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Muscle fiber-type variation in lizards (Squamata) and phylogenetic reconstruction of hypothesized ancestral states

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Summary

Previously, we found that phrynosomatid lizards, a diverse group common in the southwestern USA, vary markedly in fiber-type composition of the iliofibularis (a hindlimb muscle important in locomotion). Phrynosomatidae comprises three subclades: the closely related sand and horned lizards, and their relatives the *Sceloporus* group. The variation in muscle fiber-type composition for 11 phrynosomatid species is attributable mainly to differences between the sand- and horned-lizard subclades. Here, we expand the phrynosomatid database with three additional species and compare these results with data collected for 10 outgroup (distantly related) species. Our goal was to determine if the patterns found in Phrynosomatidae hold across a broader phylogenetic range of the extant lizards and to elucidate the evolution of muscle fiber-type composition and related traits. To allow for meaningful comparisons, data were collected from species that are primarily terrestrial and relatively small in size (3.5–65 g body mass). Results indicate that the fiber-type variation observed within the Phrynosomatidae almost spans the range of variation observed in our sample of 24 species from eight families. However, one species of *Acanthodactylus* (Lacertidae) had a consistent region of large tonic fibers (that did not stain darkly for either succinic dehydrogenase or myosin ATPase activity), a fiber-type only occasionally seen in the other 23 species examined. Many species have a large proportion of either fast-twitch glycolytic (FG; e.g. sand lizards and *Aspidoscelis*) or fast-twitch oxidative-glycolytic (FOG) fibers (e.g. horned lizards), with the slow-oxidative proportion occupying only 1–17% of the iliofibularis. Importantly, the negative relationship between FG and FOG composition observed in Phrynosomatidae appears to be a characteristic of lizards in general, and could lead to functional trade-offs in aspects of locomotor performance, as has previously been reported for Lacertidae. Reconstruction of ancestral trait values by use of phylogenetically based statistical methods indicates especially large changes in fiber-type composition during the evolution of horned lizards.

Key words: comparative method, fiber type, fiber-type composition, histochemistry, Phrynosomatidae, phylogeny, skeletal muscle.

Introduction

Most evolutionary biologists agree that it is desirable to begin comparative studies with a lineage of organisms that displays adequate diversity in the traits of interest, yet is closely enough related that one is not comparing ‘apples and oranges’ or ‘chalk and cheese’. Phrynosomatid lizards fit this bill. They are a phenotypically and ecologically diverse group of approximately 125 species (Pough et al., 2001) found in much of North and Central America, yet are closely related (e.g. Reeder and Wiens, 1996). The family comprises three subclades: the sand lizards, the horned lizards, and their relatives in the *Sceloporus* group (Fig. 1). Within a range of body sizes less than 50 g, primarily terrestrial (as opposed to arboreal or saxicolous) phrynosomatid species exhibit a diversity of locomotor performance abilities (Garland, 1994; Miles, 1994; Bonine and Garland, 1999; Irschick and Jayne, 1999) and morphologies (e.g. relative limb length) that almost matches the range found among species of several other sympatric lizard families (e.g. Bonine and Garland, 1999; Irschick and Jayne, 1999). The Phrynosomatidae also exhibit large variation in behavior and ecology (Stebbins, 1985; Conant and Collins, 1991), much of which occurs among the three subclades rather than among species within the subclades.

In a previous study of 11 phrynosomatid species, we found that the composition of muscle fiber-type proportions in the iliofibularis (IF; a hindlimb abductor important in locomotion) differs markedly among the three subclades (Bonine et al., 2001). The proportion of fast-twitch glycolytic (FG) *versus* fast-twitch oxidative-glycolytic (FOG) fibers, when considering the cross-sectional area of the IF, are strongly

