Kinematics

Postautotomic tail movements were described for the leopard gecko (Higham and Russell 2010, 2012) but not for other species. The addition of three more species of gecko revealed key similarities and differences in tail movement patterns. Flexing and extending rather than twisting are the predominant movements of the tails of the four species examined. All tails also exhibited flips and jumps similar to those reported for the leopard gecko (Higham and Russell 2010, 2012). Key differences between tail movements of these geckos relate to the frequency and amplitude of movement (fig. 3). Maximum tail velocity was greatest for *Tarentola mauritanica* but was relatively similar among the remaining species (fig. 4). All species studied exhibit variable and nonstereotyped movements of the detached tail, in contrast to the simple rhythmic swings reported for the Tokay gecko (Rumping and Jayne 1996). Our expanded sample allows us to suggest that most geckos will exhibit variable and nonstereotypical patterns of autotomized tail movement and that the leopard gecko is not unusual with respect to other geckos. The movements of all tails studied resulted in them traveling a considerable distance within the experimental arena rather than remaining in one place. This presents a unique situation among biological systems in that selection has likely favored patterns of movement that are variable and unpredictable. Although this remains to be tested directly, it would make sense given that predators would be less likely to quickly capture the tail if its movements were unpredictable and seemingly erratic.

We propose two scenarios in which fast and complex tail movement would benefit the lizard. First, lizards that are slow

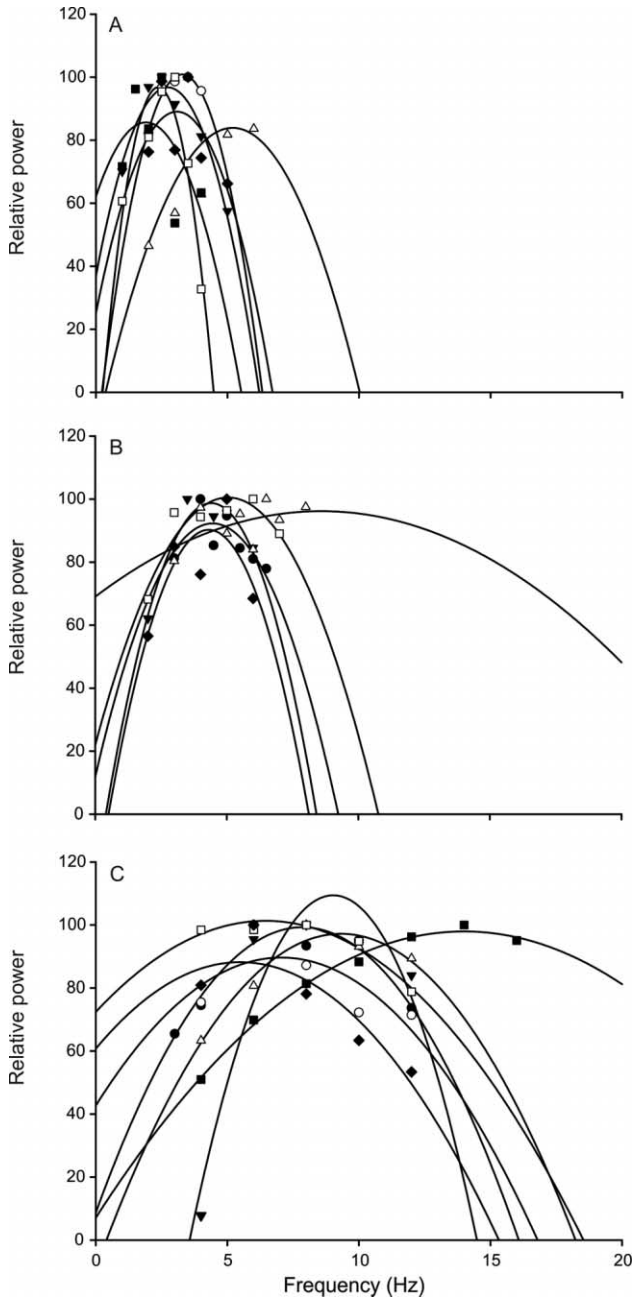

Figure 9. Net power, expressed as a percentage of the maximum produced by each preparation, developed over a range of cycle frequencies from isolated segments of original tail muscle (*A*), regenerate tail muscle (*B*), and fast iliofibularis muscle (*C*). See "Material and Methods" for specific locations from which the muscle segments were isolated. Different symbols represent data from different preparations. Curves are quadratic equations fitted to the data for each preparation.

