

Comparative Analysis of Fiber-Type Composition in the Iliofibularis Muscle of Phrynosomatid Lizards (Squamata)

Kevin E. Bonine,^{1*} Todd T. Gleeson,² and Theodore Garland Jr.¹

¹Department of Zoology, University of Wisconsin, Madison, Wisconsin

²E.P.O. Biology, University of Colorado at Boulder, Boulder, Colorado

ABSTRACT The lizard family Phrynosomatidae comprises three subclades: the closely related sand and horned lizards, and their relatives the *Sceloporus* group. This family exhibits great variation in ecology, behavior, and general body plan. Previous studies also show that this family exhibits great diversity in locomotor performance abilities; as measured on a high-speed treadmill, sand lizards are exceptionally fast sprinters, members of the *Sceloporus* group are intermediate, and horned lizards are slowest. These differences are paralleled by differences in relative hindlimb span. To determine if muscle fiber-type composition also varies among the three subclades, we examined the iliofibularis (IF), a hindlimb muscle used in lizard locomotion, in 11 species of phrynosomatid lizards. Using histochemical assays for myosin ATPase, an indicator of fast-twitch capacity, and succinic dehydrogenase, denoting oxidative capacity, we classified fiber types into three categories based on existing nomenclature: fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-twitch oxidative (SO). Sand lizards have a high proportion of FG fibers (64–70%) and a low proportion of FOG fibers (25–33%), horned lizards are the converse (FG fibers 25–31%, FOG fibers 56–66%), and members of the *Sceloporus* group are intermediate for

both FG (41–48%) and FOG (42–45%) content. Hence, across all 11 species %FOG and %FG are strongly negatively correlated. Analysis with phylogenetically independent contrasts indicate that this negative relationship is entirely attributable to the divergence between sand and horned lizards. The %SO also varies among the three subclades. Results from conventional nested ANCOVA (with log body mass as a covariate) indicate that the log mean cross-sectional area of individual muscle fibers differs among species and is positively correlated with body mass across species, but does not differ significantly among subclades. The log cross-sectional area of the IF varies among species, but does not vary among subclades. Conversely, the total thigh muscle cross-sectional area does not vary among species, but does vary among subclades; horned lizards have slimmer thighs. Muscle fiber-type composition appears to form part of a coadapted suite of traits, along with relative limb and muscle sizes, that affect the locomotor abilities of phrynosomatid lizards. *J. Morphol.* 250:265–280, 2001. © 2001 Wiley-Liss, Inc.

KEY WORDS: comparative method; fiber type; histochemistry; lizard; locomotion; phylogeny

Lizards have often served as model organisms for studies of locomotion (Bennett, 1994; Garland and Losos, 1994; Gans et al., 1997; Irschick and Jayne, 1999a). Even ignoring snakes, which are derived from lizards, lizards show a remarkable diversity of locomotor modes, morphologies, and abilities, including bipedality, gliding, limblessness, specialized toe pads, toe fringes, and the ability to run across water (Zug, 1993). Moreover, measurements of locomotor performance capacities, taken in the laboratory under controlled conditions, have demonstrated wide variation among lizard species with respect to maximal sprint-running speed, endurance, climbing, jumping, clinging, and the energetic cost of locomotion (e.g., Losos, 1990; Garland, 1994; Miles, 1994a,b; Bauwens et al., 1995; Irschick et al., 1996; Zani, 1996, 2000; Autumn et al., 1999).

In a previous study, we used a high-speed treadmill to document extensive differences in maximal sprint-running speed among 27 species of relatively small-bodied (range = 3–38 g body mass) lizards from the southwestern United States (Bonine and

Garland, 1999). The slowest species in our sample, the alligator lizard *Elgaria kingii* (Anguidae), attained an average maximum speed of only 1.08 m/s, whereas the fastest species, the teiid *Cnemidophorus tigris marmoratus*, attained an average of 6.17 m/s. Particularly noteworthy was the range of sprinting abilities observed among 17 species within the monophyletic family Phrynosomatidae—from 1.45 to 5.72 m/s (see also Irschick and Jayne, 1999a).

Phrynosomatidae includes more than 120 recognized species and is comprised of three closely related mini-radiations (see Fig. 1; Etheridge and de

Contract grant sponsors: UW-Madison Department of Zoology Davis Fund Awards, SWRS, and SICB (to K.E.B.); Contract grant sponsor: NSF; Contract grant numbers: IBN-9724140 (to T.T.G.), IBN-9723758 (to T.G.).

*Correspondence to: Kevin E. Bonine, Department of Zoology, 430 Lincoln Drive, University of Wisconsin, Madison, WI 53706. E-mail: kebonine@students.wisc.edu

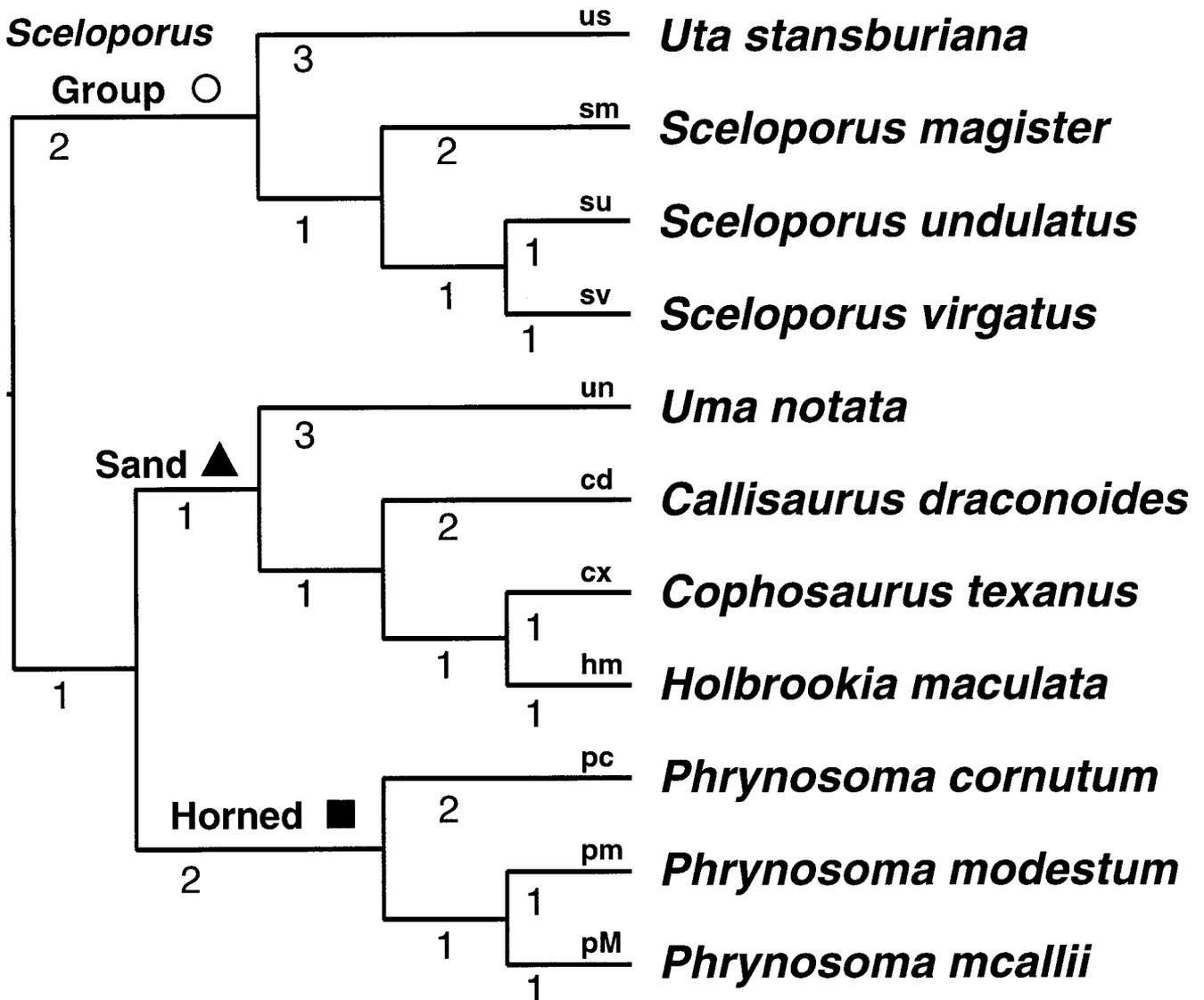


Fig. 1. Hypothesized phylogenetic relationships for 11 species of phrynosomatid lizard examined in this study. Note that we sampled representatives from each of the three distinct subclades within this family (*sensu* Frost and Etheridge, 1989; Reeder and Wiens, 1996). Topologies within each subclade: *Sceloporus* group (Wiens and Reeder, 1997), sand lizards (Wiens, 2000; Wilgenbusch and de Queiroz, 2000), horned lizards (Montanucci, 1987). Branch lengths are arbitrary (as suggested by Pagel, 1992).