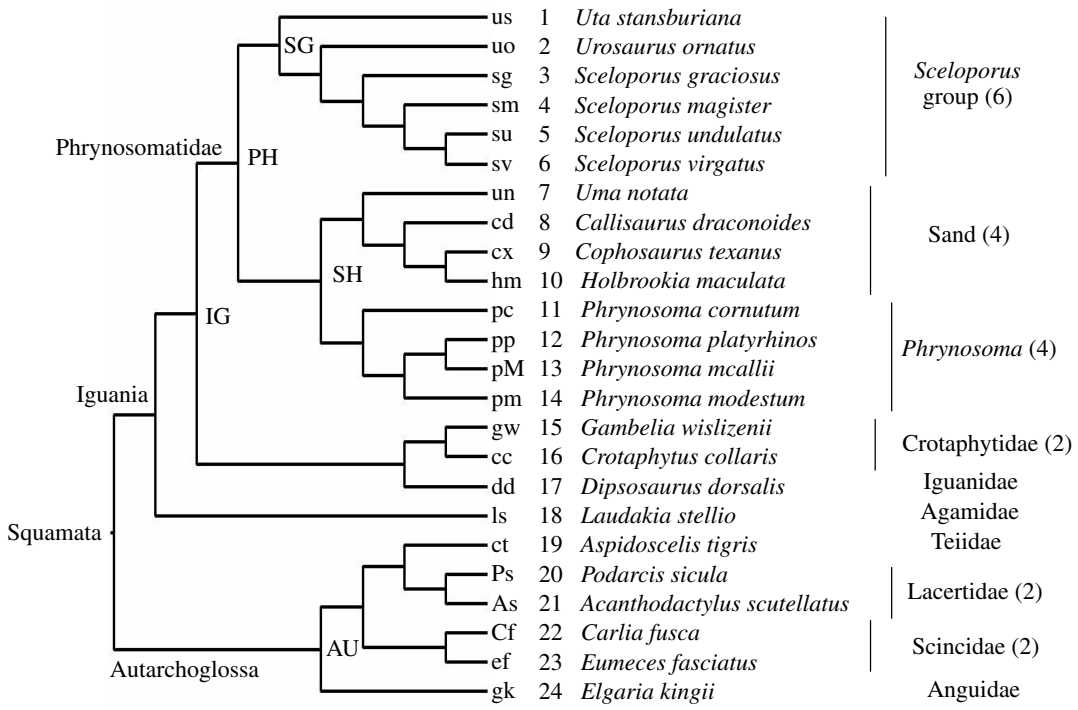

Fig. 1. Hypothesized phylogenetic relationships for 24 species of lizard examined in this study. See phylogeny section in the Materials and methods for explanation. Branch lengths are arbitrary (as suggested by Pagel, 1992).

negatively related. The sand lizards have a high proportion (64–70%) of FG fibers whereas the horned lizards have a high proportion (56–66%) of FOG fibers. The mean proportion of slow-oxidative (SO) fibers varies from less than 1% to 17% of the IF muscle cross-sectional area. The *Sceloporus* group, sister to the sand and horned lizard subclades, has intermediate proportions of FG and FOG fibers. The observed variation in the ratio of FG and FOG fiber-type proportions is driven by differences between the sand and horned lizard subclades, rather than by differences within each of the three phrynosomatid subclades. Here, we examine whether this negative relationship holds when considering a broader sample of lizard taxa. If so, then it could account for trade-offs in locomotor abilities, such as the negative relationship between speed and stamina reported for Lacertidae (Vanhooydonck et al., 2001). Additionally, we discuss the evolutionary patterns of iliofibularis muscle composition in our sample of 24 species, representing eight lizard families.