and live in a terrestrial habitat will potentially require more time to escape a predator after the tail is lost. Thus, a more distracting tail will potentially increase the chances that the prey will survive the encounter. Second, there is clear evidence that lizards, especially skinks, will consume their autotomized tails (Clark 1971). In addition to laboratory experiments, Clark (1971) also obtained field data in which skinks were located and forced to autotomize their tail. The tail was then placed in that location, and the skink was permitted to return to the site of autotomy. Each lizard was then recaptured later that same day or on a subsequent day and euthanized. Stomach contents frequently revealed pieces of a tail, suggesting that the skinks returned to the site of autotomy and ingested the tail. Such return to the site of autotomy and ingestion of the lost tail, which contains fat reserves, supports the idea that tails should not only distract predators but also evade them completely. The movements of the detached tail of an arboreal species have not been examined, but this would be an interesting next step. Given that the neuromuscular control of the tail of the arboreal Tokay gecko has been examined (Rumping and Jayne 1996), this species would be an appropriate starting point.

All of the studies of tail movements and motor control undertaken to date have been for a small taxonomic subsample of lizards, with the focus being on skinks and geckos, leaving a number of comparative aspects of autotomy unanswered. How widespread might ballistic tail movements among lizards be? How do diverse predators respond to tail movements? How many species of lizard will return to the site of autotomy to consume their lost tail? These are fruitful areas for inquiry.

Mechanical Performance

The fast portion of the iliofibularis muscle was purposely chosen for study so that measures of fiber histochemistry and mechanical performance of tail muscle could be compared with a muscle of known fast-fiber composition. Furthermore, the segments of tail muscle under study were taken from the superficial surface, similar to the samples used for histochemical staining and which histochemical staining suggests contain a small portion of highly oxidative and slow fibers (dark staining for SDH and light for ATPase), with the deeper portion being composed almost entirely of fibers staining dark for ATPase but light for SDH (FG fibers). Thus, whereas measures of mechanical performance from these preparations will not be a simple predictor of performance of the entire limb, they allow assessment of the relative speed and endurance of a population of fibers in the tail that includes the slower oxidative fibers and comparison with that of FG fibers. Differences in properties of speed and endurance can then be related to differences in the types of muscle present.

Several measures of muscle mechanics indicated that the segments of original tail muscle selected for study had slower contractile properties than the regenerate tail muscle, which in turn were slower than FG fibers (i.e., the fast portion of the iliofibularis). Contraction kinetics (fig. 7 and "Results") suggest that the fast portion of the iliofibularis is up to twice as fast as the segments of tail muscles studied, even though the tail samples contained mostly fast fibers on the basis of ATPase staining (table 1), and that the original tail muscle was the slowest of the muscles examined. Force per cross-sectional area also tends to be lower for slower and more oxidative muscle