Queiroz, 1988; Frost and Etheridge, 1989; Wiens, 1993; Reeder and Wiens, 1996; Schulte et al., 1998). In addition to variation in locomotor performance (Garland, 1994; Miles, 1994a; Bonine and Garland, 1999; Irschick and Jayne, 1999a), the family exhibits large variation in morphology, behavior, and ecology (Stebbins, 1985; Conant and Collins, 1991), much of which occurs among the three subclades. The horned lizards (14 species in the genus *Phrynosoma*) are a highly derived group of flat-bodied, ant specialists (e.g., Sherbrooke, 1981). To avoid predation, they rely primarily on crypsis, defensive morphology (spines on body and head), and the unusual ability to squirt blood from the orbital sinus (Sherbrooke, 1981, 1987; Middendorf and Sherbrooke, 1992). Their sister clade, the sand lizards, often

inhabit relatively open sandy or gravelly deserts (Belkin, 1961; de Queiroz, 1992; Howland, 1992; Degenhardt et al., 1996), have long limbs, and rely on speed to escape from predators (Dial, 1986; Bulova, 1994; Jayne and Ellis, 1998; Irschick and Jayne 1999a,b). The *Sceloporus* group (Etheridge and de Queiroz, 1988) contains many species that are more intermediate generalists in ecology, behavior, and morphology (Sites et al., 1992). The *Sceloporus* group also contains many species that are more arboreal or saxicolous (e.g., see Miles 1994a), but we focused on primarily terrestrial species in order to simplify comparisons with sand and horned lizards.

Several studies have shown that sprint-running abilities are correlated with relative hindlimb length among species of lizard (Losos, 1990; Miles, 1994a;

TABLE 1. Results from published studies of fiber-type^a composition in lizard iliofibularis muscle

Species	% Fiber-type composition			Mean cross-sectional area of single fiber ^b (μm^2)			Notes	Reference
	FG	FOG	SO	FG	FOG	SO		
<i>Dipsosaurus dorsalis</i>	75.5	17.9	6.6	12,668	3,578		called SO fibers tonic	Putnam et al., 1980; Gleeson et al., 1980a
<i>Dipsosaurus dorsalis</i>	71.1	24.4	4.5	9,111	4,056	2,644		Gleeson and Harrison, 1988
<i>Iguana iguana</i>	60.0	10.4	29.6	2,827	2,124	1,590	called SO fibers tonic, data for juveniles	Gleeson and Harrison, 1986
<i>Agama agama</i>	92	8		twitch fibers 2× size of tonic			called SO fibers tonic, mATPase stain only	Abu-Ghalyun et al., 1988
<i>Chamaeleo senegalensis</i>	50	50		tonic fibers 2× size of twitch			called SO fibers tonic, mATPase stain only	Abu-Ghalyun et al., 1988
<i>Chamaeleo jacksonii</i>	61	24	16	75,477	47,143	18,505	tonic fibers were 7% of total	Mutungi, 1992
<i>Varanus exanthematicus</i>	20	38.3	41.8	112,221	66,052	82,448	tonics fibers were 11.3% of total	Mutungi, 1990
<i>Varanus salvator</i> (red IF)	0	1	99	—	1,956	927	amount of IF that was red not reported	Gleeson, 1983
<i>Varanus salvator</i> (white IF)	92.2	7.8	0	3,314	1,956	—	amount of IF that was white not reported	Gleeson, 1983
<i>Gekko gekko</i>	42	58		not reported				Mirwald and Perry, 1991

^aNaming and staining conventions are not always parallel across studies (e.g., SO and tonic fibers grouped in table, see notes column).

^bExcept for *D. dorsalis* (Gleeson and Harrison, 1988) and *V. salvator*, diameter was reported and here converted to cross-sectional area assuming a circular cell.

Bauwens et al., 1995; Bonine and Garland, 1999; Irschick and Jayne, 1999a). Another probable cause of interspecific variation in sprinting abilities is muscle properties. A standard way to characterize muscles is via histochemical analysis to determine fiber-type composition. All else being equal, one would predict that fast species should have a high percentage of fast-twitch glycolytic (FG) fibers in their locomotor muscles. Whether this prediction holds true is unclear. For example, the cheetah has 61% fast-twitch fibers in its gastrocnemius and 83% in the vastus lateralis muscle (Williams et al., 1997), but these values are no greater than found in some of the five species studied by Ariano et al. (1973; guinea pig, rat, cat, lesser bushbaby, slow loris). In human athletes, the vastus lateralis is known to vary from up to 70% fast twitch to as low as 15%, and sprint speed is positively correlated with the amount of fast-twitch fibers (Schele and Kaiser, 1982; references in Wilmore and Costill, 1994). With respect to lizards, the results of several previous studies, taken together, demonstrate wide interspecific variation in fiber-type composition (e.g., see Table 1 for data on the iliofibularis muscle), but no multispecies comparative study has been attempted.

Here we report on variation in fiber-type composition of the iliofibularis (IF) muscle among 11 species of Phrynosomatidae (see Fig. 1). The IF is a parallel-fibered or unipennate muscle that spans both the knee and hip joints. It is active during the swing phase (when the femur is being abducted and the knee bent) of both graded and burst locomotion in lizards (Jayne et al., 1990). The IF is relatively easy

to find in a cross-sectional segment of lizard limb, contains discrete red and white regions, and has been extensively studied in lizards (e.g., Gleeson et al., 1980a, 1984; Putnam and Bennett, 1982; Gleeson, 1983; Johnston and Gleeson, 1984; Gleeson and Harrison, 1986; Gleeson and Johnston, 1987; Gleeson and Dalessio, 1990; Mutungi, 1990; Mirwald and Perry, 1991; see references in Table 1). In *Varanus exanthematicus*, the savanna monitor lizard, electromyographic studies show that the red region is active at both low and high locomotor speeds, with regular bursts of activity, whereas the white region is active only above some threshold speed and with often irregular activity (Jayne et al., 1990). For several lizard species the IF muscle has been characterized for fiber-type composition (see references in Table 1) and for fiber-type recruitment patterns (Jayne et al., 1990; see also Marsh and Bennett, 1985, 1986; Marsh, 1988; Johnson et al., 1993; Swoap et al., 1993).

Based on existing knowledge of locomotor performance, behavior, and habitat, we hypothesized that, among the 11 species of Phrynosomatidae sampled, the sand lizards would have the highest proportion of fast-twitch glycolytic (FG) fibers in the IF muscle, the horned lizards (*Phrynosoma*) would have the lowest, and the *Sceloporus* group would be intermediate.

MATERIALS AND METHODS

Animal Collection

The data included in this study were collected from animals captured and measured in three different years. In 1996 and 1997, we collected lizards

from populations in southern Arizona and western New Mexico, using the conveniently located Southwestern Research Station (SWRS; Portal, AZ) as a base of operations. In 1999, lizards were captured in the field from targeted populations throughout the United States and shipped alive to Madison, Wisconsin. Because of potential seasonal differences in metabolism and performance (e.g., Garland and Else, 1987), we restricted animal collections to late May through early August. For each species (with a few unavoidable exceptions), individuals were collected from a restricted geographic area, because populations may differ in physiological characteristics (Garland and Adolph, 1991). To avoid complications from comparing widely divergent locomotor modes, we focused on species that are largely terrestrial (as opposed to arboreal or saxicolous) and occur in arid or semiarid habitats. All lizard species included in this study are diurnal and primarily insectivorous (Stebbins, 1985; Conant and Collins, 1991). To avoid possible sex and ontogenetic differences, we used only adult males. During captivity, we kept individual lizards isolated in cloth bags or plastic containers (depending on size), with periodic access to water but no food. Individuals were sacrificed within 14 days of capture. Animal care protocols were approved by all relevant educational and research institutions.