We analyzed the iliofibularis (IF), a hindlimb muscle that has many characteristics conducive to comparative study. It is parallel-fibered, or unipennate, and 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 and Dalessio, 1990; Gleeson et al., 1980; Putnam and Bennett, 1982; Gleeson, 1983; Gleeson et al., 1984; Johnston and Gleeson, 1984; Gleeson and Harrison, 1986; Gleeson and Johnston, 1987; Mutungi, 1990; Mirwald and Perry, 1991; see references in table 1 of Bonine et al., 2001). For several lizard species the IF muscle has been characterized for fiber-type composition (see Bonine et al., 2001 and references therein) and for fiber-type recruitment patterns (Jayne et al., 1990). In *Varanus exanthematicus*, the Savannah 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 a threshold speed and with often irregular activity (Jayne et al., 1990). Moreover, this threshold speed is consistent with the maximal aerobic speed of *V. exanthematicus*, indicating an important role for the red region during sustained, aerobic activity, and increased recruitment of predominantly anaerobic fibers of the white region at higher speeds (Jayne et al., 1990). A logical prediction from these data would be that species with higher aerobic locomotor capacities should have more red fibers, whereas species with greater anaerobic burst capabilities should have a greater proportion of white fibers. The IF is active during the recovery phase of hind-limb cycling and therefore could be rate-limiting in stride frequency during sprinting, or partly determine fatigue resistance during sustained locomotor bouts.

Within the lizard species we studied, many use bipedalism frequently and some, like the horned lizards (*Phrynosoma*), are not known to use bipedalism at all. Because we studied the IF, a muscle found only in the hindlimb, quadrupedal *versus* bipedal locomotion may be important. However, data on

locomotor abilities within species that are known to run using both two and four limbs indicate that bipedalism does not confer a speed advantage relative to quadrupedalism (Irschick and Jayne, 1999). We, therefore, believe detailed examination of the IF is relevant for all the species included herein to initiate detailed comparison of muscle morphology and physiology across a broad taxonomic range, especially in the context of potential explanatory relationships for variation in locomotor performance abilities.

Here, we report data for 14 phrynosomatid species [11 first reported by Bonine et al. (2001) and an additional three] as well as 10 species from other lineages (outgroups). This allows us to test the hypothesis that the negative FG–FOG relationship in the IF is a general characteristic of lizards (ignoring snakes, which lack IF muscles). We also use phylogenetic methods to estimate ancestral states of muscle-fiber composition, and then test whether large evolutionary changes (high rates of evolution along particular phylogenetic branches) have occurred within the Phrynosomatidae. For the latter analyses, we employ recently developed methods that incorporate information on within-species variation (Garland et al., 2004).

Materials and methods

Species examined

We examined the following 24 species. Taxonomic authority (as cited in Crother, 2000 and elsewhere) is given in parentheses. Tip number and species code as in Fig. 1.

Iguania

Phrynosomatidae
1 us. *Uta stansburiana* (Baird and Girard, 1852)
2 uo. *Urosaurus ornatus* (Baird and Girard, 1852)
3 sg. *Sceloporus graciosus* (Baird and Girard, 1852)
4 sm. *Sceloporus magister* (Hallowell, 1854)
5 su. *Sceloporus undulatus* (Bosc and Daudin in Sonnini and Latrielle, 1801)
6 sv. *Sceloporus virgatus* (Smith, 1938)
7 un. *Uma notata* (Baird, 1859 “1858”)
8 cd. *Callisaurus draconoides* (Blainville, 1835)
9 cx. *Cophosaurus texanus* (Troschel, 1852 “1850”)
10 hm. *Holbrookia maculata* (Girard, 1851)
11 pc. *Phrynosoma cornutum* (Harlan, 1825)
12 pp. *Phrynosoma platyrhinos* (Girard, 1852)
13 pM. *Phrynosoma mcallii* (Hallowell, 1852)
14 pm. *Phrynosoma modestum* (Girard, 1852)
Crotaphytidae
15 gw. *Gambelia wislizenii* (Baird and Girard, 1852)
16 cc. *Crotaphytus collaris* (Say, 1823)
Iguanidae
17 dd. *Dipsosaurus dorsalis* (Baird and Girard, 1852)
18 ls. *Laudakia stellio* (Linnaeus, 1758)