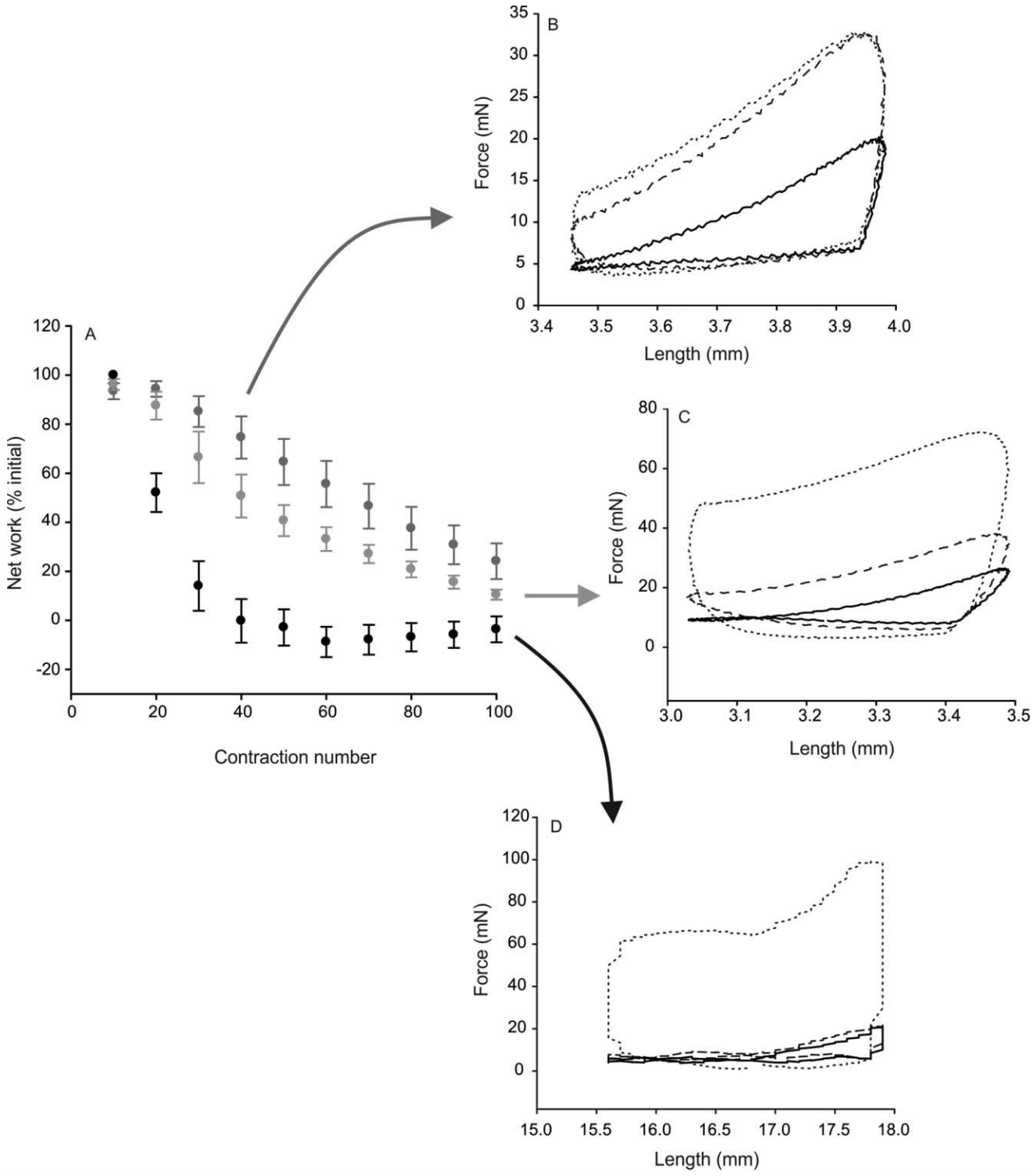

Figure 10. Net work done by isolated segments of original tail muscle (dark gray), regenerate tail muscle (light gray), and fast iliofibularis muscle (black) during a series of 100 contractions causing fatigue (*A*). The work done every tenth cycle during the series is shown. See "Material and Methods" for specific locations from which the muscle segments were isolated. Work is expressed relative to that during the first cycle. $n = 7$, 6, and 9 for original tail muscle, regenerate tail muscle, and iliofibularis muscle, respectively. Values are mean $\pm$ SEM. The cycle frequency was 3 Hz for tail muscles and 8 Hz for iliofibularis muscle, chosen to be near the frequencies resulting in maximal power for each type of muscle. Also shown are representative plots of force versus muscle length (work loops) during complete lengthening/shortening cycles from muscle segments isolated from the original tail (*B*), regenerate tail (*C*), and iliofibularis (*D*) muscles. Work loops are shown from three different points during the series of contractions that resulted in muscle fatigue; small dashed lines show the tenth contraction, large dashed lines show the fiftieth contraction, and solid lines show the one hundredth and final contraction in the series. A color version of this figure is available online.