Morphometrics

In addition to quantifying muscle morphology of the iliofibularis, we measured morphometric variables and locomotor performance abilities (sprint speed and endurance; see Bonine and Garland, 1999; Garland, 1994, for details) for all animals. In 1996 and 1997 these measurements were made at SWRS. For lizards captured in 1999 measurements were made at the University of Wisconsin-Madison. Limb and body proportions were measured to the nearest 0.5 mm using a clear plastic ruler. Body mass was measured to the nearest 0.001 g within a few days of capture using a Mettler balance (model PM200) in 1996 and 1997 and a Sartorius balance (model L420) in 1999.

Tissue Preparation

In preparation for histochemical analyses, lizards were decapitated after we warmed them (overnight in 1996 and 1997, for at least 1 h in 1999) to their approximate field-active body temperature, as determined from the literature. Each hindlimb was quickly removed intact along with a portion of the pelvis. Limbs were mounted with pins above a Styrofoam block with knee and ankle joints flexed at 90° to ensure comparable muscle lengths among individuals. Muscle and mounting block were then plunged into isopentane cooled in liquid nitrogen. This technique allowed quick freezing of the tissues and cre-

ated uniform dissection and length conditions for muscles from both small- and large-bodied species. In 1996 and 1997, animals were shipped alive from SWRS to TT Gleeson in Boulder in preparation for muscle composition measurements. In 1999, lizards were sacrificed and tissues prepared in Madison. Tissues were stored at -80°C. Ultimately, all individuals will be deposited in the frozen or alcohol collection of the University of Wisconsin-Madison Zoological Museum.

Histochemical Analyses

In summer 1997 and spring 1999, frozen limbs were cut in half just distal of mid-thigh and the proximal portion of the thigh was mounted on cryostat chucks using Tissue Tek® embedding medium. Using a cryostat microtome (AO 855), we sectioned hindlimb muscles at mid-thigh in a plane perpendicular to the femur in 10 µm sections at -22°C. Serial sections, captured on glass coverslips and air-dried for at least 30 min, were used for histochemical identification of fiber types, as done previously in our laboratory (e.g., Putnam et al., 1980; Gleeson, 1983; Gleeson and Harrison, 1986, 1988; Garland et al., 1995). Histochemical activities of alkaline-stable myosin ATPase (mATPase; pH 8.7, 30 min) and succinic dehydrogenase/NADH diaphorase (SDH; 2 h, pH 7.4) were used to identify fibers as slow-oxidative (SO; light mATPase, dark SDH), fast twitch-glycolytic (FG; dark mATPase, light SDH), or fast-twitch oxidative glycolytic (FOG; dark mATPase and dark SDH). See Discussion for further comments on fiber-type terminology and comparison with "the mammalian standard." The only procedural alteration relative to the above references is that all sections were preincubated 30 sec in a 5% formaldehyde solution and rinsed 1 min in a 100 mM Tris buffer at room temperature (buffer calibrated to pH 7.4 at 37°C) prior to incubation for mATPase or SDH activity. This addition is a modification of the original Guth and Samaha (1969) description for fiber-type characterization. Preliminary work with *Cophosaurus* and *Phrynosoma* determined that this is necessary for uniformity across species with regard to characterization of fiber-type dimensions, as sectioned tissue from some species was inclined to contract radially during incubation.

Incubation for mATPase activity often results in a family of darkly stained fibers ranging from medium tan to dark brown, in addition to the light caramel-colored fibers that we have shown to have slow contractile characteristics in *Dipsosaurus dorsalis* (Johnston and Gleeson, 1984). This variation (tan-brown) corresponds to populations of FG and FOG fibers, but subjective judgment is sometimes required to distinguish tan from caramel fibers. In practice, muscle biologists have minimized this subjectivity by slightly altering pH and other incubation conditions for each muscle and each species studied.

This approach was impractical for the number of species being examined here. Instead, we used constant incubation conditions that optimized fiber characterization in tissues of the well-studied lizard, *D. dorsalis* (Gleeson et al., 1980a; Putnam et al., 1980; Gleeson and Harrison, 1988). *Dipsosaurus dorsalis* iliofibularis tissue was incubated alongside the tissue of interest and thus served as both a staining reference and internal incubation control.

After incubation, sections were allowed to dry thoroughly and then mounted on microscope slides using Histomount® (National Diagnostics, Manville, NJ). The IF, which is located posteriorly and dorsally in the hindlimb, was identified in each cross-section, with the sciatic nerve being a useful landmark. Multiple 35 mm photographs were taken of each stained iliofibularis muscle using Kodak EliteChrome film (ASA 100 and 200) and an Olympus (C-35 AD-4) camera mounted on a compound microscope (Olympus, BH-2). Objectives used were primarily 4× and 10×. Photographic slides were scanned into PhotoShop 5.5 using NikonScan 2.1 software and a Nikon slide scanner (model LS-2000). Within PhotoShop, images of both mATPase and SDH stained cells were simultaneously displayed to determine muscle fiber-type classification; individual cells were marked with different colored dots representing different fiber types. NIH Image (v. 1.62) was used to count fibers of each type and to measure fiber cross-sectional areas by tracing the perimeter of each cell in both the oxidative and nonoxidative regions within the muscle. In general, we tried to measure all of the fibers in the IF for a given individual. When it was not possible to measure all fibers (because some individual muscles were too large, or the entire muscle was not contained in the serial section being measured), we determined regions of like composition and measured a large number of fibers within that region. Depending on the individual, we measured 60–100% of the fibers (average 235) in the oxidative region located medially—this appears red in fresh tissues. For the lateral and more homogeneous white portion of the muscle, we measured 40–90% of the fibers (average 164). We assumed the remainder of a given region was comprised of similarly sized fibers in the same relative proportion of fiber types. Data were collected from four individuals of each species using only the mATPase images for dimension measurements to control for variation in cell deformation caused by the two different histochemical stains. Previously, fiber cross-sectional areas have been estimated by this technique with an error of approximately 1% (TT Gleeson, unpublished data).

To assess the relative size of the iliofibularis muscle, we used a dissecting scope (Olympus SZX12) with a digital camera (Olympus DP10) mounted above to capture lower magnification images of the whole thigh. Images were saved on a Smart Media

card (Simple Technology) and transferred to a Macintosh computer using a Camedia Floppydisk Adapter (Olympus). Whole-thigh and iliofibularis areas were also measured, by tracing, using NIH Image. Both the dissecting scope images and the compound microscope images were calibrated using a stage micrometer.

Statistical Analyses

We simultaneously determined whether species and subclades differed by performing conventional nested analysis of variance (ANOVA) and covariance (ANCOVA) using values for each of the four measured individuals per species and values for species nested within subclade (SAS PROC GLM with Type III sums of squares). Except for the three percentage fiber-type traits and the percent of thigh muscle that was IF, all of the morphometric traits were \log_{10} transformed prior to analyses. The ANCOVA analyses were conducted using either log body mass or log snout–vent length as a covariate.

Variance components attributable to clade, species within clade, and individuals within species (error) were computed following Sokal and Rohlf (1991). Because several of the traits were strongly correlated with body size, we ignored the mean squares attributable to this covariate when computing variance components (i.e., percent variance).

We then used arithmetic mean values for each species for phylogenetically based statistical analyses. Again, the morphometric traits were \log_{10} transformed prior to analyses. We employed Felsenstein's (1985) method of phylogenetically independent contrasts and phylogenetic analysis of covariance by computer simulation (Garland et al., 1993). For both phylogenetic methods, we used the topology and the arbitrary branch lengths shown in Figure 1. The general evolutionary relationships within Phrynosomatidae are well supported (Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989; Wiens, 1993; Reeder and Wiens, 1996; Schulte et al., 1998). Within the *Sceloporus* group (which includes *Petrosaurus*, *Urosaurus*, *Uta*, and *Sceloporus*, but is represented here by only the latter two genera; Reeder and Wiens, 1996), we used the most recent topology as described by Wiens and Reeder (1997). The topology within the sand lizards is supported by several researchers (Changchien, 1996; Reeder and Wiens, 1996; Wiens, 2000; Wilgenbusch and de Queiroz, 2000). The *Phrynosoma* (horned lizards) topology follows Montanucci's (1987) cladistic analysis of morphology. As information on divergence times or some other metric was unavailable, we used the arbitrary branch lengths suggested by Pagel (1992), as shown in Figure 1. We checked diagnostic plots (Garland et al., 1992; Diaz-Uriarte and Garland, 1998) of the absolute values of standardized contrasts vs. their standard deviations (square roots of sums of corrected branch lengths) and they showed

no obvious trends, with the exception that the contrast between sand and horned lizards was often very large (see Results).