Autarchoglossa

Teiidae
19 ct. *Aspidoscelis tigris* (Baird and Girard, 1852)
Lacertidae
20 Ps. *Podarcis sicula* (Rafinesque, 1810)
21 As. *Acanthodactylus scutellatus* (Boulenger, 1918)
Scincidae
22 Cf. *Carlia fusca* (Dumeril and Bibron, 1839)
23 ef. *Eumeces fasciatus* (Linneaus, 1758)
Anguidae
24 gk. *Elgaria kingii* (Gray, 1838)

Animal collection

To avoid complications from comparing widely divergent locomotor modes, we focused on species that are largely terrestrial (as opposed to saxicolous or arboreal) and occur in arid or semi-arid habitats. All lizard species included in this study are diurnal, and the majority are insectivorous (Stebbins, 1985; Conant and Collins, 1991). The two representative crotaphytids are carnivorous, whereas *Dipsosaurus dorsalis* (Iguanidae) is primarily herbivorous (Stebbins, 1985). To avoid possible sex and ontogenetic differences, we studied only adult males.

In 1996 and 1997, we collected lizards from populations in southern Arizona and western New Mexico while based at the Southwestern Research Station (SWRS) near Portal, Arizona, USA. In 1999, lizards were captured in the field from targeted populations throughout the United States, Guam and Israel, and shipped alive to Madison, Wisconsin, USA. We restricted animal collections from these northern-hemisphere localities to late May through to early August because of potential seasonal differences in metabolism and physiology (e.g. Garland and Else, 1987). Individuals of a given species (with a few unavoidable exceptions) were collected from a restricted geographic area because populations may differ in physiological characteristics (Garland and Adolph, 1991). During captivity, we kept individual lizards isolated in either plastic containers or cloth bags (depending on size), with periodic access to water but no food. Individuals were sacrificed within 2 weeks of capture. Variation in time in captivity prior to sacrifice was randomly associated with species and lineage, and preliminary analyses indicated no apparent relationship between time in captivity and fiber-type traits. Furthermore, attempts to train lizards have not been successful (Gleeson, 1979; Garland and Else, 1987; A. Szucsik, personal communication), suggesting that detraining is also unlikely. Relevant animal care and use committee protocols were followed in Boulder, Madison, and at SWRS.

Morphometrics

In addition to quantifying muscle morphology of the iliofibularis, we measured morphometric traits for all animals. 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. In order to include variation in the width of the pelvic and pectoral girdles among species, our measure of limb span was from toe-tip to toe-tip (excluding the nail) with each limb held perpendicular to the axis of the body in the same lateral plane

as the body (Garland, 1984, 1985; Bonine and Garland, 1999). Limb and body proportions were measured to the nearest 0.5 mm using a clear plastic ruler.

Tissue preparation and histochemical analyses

Preparation of tissues and histochemical analyses were as described in detail by Bonine et al. (2001). Briefly, in accordance with established protocols and guidelines for physiological research, lizards were decapitated after we warmed them to their approximate field-active body temperature. Warming animals prior to sacrifice served three functions: (1) facilitated collection of blood quickly and in sufficient quantities; (2) allowed for examination of physiological variables under more ecologically relevant conditions; and (3) facilitated comparison with previous research using similar protocols. Each hindlimb was quickly removed intact along with a portion of the pelvis. Limbs were mounted 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. In 1996 and 1997, animals were shipped alive from SWRS to T. T. 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.

Serial, 10 μm-thick sections from frozen limbs were taken at mid-thigh and used for histochemical identification of fiber types (e.g. Putnam et al., 1980; Gleeson, 1983; Gleeson and Harrison, 1986, 1988; Garland et al., 1995; Bonine et al., 2001). Histochemical activities of alkaline-stable myosin ATPase and succinic dehydrogenase/NADH diaphorase (SDH) were used to identify fibers as slow-twitch 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). For further comments on fiber-type terminology and comparison with 'the mammalian standard' see the discussion in Bonine et al. (2001).