(Medler 2002), where more of the intracellular volume of slower muscles is dedicated to structures such as sarcoplasmic reticulum and mitochondria, which support sustained oxidative metabolism, leaving less space for contractile myofibrils within the muscle mass (Josephson 1993). The area-specific force output of the three muscles (fig. 8*A*) reflects a pattern suggesting that the original tail muscles are a slower phenotype, the regenerate tail muscles slightly faster, and the white iliofibularis fibers the fastest. The relatively low force production in tail muscles, particularly the original tail, may reflect an unusually high volume density of structures that metabolically support sustained activity following autotomy, similar to what is observed in muscles of animals that sustain muscle contraction for long periods (Syme and Josephson 2002). It is noteworthy that the tail muscles exhibited low force despite being predominantly fast on the basis of ATPase staining (figs. 5, 6; table 1). However, the differences in area-specific force between muscles from tail and limb may also be due, in part, to a higher proportion of nonviable tissues in the original and regenerate muscle preparations resulting from the difficulty of their dissection, which would overestimate the mass and cross-sectional area of the fibers contributing to work and force. While histochemical procedures can be used to identify and account for dead fibers in a muscle preparation, the extensive fatigue trial at the end of each experiment likely damaged some of the preparations, making it impossible to interpret the results of such an analysis.

Work and power reflect the ability of a muscle to shorten against a load, as occurs during movement of the tail and limbs (Josephson 1985). Maximum mass-specific work and power are typically higher for faster muscles because they are capable of sustaining higher forces while shortening (Altringham and Young 1991; Josephson 1993). Likewise, the frequency at which power is maximal tends to be higher for faster muscles (Altringham and Young 1991), such that slower muscles tend to operate at lower frequencies than do faster muscles (Rome et al. 1988). The fast portion of the iliofibularis had the highest maximum work and power output and the highest frequency at which power was maximal, followed by the segments of regenerate tail muscle and then the original tail muscle (figs. 8*B*, 8*C*, 9). These patterns also support the conclusion that the fibers in the segments of original tail muscle tested had a substantially slower phenotype than did the fast fibers of the limb, even though most of the fibers present in the tail expressed high ATPase activity (table 1; fig. 5).

Stride frequency of *Eublepharis macularius* is approximately 2 Hz when walking and up to 4 Hz when running (Fuller et al. 2011), which closely matches the frequency of tail movement during these activities and the frequency at which power was maximal in the tail preparations under study (fig. 9). However, the frequency of tail movement following autotomy is approximately 8 Hz in *E. macularius* (Higham and Russell 2010, 2012), which is faster than the frequency for maximal power. This likely reflects the autotomized tail simply moving at a frequency higher than that yielding maximal power, as speed of movement is likely of greater importance than power in attracting the attention of a potential predator. In addition, the tail muscle preparations were selected from the superficial surface of the tail, which appears to include some relatively slow fiber types (figs. 5, 6). Thus, we might expect somewhat faster frequencies for movement and maximal power in the intact tail.

Resistance to Fatigue

Muscles with slower phenotypes have a higher resistance to fatigue (Fitts 1994). In agreement with the measures of contractile performance, all of which suggest that the original tail preparations had the slowest contractile phenotype, the original tail preparations maintained work output throughout the 100 cycles of contraction to a greater extent than did the regenerate tail muscles and the fast iliofibularis fibers (fig. 10*A*). Although the faster fibers in the tail may provide rapid and forceful movements initially, they likely fatigue rapidly following autotomy, as suggested by results from the FG iliofibularis fibers. Yet the slower and oxidative layer of superficial fibers in the tails, even though relatively few in number (figs. 5, 6), may endow the tail with the ability to continue moving for extended periods following autotomy. The energy available to move the tail following autotomy is the product of relative performance, which is better sustained in tail muscle (fig. 10*A*), and the mass-specific work capacity of the muscles, which is lowest in the original tail muscle and greater in the regenerate and the fast fibers of the iliofibularis (fig. 8*B*). The net effect appears to be a very poor capacity to sustain movement in FG fibers, which produced zero or less net work after only about 50 contractions despite of high power output initially, but a sustained ability to produce work at a relatively low rate in original and regenerate tail muscle. This performance was attained in atmospheric oxygen, and so questions still remain about the capacity of tail muscle to sustain performance at the as-yet-unknown oxygen pressures experienced during ischemia following autotomy.