We used the MS-DOS computer program PDTREE (Garland et al., 1993, 1999; Garland and Ives, 2000) to enter trees and to compute independent contrasts (Felsenstein, 1985). Independent contrasts were analyzed either within PDTREE or exported to a conventional statistical program for analysis by correlation or regression through the origin. We used PDSIMUL to simulate bivariate character evolution under Brownian motion and PDANOVA to analyze the simulated data, both as described in Garland et al. (1993). All of these programs are available on request from T. Garland.

For the simulations we used a model of Brownian motion with limits to trait evolution. (Limits were used to avoid biologically impossible values, e.g., negative % fiber-type compositions.) For %FOG and %FG we set upper limits of 99.5% and lower limits of 0.5%, and used the "Replace" option of PDSIMUL (see Garland et al., 1993). For thigh cross-sectional area, with body mass as a covariate, we used limits of 4 and 80 mm² and 1 and 100 g, respectively. Thigh cross-sectional area and body mass were simulated on the log scale. For all four traits in both sets of simulations, for both initial values (starting at the root of the phylogeny) and final means, we used the simple mean of the trait value for all 11 species. For variances of the simulated tip values we used the variances of the actual data. We used $\alpha = 0.05$ as the critical value in all statistical tests.

RESULTS

Within each IF muscle, we found an oxidative core and a fast-twitch glycolytic perimeter (e.g., see Fig. 2), as has also been found in all other species examined (see references in Table 1). Furthermore, the oxidative portion of the IF was always located medially within the muscle, nearest to the femur, and the more lateral portion of the muscle was the predominantly fast-twitch region. FOG and FG made up the largest proportion of the muscle for all 44 individuals.

Occasionally, we found fibers that did not stain darkly for either mATPase or SDH (5 of 44 individuals; less than 1% in one individual each of *Uta stansburiana*, *Cophosaurus texanus*, *Phrynosoma cornutum*; 3% in one individual *P. modestum*; a second individual of *P. cornutum* was 6% tonic, but this may be a result of unusually light staining overall for that individual). These fibers have been termed "tonic" by previous researchers. Because we only rarely found these fibers and because their twitch properties are undetermined, we grouped these fibers in with the SO category for all analyses.

Nested ANCOVA

Descriptive statistics for each species are presented in Tables 2 (body and limb dimensions) and 3 (muscle morphometric and histochemical data). Nested ANOVA of the 44 values for individual lizards (four per species) indicated, not surprisingly, that species differ significantly in body mass and in snout-vent length (SVL), on both the raw and log-transformed scales (all $P < 0.001$). Of more interest, nested ANCOVA with log body mass as the covariate (Table 4) indicated that species differ significantly in log SVL, hindlimb span, and forelimb span (all $P = 0.0001$). In addition, the three subclades differ significantly for both SVL and hindlimb span (see Fig. 3). Results of ANCOVA with log SVL as a covariate were similar, except that log forelimb span is significantly different at the subclade level and log IF area is not different at the species level (see footnotes of Table 4).

Nested ANCOVA with log body mass as a covariate (Table 4) indicated that the percentage of each of the three fiber types differs significantly among subclades (Fig. 4A,B). (We recognize that the three fiber-type proportions are not independent pieces of information because they must sum to one, but we present results for all three for completeness.) As a subclade, horned lizards have a low proportion of FG fibers (25–31%), species from the *Sceloporus* group are intermediate (41–48%), and sand lizards have a relatively high FG proportion (64–70%); the proportion of FOG fibers shows the converse pattern (Table 3). Among species within subclades, only %SO varies significantly (range = 0.8–17%).

Considering absolute cross-sectional areas, mean cross-sectional area of individual fibers does not vary at the subclade level (Table 4; Fig. 4C,D), and only SO cross-sectional area differs among species within subclades ($P = 0.0015$). Thigh muscle cross-sectional area (excluding femur) varies significantly among the three subclades (Table 4, $P = 0.0005$); *Phrynosoma* has relatively thin thighs (Fig. 4E). Log iliofibularis muscle alone differs marginally among species ($P = 0.0326$), but not among subclades ($P = 0.1550$). When expressed as a percentage of total thigh muscle cross-sectional area (Fig. 4F), IF area again differs among species ($P = 0.0051$), but not among subclades ($P = 0.1055$). For both log total thigh and log IF muscle areas, log body mass is a highly significant covariate ($P = 0.0001$ and 0.0005 , respectively).

Analysis of Mean Values for Species

Considering only the 11 species' mean values, conventional ANOVA also indicates highly significant differences among the three subclades for %FG ($F = 146.9$, d.f. = 2,8, $P < 0.001$) and %FOG ($F = 74.6$, $P < 0.001$), but not for %SO ($F = 3.5$, $P = 0.081$). When the foregoing F statistics are compared with

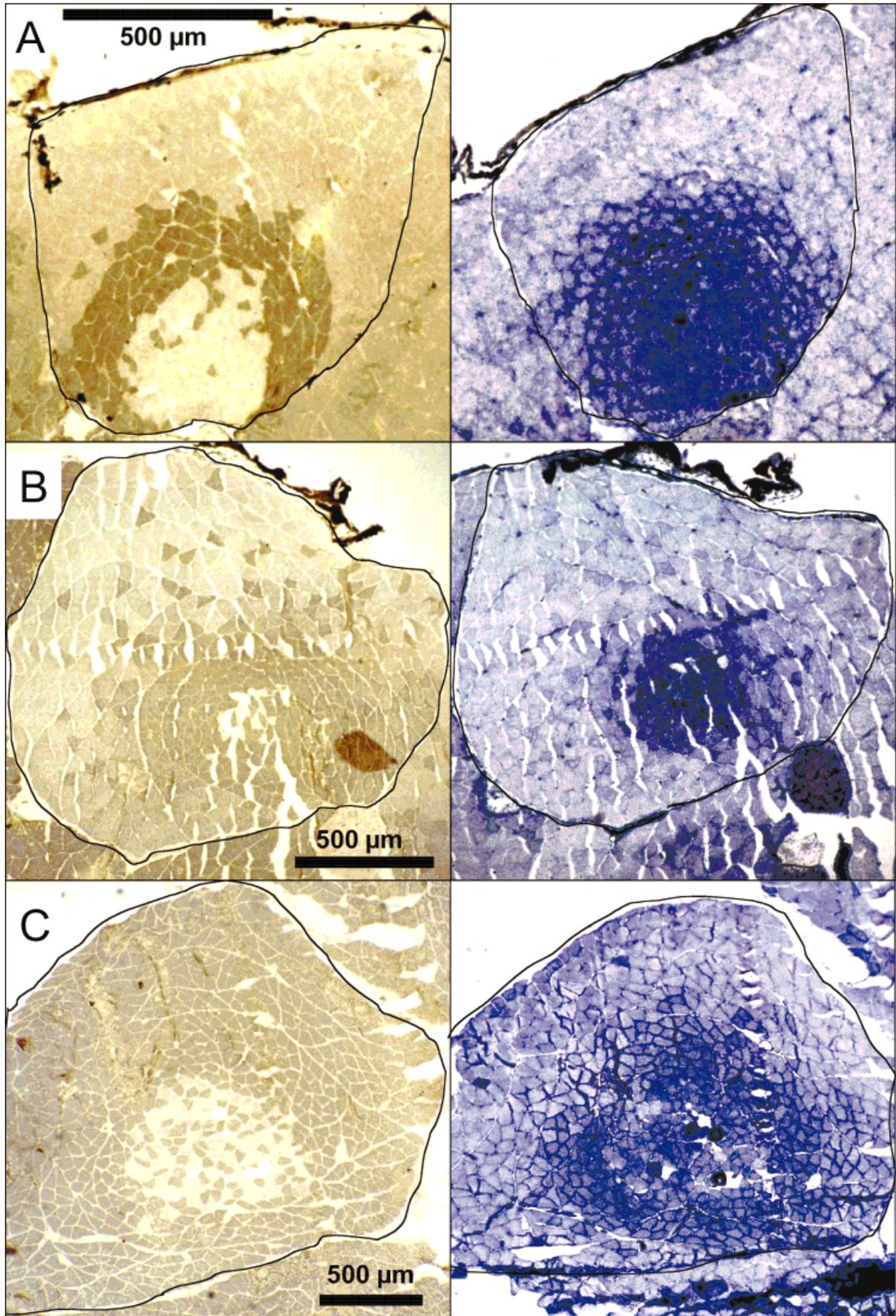


Fig. 2. Serial sections of iliofibularis muscle histochemically stained for activity of two enzymes. The lower portion of the IF in each panel is also the medial portion. Fibers staining tan to brown for myosin ATPase activity (left) indicate fast-twitch capacity, whereas fibers staining dark blue for succinic dehydrogenase (right) indicate high oxidative capacity. A fiber that stains darkly only on the left would be FG, only on the right would be SO, and staining darkly on both is classified FOG. See Methods for details. **A:** *Sceloporus undulatus*, in the *Sceloporus* group, has intermediate proportions of both FG and FOG fibers. **B:** *Callisaurus draconoides*, a sand lizard, has high FG content, low FOG content, and only a few SO fibers. **C:** *Phrynosoma cornutum*, a horned lizard, has a low percentage of FG fibers and a high percentage of FOG fibers.