After incubation, sections were mounted on microscope slides and magnified images were digitized. The IF, which is located posteriorly and dorsally in the hindlimb, was identified in each cross section, and images of both mATPase- and SDH-stained cells were simultaneously compared to determine muscle fiber type. We counted fibers of each type and measured fiber cross-sectional areas by tracing the perimeter of each cell in both the oxidative and non-oxidative 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 approximately 60–100% of the fibers in the oxidative region (this appears red in fresh tissue) located medially. For the lateral and more homogeneous white portion of the muscle, we measured approximately 40–90% of the fibers. We assumed that the remainder of a given region comprised similarly sized fibers in the same relative proportion of fiber types. On average, we measured 439 fibers per individual. Cross-sectional areas were measured for four individuals of each species using the mATPase images (not the SDH images) to control for variation in cell deformation caused by the two different histochemical procedures. To assess the relative size of the iliofibularis muscle, whole-thigh and iliofibularis muscle areas were also measured by tracing digitized, lower-magnification mATPase images. All measurements were made using NIH Image (version 1.62) software.

Literature values

Data on iliofibularis fiber-type composition are available for several species (see references in table 1 of Bonine et al., 2001). Unfortunately, many of these data are not comparable because the same morphometric traits are not consistently reported (e.g. *Agama agama*; Abu-Ghalyun et al., 1988; *Gekko gecko*; Mirwald and Perry, 1991). Appropriate data for *Iguana iguana* are from juveniles (Gleeson and Harrison, 1986), and significant ontogenetic changes can occur in lizard muscle [e.g. in *Ctenosaura similis*, thigh lactate dehydrogenase (LDH) activity increases ontogenetically (Garland, 1984), in *Amphibolurus nuchalis*, thigh LDH and citrate synthase activities increase ontogenetically (Garland, 1985)]. Other comparable data are for much larger lizards (*Varanus* spp.; Mutungi, 1990; Gleeson, 1983) than those studied herein, or for species that are not typically terrestrial (*Chamaeleo* spp.; Abu-Ghalyun et al., 1988; Mutungi, 1992). Hence, the only literature data we include in our current analyses are for *Dipsosaurus dorsalis* (mean values for 20 individuals; from Gleeson and Harrison, 1988). As some of the morphometric traits we measured, specifically hindlimb span and forelimb span, were not reported by Gleeson and Harrison (1988), we calculated regressions on body mass for these variables from a set of nine different *D. dorsalis* individuals (Garland, unpublished data) and then calculated, from the regression equation, the hindlimb and forelimb spans at the mean body mass reported by Gleeson and Harrison (1988). Overall, the analyses and results are restricted to comparable data (generated from the same laboratory) for adult male lizards of 65 g or less with similar locomotor modes (primarily terrestrial).

Phylogeny

We attempted to sample broadly among extant lizards, while restricting species studied to those available that we felt would provide meaningfully comparable results (i.e. among small, terrestrial lizards). The included species span much of the range of phylogenetic diversity of lizards, yet are reasonably similar to the Phrynosomatidae in terms of body size and ecology, thereby removing some of the complications that would be inherent in comparing fossorial, or strictly arboreal species, with terrestrial ones (cf. discussion in Garland et al., 1997). Direct comparisons with snakes or other limbless

lizards is not feasible given the absence of the IF muscle in these groups. Furthermore, their limbless mode of terrestrial locomotion is not comparable to limbed locomotion in the terrestrial lizards on which we focused. However, we did include two species of Scincidae and one species of Anguidae; both groups that have repeatedly lost limbs in several lineages (e.g. Pough et al., 2001; Wiens and Slingluff, 2001).