Comparisons of Histochemical Staining and Mechanical Performance

Histochemical staining of the complete tail muscle, while not assessed quantitatively, appeared mostly fast (fig. 5), with more than 90% of fibers showing high ATPase activity even in the superficial samples that contained some slow fibers (table 1). Furthermore, a 50 : 50 mix of the red and white regions of the iliofibularis had a higher proportion of oxidative fibers than did the regions of the tail muscles that included the slower, more oxidative fibers (table 1). While these findings are based on only a select portion of the muscles, it is interesting that one predominantly fast muscle with relatively few oxidative fibers (the tail) had such a high endurance compared with the fast portion of the iliofibularis, suggesting either different fast phenotypes or that the inclusion of even a relatively few SO fibers has a substantial impact on fatigue resistance. Another potential contributor to the slow phenotype of the tail musculature, despite histochemical evidence that the tail muscles are primarily fast, could be their activation patterns in vivo.

Muscles develop slower contractile properties when they are activated more frequently (Hoyle 1983), while there is evidence that the histochemical properties of the fibers remain unchanged (Tollbäck et al. 1992). The tail musculature, in supporting the tails as a postural structure, may experience chronic and tonic activation that could bias its phenotype to that of a slower muscle. This may also account for the original tail muscles expressing a slightly slower phenotype than the regenerate tail muscles, because a regenerate tail has experienced a shorter period of activity than an original tail and may thus show less transition toward a slower phenotype. Additionally, the regenerate tails of all lizards examined to date are unsegmented and have a cartilaginous rod in place of the vertebrae, which could influence the performance of the tail on autotomy (Russell and Bauer 1992; Meyer et al. 2002).

Conclusion

Histochemical measures show the tail musculature of the leopard gecko to consist primarily of fast and anaerobic fibers. However, a layer of slow and highly oxidative fibers is present in the most superficial subdermal region of the tail. Muscle preparations that include this layer demonstrate a notably slower contractile phenotype than do fast fibers from the limb, which may enable the tail to sustain contractions for an extended period following autotomy before it fatigues. The layering that we observed in the tail of the leopard gecko might reflect the behavioral repertoire of the tail itself, which includes both rhythmic swings and jumps and flips that are more ballistic (Higham and Russell 2010). The oxidative layer might actuate the less powerful rhythmic swings and endow the tail with the ability to sustain contractions for long periods following autotomy, whereas the deeper, less oxidative muscle might drive the more powerful but short-lived ballistic behaviors. In addition to linking this fiber distribution to in vivo function, further investigation of the fiber-type distributions and contractile properties of tail muscles in other reptilian species that undergo caudal autotomy is required to test the uniqueness of these traits in leopard geckos. Whether a comparable distribution of fibers exists in species that do not exhibit ballistic tail movements is not known. It is possible that differences in tail behavior, if they exist, are related to the distribution of muscle fibers and tail morphology.

Unanswered Questions and Future Directions

Several factors likely contribute to the behavior and performance of the tail following autotomy. First, the behavior of the tail when lost will influence its ultimate fate, so selection for this fate may act as a constraint on how the tail behaves when cast off. For example, Clark (1971) suggested that blood loss is minimized from the autotomized tail to minimize attraction of the predator to the tail, allowing the tail to be consumed by the lizard (original owner of the tail) at a later time.

We have revealed that different species of gecko exhibit tails that move differently once autotomized but that these movements are based on the same underlying patterns of movement (rhythmic swings with interpolations of jumps and flips). This might simply be related to the morphology of the tail (both internal and external), but larger-scale differences between groups of lizards likely result from other factors and perhaps selective pressures for particular movements. One possibility is that tail movements reflect the sensory or motor abilities of the predator, including impeding or enhancing their attraction to the tail or their ability to seize it. The frequency of movement—or even the presence of intermittent ballistic behaviors—might be linked to selection related to the specific predators of the lizard. Lizards experience a diverse array of predators depending on their habitat, including snakes, small mammals, other lizards, birds, and even invertebrates. It would be informative to explore the dynamics of interactions between natural predators and tails following autotomy. There has been some study of these relationships (see below), but details of the kinematics and mechanics involved are unknown, including their relationship to the behavior of the predator.