TABLE 2. Mean and standard deviation of body and limb linear dimensions* for 11 species of *Phrynosomatidae*

	Body mass (g)	Snout-vent length (mm)	Hindlimb span (mm)	Forelimb span (mm)
Sceloporus Group				
us <i>Uta stansburiana</i>	3.48 0.60	52.4 3.5	82.9 3.1	56.8 2.2
sm <i>Sceloporus magister</i>	34.01 11.93	104.6 10.5	150.8 10.5	119.8 9.0
su <i>Sceloporus undulatus</i>	4.50 0.27	59.0 2.2	86.9 1.2	63.8 0.5
sv <i>Sceloporus virgatus</i>	5.52 0.86	60.4 2.0	89.6 2.4	67.3 2.3
Clade Mean (n = 4) s.d.	11.88 14.78	69.1 23.9	102.5 32.3	76.9 28.9
Sand Lizards				
un <i>Uma notata</i>	31.54 3.09	110.1 5.7	165.6 6.6	122.8 4.9
cd <i>Callisaurus draconoides</i>	10.62 6.21	75.8 11.1	145.8 18.2	97.5 12.2
cx <i>Cophosaurus texanus</i>	11.26 3.09	76.0 7.2	143.6 12.4	96.0 7.2
hm <i>Holbrookia maculata</i>	3.91 0.95	50.3 2.7	81.5 2.4	59.4 1.9
Clade Mean (n = 4) s.d.	14.33 11.94	78.0 24.6	134.1 36.5	93.9 26.1
Horned Lizards				
pc <i>Phrynosoma cornutum</i>	38.51 7.86	97.1 9.4	135.6 7.8	118.4 5.3
pm <i>Phrynosoma modestum</i>	5.33 0.94	50.6 3.8	76.1 5.0	66.0 3.9
pM <i>Phrynosoma mcallii</i>	12.68 2.50	72.8 3.8	109.1 4.0	92.4 3.4
Clade Mean (n = 3) s.d.	18.84 17.43	73.5 23.3	107.0 29.8	92.3 26.2

*For each trait, n = 4 individuals, except forelimb span of *Sceloporus virgatus* (n = 3).

the phylogenetically informed distributions generated by analysis of simulated data, the same picture emerges. The 95th percentiles of the distributions of F statistics for the group effect were 25.53 for %FOG and 25.03 for %FG. Only four of the simulated F statistics were greater than the real F for %FOG, and none were greater than the real F for %FG. Hence, the three subclades show highly significant differences for %FOG and %FG irrespective of type of analysis. Moreover, these differences among subclades are greater than for any other characteristics studied here, as demonstrated by the variance components reported in Table 4.

The %FG and %FOG fibers showed a very strong negative relationship (Fig. 5; $r = -0.951$, 2-tailed $P < 0.001$; $r_{ic} = -0.890$, $P < 0.001$). When the conventional correlation coefficient was compared to values for 1,000 phylogenetically simulated data under the null hypothesis of no correlation, none of the correlation coefficients (range = -0.869 to 0.872) for the simulated data was as large as the real r .

Several interesting differences in body proportions are evident in these species. Horned lizards are

heavy for their SVL (Fig. 3A), but that does not fully account for their especially short hindlimbs (Fig. 3B). The hindlimb span shows great variation and is much more variable than the forelimb span (Fig. 3C). The "chubbiness" of horned lizards also does not account for their thighs being so much smaller in cross-sectional area than the other phrynosomatids studied (Fig. 4E). Conventional ANCOVA of thigh muscle cross-sectional area indicated significant differences among the three subclades ($F = 19.2$, $P = 0.001$); when compared with simulated data, the P for subclade was 0.069.

DISCUSSION

Phrynosomatid lizards show striking differences in fiber-type composition of the iliofibularis muscle. This variation occurs almost entirely among the three subclades (see Fig. 1). The sand lizards have a high proportion of FG fibers but relatively few FOG fibers, whereas the closely related horned lizards show the opposite pattern. Members of the sister group to the sand and horned lizards, the *Sceloporus*

TABLE 3. Mean and standard deviation of muscle cross-sectional areas and histochemical traits* for 11 species of Phrynosomatidae

	Muscle cross-sectional area (mm ²)		Iliofibularis (% of thigh muscle)	% Fast-glycolytic	% Fast-oxidative glycolytic	% Slow-oxidative	Mean individual fiber area (μm ²)		
	Iliofibularis	Thigh					FG	FOG	SO
Sceloporus Group									
us <i>Uta stansburiana</i>	0.67	11.76	5.9	48.0	45.5	6.5	3,166	1,789	636
	0.18	1.92	0.7	6.6	10.0	4.0	397	220	205
sm <i>Sceloporus magister</i>	4.10	53.32	8.0	41.0	41.8	17.0	5,263	3,349	2,441
	0.79	16.25	1.6	6.8	6.3	6.6	855	629	680
su <i>Sceloporus undulatus</i>	1.32	15.82	8.3	42.7	42.7	14.6	3,973	2,450	1,150
	0.45	4.45	1.7	6.3	5.8	1.6	1,240	723	352
sv <i>Sceloporus virgatus</i>	1.55	19.17	8.0	44.2	44.5	11.4	4,015	2,871	1,508
	0.37	2.53	0.9	3.3	4.1	0.9	305	348	110
Clade Mean	1.92	25.02	7.6	44.0	43.6	12.4	4,104	2,615	1,434
(n = 4) s.d.	1.50	19.11	1.1	3.0	1.7	4.6	866	662	761
Sand Lizards									
un <i>Uma notata</i>	3.60	56.34	6.4	63.9	25.4	10.7	5,598	3,198	2,464
	1.24	7.59	1.7	8.7	4.8	3.9	1,306	500	302
cd <i>Callisaurus draconoides</i>	2.50	25.92	9.4	70.1	29.2	0.8	4,298	2,537	703
	1.09	9.05	0.8	9.6	9.6	0.4	1,759	1,164	281
cx <i>Cophosaurus texanus</i>	2.13	29.25	7.2	64.5	32.7	2.8	4,569	2,779	792
	1.04	12.19	0.9	4.9	6.3	2.0	1,853	831	132
hm <i>Holbrookia maculata</i>	0.81	13.13	6.2	65.1	32.1	2.8	2,661	1,612	701
	0.20	3.21	0.6	3.2	2.6	0.6	817	364	232
Clade Mean	2.26	31.16	7.3	65.9	29.8	4.3	4,281	2,531	1,165
(n = 4) s.d.	1.15	18.17	1.5	2.8	3.4	4.4	1,217	671	867
Horned Lizards									
pc <i>Phrynosoma cornutum</i>	3.06	36.95	8.4	25.3	66.3	8.4	5,385	3,510	2,061
	0.42	5.48	1.2	12.3	10.4	3.6	491	406	498
pm <i>Phrynosoma modestum</i>	0.70	6.66	10.5	29.5	64.3	6.2	2,635	1,845	973
	0.19	1.06	2.4	7.1	6.6	1.5	491	306	367
pM <i>Phrynosoma mcallii</i>	1.85	19.58	10.1	30.8	56.3	12.9	4,305	2,706	1,678
	0.69	10.45	1.7	9.9	13.9	5.2	1,977	898	714
Clade Mean	1.87	21.06	9.6	28.5	62.3	9.2	4,108	2,687	1,571
(n = 3) s.d.	1.18	15.20	1.1	2.9	5.3	3.4	1,386	832	552

*For each trait, n = 4 individuals.

group, contain intermediate amounts of both FOG and FG fibers (Table 2; Fig. 4A).

For all 11 species examined FG and FOG fibers comprise more than 80% of the IF, but the representation of these two fiber types is negatively related across species (Fig. 5A). However, the among-clade variation in percentage fiber-type composition is much greater than the within-clade variation, and within each subclade we see no obvious relationship between %FG and %FOG fibers. Hence, the negative relationship between %FG and %FOG is attributable entirely to the wide divergence between the sand and horned lizards (Fig. 5B).

The among-clade variation in %FG and %FOG fibers is substantially greater than for any of the other traits studied herein (Figs. 3, 4; Table 4), and is also greater than was observed for maximal sprint-running speed (Bonine and Garland, 1999) or treadmill endurance running capacity (Garland, 1994). Moreover, the differences appear greater than for home range areas (Perry and Garland, in press) or life-history traits (Pianka, 1986; Dunham et al., 1988; Clobert et al., 1998). Thus, fiber-type

composition of the IF muscle shows what may be considered a very large "phylogenetic effect" within this family of lizards. Indeed, of the traits shown in Figures 3 and 4 only %FG (and %FOG) show statistically significant differences among the three subclades when a phylogenetically based statistical analysis is performed. And from an even broader perspective, fiber-type composition as reported herein is one of the relatively few quantitative traits that has been shown to differ significantly among clades when such analyses are conducted phylogenetically (e.g., see Brashares et al. [2000] and references therein).

Some other studies of vertebrate lineages have compared multiple species with respect to fiber-type composition of a particular muscle. Among five species of distantly related mammals, variation in the fiber-type composition of many different hindlimb muscles, both among muscles and among species (Ariano et al., 1973), was only slightly greater than we have found in phrynosomatids. Among pectoralis muscles of six species of woodpecker, Tobalske (1996) found an increase in fiber-type heterogeneity

TABLE 4. Results of nested ANCOVA, with log body mass as a covariate^a for individual traits among 11 phrynosomatid species nested within three subclades (four individuals per species)

Trait	Species effect d.f. 8, 32			Subclade effect d.f. 2, 8		
	F	P	% variance	F	P	% variance
Body and limb linear dimensions						
log snout-vent length	10.30	0.0001	32.6	6.43	0.0217	53.4
log hindlimb span	46.76	0.0001	35.4	7.23	0.0161	61.5
log forelimb span ^b	33.12	0.0001	62.0	2.74	0.1244	30.2
% of fiber type in IF muscle cross-sectional area						
FG	0.37	0.9308	0 ^d	220.24	0.0001	86.8
FOG	0.68	0.7034	0 ^d	75.55	0.0001	79.0
SO ^c	5.88	0.0001	28.4	6.18	0.0239	48.3
Cross-sectional areas						
log mean FG fiber	1.12	0.3778	2.4	3.64	0.0751	16.4
log mean FOG fiber	2.23	0.0512	19.9	2.58	0.1370	15.5
log mean SO fiber	4.23	0.0015	35.0	2.74	0.1240	21.7
log IF	2.48	0.0326	23.1	2.37	0.1550	14.5
log total thigh muscle	1.56	0.1769	4.1	22.50	0.0005	66.7
IF as % of thigh muscle cross-sectional area	3.51	0.0051	29.7	3.02	0.1055	22.9

Bold indicates significant *P*-values.

^aLog body mass was a significant covariate for the eight dimension and muscle morphometric traits (all *P* < 0.0016), but for none of the four percentage traits (%FG, %FOG, %SO, IF as % of thigh; all *P* > 0.23). Results with log SVL as a covariate were qualitatively similar except for log forelimb span at the subclade level (*P* = 0.0189) and log IF area at the species level (*P* = 0.1342).

^bFor log forelimb span, only 43 individuals were measured (d.f. 8, 31 for species effect).

^c%SO was square-root transformed.

^dFor %FG and %FOG, the estimates of species variance components were actually negative (-2.5 and -1.8, respectively).

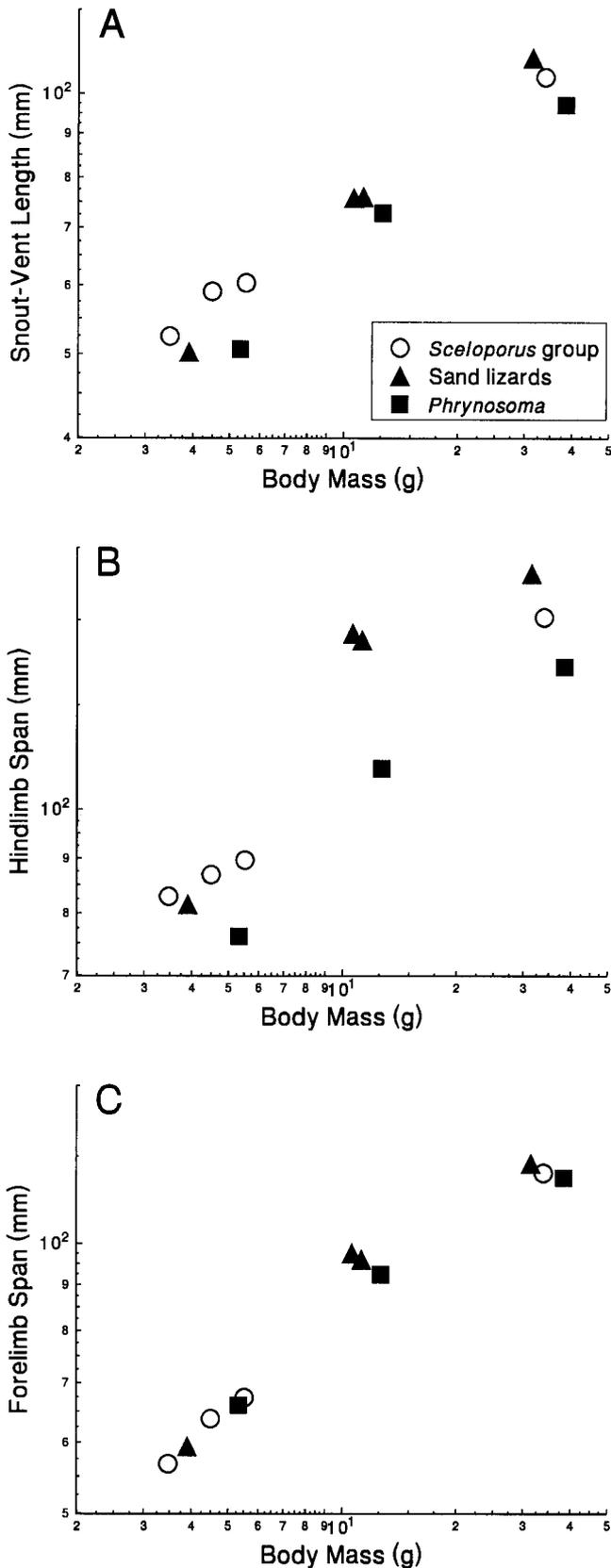
(from one to two fiber types; the fiber-type classifications were not the same as those used herein) with increasing body mass. Smaller birds had muscles with more fast-twitch oxidative area than the larger birds, which had increasing amounts of fibers approaching the FG classification. Based on his data and those of previous workers, Tobalske (1996, p. 172, and references therein) argues that the "phylogenetic effect" in muscle fiber-type composition is also strong for birds.

Previous reports of lizard IF fiber-type composition are presented in Table 1. The genera most closely related to the phrynosomatids, *Dipsosaurus*, *Iguana*, and *Agama*, all have a high proportion of FG fibers, similar to the sand lizard subclade studied herein. Comparisons with the more distantly related species are problematic because of the different methods used by other researchers and the different data reported.

The proportion of SO fibers in lizards may be positively correlated with body mass. This trend (not significant from ANCOVA with log body mass as covariate, *P* = 0.23) can be seen in our data (Fig. 4B) and from Table 1 (*Iguana* and the three *Varanus* species are much larger than any of the species studied herein). Whether this variation in %SO reflects differences among phylogenetic lineages and/or an effect of body size remains to be determined. Ontogenetically, over an almost 10-fold range in body mass, larger juvenile *Iguana iguana* had a greater area of oxidative fibers in the IF and greater IF cross sectional area than would be expected from geometric scaling principles (Gleeson

and Harrison, 1986). In a related species, *Ctenosaura similis*, mass-specific citrate synthase activity of mixed thigh muscle did not increase ontogenetically (Garland, 1984), but in an agamid lizard (*Ctenophorus nuchalis*) it showed positive allometry (Garland and Else, 1987).