Snakes are a common predator of lizards, and previous work has explored the ability of three species of skink (*Eumeces*) to escape from scarlet king snakes (*Lampropeltis triangulum elapsoides*) when the skinks retained the tail and when the tail was previously removed (Vitt and Cooper 1986). Compared with tailed individuals, tailless individuals were much more likely to suffer a body bite (100% vs. 61%), less likely to escape (9% vs. 54%), and more likely to be killed (91% vs. 46%). Although this does not directly relate to the specific movements of the tails themselves, it does highlight the cost of losing a tail, at least during a subsequent predator-prey interaction. We still do not know whether the behavior of the tail, if lost during the encounter, is sufficient to distract the predator and allow the lizard to escape or how specific movements of the tail after autotomy may influence that outcome.

The best evidence that tail movements themselves can successfully distract predators comes from a study involving feral cats (*Felis catus*) and two species of lizard (*Scincella lateralis* and *Anolis carolinensis*; Dial and Fitzpatrick 1983). Freshly autotomized (moving) and exhausted (not moving) tails were presented to the cats to determine the efficacy of freshly autotomized tails versus stationary tails to distract the predator. In all of the trials involving *A. carolinensis* (whether the tail was freshly autotomized or exhausted), the cat pursued the lizard and ignored the tail. The postautotomic movements of these tails are modest and do not involve ballistic behaviors. In contrast, freshly autotomized tails of *S. lateralis* thrash around and, in every case, distracted the cat. These same authors also examined whether tail movements significantly increased handling time by presenting either fresh or exhausted tails of *S. lateralis* to a scarlet king snake. Handling time was significantly longer for freshly autotomized (and thrashing) tails compared with exhausted (nonmoving) tails (Dial and Fitzpatrick 1983). Thus, tail movements that are ballistic and fast both distract predators and increase handling time. Key questions that have not been addressed relate to exactly what movements are necessary to achieve these outcomes. Does this de-

pend on the type of ballistic movements? Does this depend on the frequency and amplitude of the movements? Does the anatomy and color of the tail impact the ability to distract? Does the ability to distract depend on the specific predator that is attacking the tail? How does habitat structure impact tail movement and distraction ability? The answers to such questions are paramount for understanding why and how tail autotomy has evolved as a successful survival strategy.

Another question that has not been addressed is whether tail morphology, physiology, and behavior reflect selective pressures on function while the tail is attached to the body, when it is disconnected from the body, or both. It is possible that the patterns of postautotomic movement are solely a by-product of evolutionary pressures for tail function during locomotion. Although we suspect that this is not the case, no study has formally addressed this question. A systematic analysis of tail movement across species before and after autotomy is needed to explore these relationships.

Most lizards will regenerate their tail after autotomy. Although the anatomy of the regenerate tail is different from that of the original (Fisher et al. 2012; Ritzman et al. 2012), it still retains the ability to move. That said, it is commonly assumed that lizards drop their entire tail only once, with the regenerate being incapable of severing due to the lack of autotomy planes. A detailed examination of neural control, neural network patterning, and movement patterns of original and regenerate tails is needed to fully understand the functional changes that occur after regeneration.

As mentioned earlier, the mechanism underlying the switch between rhythmic tail movements and more ballistic movements may be reminiscent of gait transitions during locomotion. Identification and recordings from spinal motoneurons responsible for tail movement before and after autotomy are necessary for understanding how the tail is controlled. Furthermore, the mechanics of tail musculature has to date been examined only in a single species. Given the dramatic differences in tail composition between species, there are likely some important mechanical differences that relate to tail movement and effectiveness in evading predators in different species. There is little doubt that many important advancements will emerge with the exploitation of new techniques that enable the facilitation, quantification, and evaluation of tail function.

Acknowledgments

This contribution stems from a symposium at the 2012 World Congress of Herpetology. We are very thankful for the generous financial support from Kubtec, the Company of Biologists, and Nelson Education. Three reviewers provided helpful comments that improved the manuscript. We are grateful to Meghan Rock for providing artwork for figures 1 and 2. This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to D.A.S. (203190-2012), an NSERC Discovery Grant to A.P.R. (9745-2008), and a National Science Foundation grant (IOS 1147043) to T.E.H.

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