Since the mid-1970s mammalian skeletal muscle has been considered to be composed of three basic fiber types (but see Schiaffino and Reggiani, 1994). The representation and distribution of these fiber types within a muscle are often predictive of its contractile and metabolic function (Gleeson et al., 1980a). Using the nomenclature of Peter et al. (1972), these fiber types are described as fast twitch-glycolytic (FG), fast-twitch oxidative glycolytic (FOG), and slow-twitch oxidative (SO). Muscles composed primarily of FG fibers are rapid-contracting but fatigue rapidly, whereas muscles composed primarily of SO fibers are of high endurance, but slow; FOG fibers confer both speed and endurance (Saltin and Golnick, 1983; Brooks et al., 1996). As in mammals, lizard muscles that function during brief, intense activity (e.g., caudifemoralis longus, white [glycolytic] iliofibularis) have enriched populations of FG fiber types. Likewise, muscles used during lower intensity activity or for extended periods of time (red [aerobic] iliofibularis, penis retractor) are composed primarily of oxidative fiber populations (Gleeson, 1983; Jayne et al., 1990). The power output of these fiber types is dependent on the frequency of the limb cycle and slow fibers are designed to produce power at slow gaits, whereas fast



fibers function optimally at frequencies corresponding to higher running speeds (James et al., 1995).

Previous researchers have assumed that the lizard IF is important in crural flexion and femoral retraction at the beginning of the propulsive stroke (e.g., Snyder, 1954; Abu-Ghalyun, 1991). However, EMG analysis revealed that the IF is recruited during the recovery phase, not when the limb is on the ground (Jayne et al., 1990). Nevertheless, increased stride frequency is an important contributor to high sprint speed in lizards (e.g., Irschick and Jayne, 1999a), and recovery after the propulsive stroke is therefore also important (Jayne et al., 1990). In *Varanus exanthematicus*, fibers in the red region (SO and FOG fibers) are used increasingly with increasing speed and their recruitment plateaus at about 1.5 km/h at 35°C (Jayne et al., 1990). The white region (predominantly FG with some FOG) is used in higher-speed locomotion, above a threshold speed (1.3 km/h; Jayne et al., 1990) equivalent to the reported maximum aerobic speed (1.2 km/h; Gleeson et al., 1980b) for *V. exanthematicus*.

Other types of vertebrate skeletal muscle fibers exist. For example, most vertebrates also possess a multiterminally innervated (and in some cases multiply innervated) fiber that has low myosin ATPase activity. These fibers may twitch or may be "tonic" in their contractile properties and they may be low oxidative, as in amphibians (Putnam and Bennett, 1983), or high oxidative, as in birds (Morgan and Proske, 1984). As reviewed in Guthe (1981), reptilian skeletal muscle seems generally to be consistent with mammalian classifications for FG and FOG fibers, but may be different with respect to SO fibers. Studies of *Dipsosaurus dorsalis* muscle used these same classifications but results indicated that SO fibers in mammals are not the same as the "tonic" fibers in this lizard (Gleeson et al., 1980a; Putnam et al., 1980). The slow fiber type in *Dipsosaurus* is highly oxidative, with high mitochondrial densities, like bird slow fibers, and is multiterminally innervated (Gleeson et al., 1984). Whether the fibers are twitch and/or tonic has never been definitively determined (Gleeson et al., 1980a; Gleeson and Johnston, 1987). Several previous researchers used the term "tonic" for these slow fibers in lizards because they assumed the IF played a static postural role (e.g., Putnam et al., 1980). However, according to EMG analysis the IF in *Varanus exanthematicus* is not used when standing on level or angled surfaces (up to 90°), whether the abdomen is held up or down (Jayne et al., 1990). However, as Carrier (1989) pointed out, slow (or tonic) muscle may function at very low frequencies—frequencies that may

Fig. 3. Bivariate scatterplots of body and limb linear dimensions in relation to body mass and subclade (see Fig. 1) for 11 species of phrynosomatid lizard, using mean values reported in Table 2.

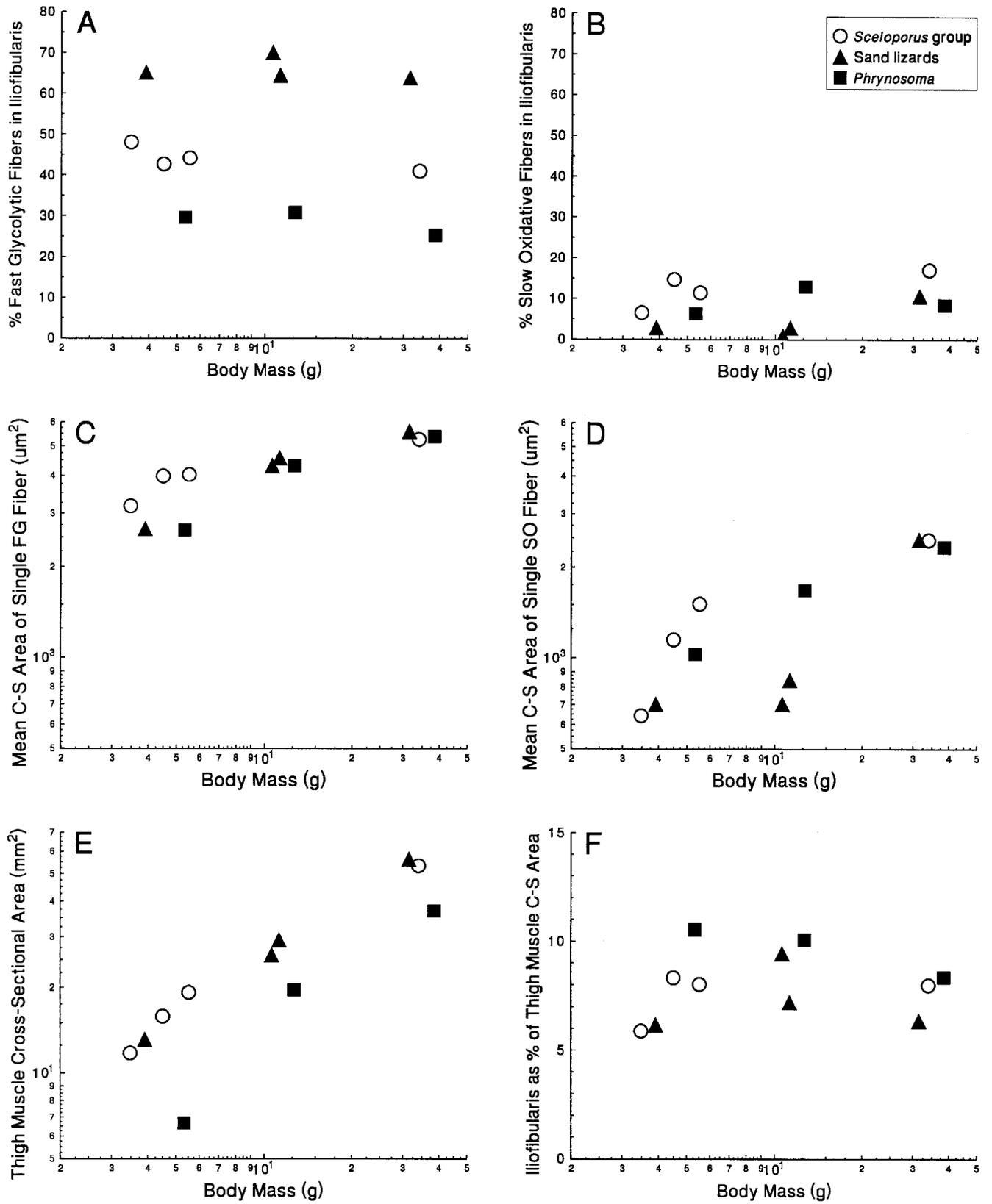


Fig. 4. Bivariate scatterplots of histochemical and muscle morphometric traits in relation to body mass and subclade (see Fig. 1) for 11 species of phrynosomatid lizard, using mean values reported in Table 3.

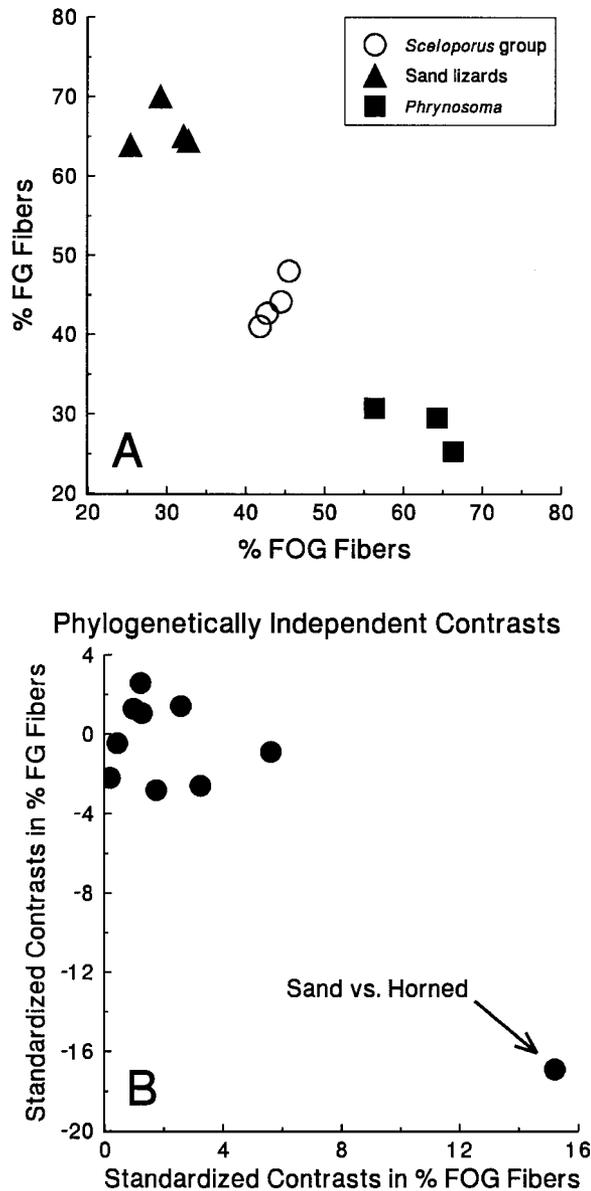


Fig. 5. **A:** Percent fast-twitch glycolytic and fast-twitch oxidative glycolytic muscle fiber-types in 11 species of phrynosomatid lizard. Across all 11 species, the correlation is $r = -0.951$ (two-tailed $P < 0.001$). **B:** Phylogenetically independent contrasts of data presented in **A**, using topology and branch lengths shown in Figure 1 (number of independent contrasts is always one less than the number of species). Correlation (computed through origin) = -0.890 ($P < 0.001$). Note that this relationship is almost entirely determined by the contrast between the sand- and horned-lizard subclades; as can also be seen from **A**, little relationship between %FG and %FOG is evident within each of the three phrynosomatid subclades.

be missed by researchers if the high-pass filter on the recording equipment is set at 80 or 100 Hz (Jayne et al. [1990] used 60 Hz for their high-pass filter). High-pass filters are used because most modern buildings have persistent noise in the 50–60 Hz range. Further study of the true characteristics of

slow lizard skeletal muscle fibers is warranted. Herein, we have chosen to use the term SO for those fibers with high SDH activity but low mATPase activity, thus adhering to the standard mammalian naming convention. We do this with the understanding that they are not strictly homologous to mammalian SO fibers. The relatively small proportion of SO fibers may be important in future studies of the mechanistic bases of lizard endurance capacity.

For these phrynosomatids we found only the occasional fiber that did not fit into the FG, FOG, SO schema and would likely be termed “tonic” (staining lightly for both mATPase and SDH), and we lumped these fibers in with the SO category. Preliminary examination of fiber types in the IF of the distantly related *Acanthodactylus* spp. (Lacertidae) indicate that at least some members of this genus have a large proportion of fibers in the medial region of the muscle that do not stain darkly for either mATPase or SDH, and thus would likely be termed “tonic.” Future work in divergent lizard families may unveil very different fiber-type characteristics for the IF across lizard taxa.

In this report, we are primarily concerned with how muscle fiber composition in a locomotor muscle may affect speed, but speed could also be affected by the size of the fibers in the IF, IF muscle area and its proportion in the thigh, or the area of all muscles in the thigh. In these phrynosomatids the mean cross-sectional area of individual fibers is significantly correlated with body mass but does not differ among subclades. However, the size of SO fibers does differ among species. In *Iguana iguana*, mean area of individual FOG and “tonic” fibers increase with body mass more than would be predicted by geometric scaling principles (Gleeson and Harrison, 1986). Body mass and individual fiber area are not correlated for *Dipsosaurus dorsalis* (Gleeson and Harrison, 1988). However, fiber size and sprint speed are negatively correlated in *D. dorsalis* (Gleeson and Harrison, 1988). Here, the mass-adjusted log cross-sectional area of the IF, and its proportion of the thigh, both vary significantly among species, but not among subclades (Table 4), so neither of these measures help explain the speed differences among members of the three subclades. The mass-adjusted total cross-sectional area of all muscle in the thigh does not vary among species, but does vary among subclades ($P = 0.069$ in phylogenetic ANCOVA)—the horned lizards may have smaller thighs and this may explain, in part, their relatively slow sprint speeds. Horned lizards are also relatively heavy for their body length (Fig. 3A). Previous studies have found that total thigh muscle mass is a significant predictor of individual differences in endurance capacity (Garland, 1984; unpublished results for *Callisaurus draconoides* and *Cnemidophorus tigris*). However, for *Ctenosaura similis* and *Ctenophorus nuchalis*, thigh muscle mass did not predict individual differences in speed (Garland, 1984, 1985). As

more distantly related species are examined, we might expect to find more variation in all of the possible traits that can influence locomotor performance.

Figure 3 shows that hindlimb span is much more variable than forelimb span, both among species and among the three subclades. Presumably, hindlimb span has been subjected to greater diversifying selection than forelimb span. Phylogenetic reconstruction techniques (e.g., Garland et al., 1999), using a broader range of lizard taxa, will be necessary to infer ancestral values of limb lengths, but we speculate that the closely related sister groups (sand + horned) have derived limb proportions, whereas the condition evident in members of the *Sceloporus* group studied herein (see also Bonine and Garland, 1999) is more similar to ancestral values for the Phrynosomatidae as a whole. We found previously that sprint speed and hindlimb span are correlated across 27 species of lizards, 17 of which were phrynosomatids (Bonine and Garland, 1999). As most of these species run bipedally at high speed, with the apparent exception of *Phrynosoma* (Irschick and Jayne, 1999a), the length of forelimbs may be more affected by other selective factors and/or stabilizing selection. For some desert lizards, including *Callisaurus draconoides*, limb length may also be affected by thermoregulatory considerations (Muth, 1977). Surprisingly, the *Holbrookia maculata* studied here have rather short hindlimbs for a sand lizard (compare Fig. 3B with fig. 3 of Bonine and Garland, 1999). The *H. maculata* studied here were from a population in Arthur County, Nebraska, whereas the longer-limbed animals in our earlier study were from a population in southeastern Arizona and southwestern New Mexico; hence, the possibility of population differences may warrant further study (Garland and Adolph, 1991; Dohm et al., 1998). Indeed, recent analyses of *H. maculata* indicate that this species may soon be divided into two species (Wilgenbusch and de Queiroz, 2000).

Variation in whole-organism functional traits, such as locomotor abilities, results from multivariate interactions of underlying morphological, physiological, and biochemical traits (e.g., Bennett, 1989; Garland and Losos, 1994; Feder et al., 2000). Results presented here indicate that muscle fiber-type composition appears to form part of a coadapted suite of traits that affect the locomotor abilities of phrynosomatid lizards (see also Bonine and Garland, 1999; Irschick and Jayne, 1999a), a closely related group of lizards that is nonetheless very diverse in ecology, behavior, and body plan. Additional focus on this group of lizards, and studies of other traits, such as gait, muscle architecture, or contractile properties, should provide unique information concerning the multiple mechanisms by which performance abilities have evolved.

ACKNOWLEDGMENTS

We thank the staff and volunteers at the AMNH Southwestern Research Station (SWRS) for logistical support, and the following researchers for assistance with animal collections: Royce Ballinger, Steve Jones, Jon Sandridge, Arjun Sivasundar, Barney Tomberlin, and Kevin and April Young. The Arizona and New Mexico Departments of Game and Fish provided scientific collecting permits. Emily Baker, Anna Hansen, Alan Peterson, and David Scholnick assisted with histochemical analyses. Paul Berry, Mike Clayton, Bill Feeny, Jeff Houser, Ray Lord, and Alan Wolf provided technical support. Hobart Smith generously provided temporary housing to KEB. Two anonymous reviewers provided helpful comments on an earlier draft.

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