The evolutionary hypothesis presented here (Fig. 1) is based on recent and/or comprehensive available phylogenies (see also Perry and Garland, 2002). The large-scale tree topology follows Estes et al. (1988), rather than the single-gene-based analysis of Harris et al. (1999). Within Iguania, we followed the family-rich nomenclature of Frost and Etheridge (1989), although some relationships were clarified by Wiens and Hollingsworth (2000) [iguanids (e.g. *Dipsosaurus*) basal within Iguania], Schulte et al. (1998; agamid placement within Iguania) and Macey et al. (1997; crotaphytids sister to phrynosomatids). 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 is represented here by species of *Urosaurus*, *Uta* and *Sceloporus*; 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 the synthetic analysis of both mitochondrial DNA and morphology of Reeder and Montanucci (2001). Importantly, the sand lizards and horned lizards are sister clades, yet exhibit remarkable differences in general ecology, locomotor and antipredator behavior (Norris, 1958; Sherbrooke, 1981; Dial, 1986; Middendorf and Sherbrooke, 1992; Bulova, 1994), sprint speed, relative hindlimb length (Bonine and Garland, 1999) and muscle fiber-type composition (Bonine et al., 2001). For the other half of the lizard tree, within Autarchoglossa, the placement of teiids, lacertids, scincids and anguids is based, again, on Estes et al. (1988). As information on divergence times or some other common metric was generally unavailable, we used arbitrary branch lengths as described below.

Statistical analyses

In general, estimates of mean trait values for species cannot be considered to represent statistically independent data points because of differing amounts of shared phylogenetic history. Therefore, we employed the method of Felsenstein (1985) of phylogenetically independent contrasts, which is well understood and mathematically equivalent to generalized least squares approaches in the applications employed here (Garland et al., 1999; Garland and Ives, 2000; Rohlf, 2001).

We used arithmetic mean values for each species to compute independent contrasts (always one less than the number of tip species included). Species' mean values of morphometric traits were $\log_{10}$ transformed (except for total thigh cross-sectional area, which was square-root transformed) prior to analyses, but proportion traits were not. For computing contrasts, we originally considered many arbitrary branch lengths, including Nee's (Purvis, 1995), those described below, and various modifications of each. After checking diagnostic plots (Garland et al., 1992; Diaz-Uriarte and Garland, 1998) of the absolute values of standardized contrasts versus their standard deviations (square roots of sums of corrected branch lengths), three different branch lengths were used for different traits in order to satisfy assumptions of the independent contrasts analyses. Pagel's arbitrary branch lengths (Pagel, 1992), as shown in Fig. 1, were used for proportions of each of the three fiber types in the IF, log total cross-sectional areas of SO and FOG, the percentage of the IF area consisting of oxidative fibers, and the proportion of the thigh consisting of IF muscle; Pagel's modified by Grafen's (Grafen, 1989) rho [rho is a parameter that transforms branch lengths by altering the depths of the nodes relative to the tips of a phylogenetic tree. In the present case, a rho of 0.5 pulls the nodal depths down towards the root of the tree, thus making the overall tree less hierarchical (more like a star)] with a value of 0.5 were used for log hindlimb span, log forelimb span, and for log total cross-sectional area of FG; constant (all=1) branch lengths were used for log body mass, log snout-vent length, the log of mean individual fiber size for each fiber type, log IF cross-sectional area, and the square root of total thigh cross-sectional area. A recent review paper with worked examples (Garland et al., 2005), as well as the references cited above, provides a more detailed description of the use of independent contrasts, including selection of branch lengths.

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). Contrasts were analyzed either within PDTREE (available on request from T.G.) or exported to a conventional statistical program (e.g. Statistical Package for the Social Sciences; SPSS, Inc., Chicago, IL, USA) for analysis by correlation or regression through the origin. We used α=0.05 as the critical value in all statistical tests.

Ancestor reconstruction

Hypothetical ancestral trait values at nodes within a phylogeny can be estimated while computing independent contrasts via a rerooting procedure (Garland et al., 1999; see Johnston et al., 2003 for an example). The values calculated via this method in PDTREE are identical to those calculated using generalized least squares models or the methods of Schluter et al. (1997). We used the program PDTREE to calculate ancestral values and 95% confidence intervals at the root of our entire 24-species tree, at the base of the phrynosomatids, and at the base of the *Sceloporus* group for the two most variable fiber-type proportions, FG and FOG.

In an additional analysis, we included the standard error for each species' mean value (as calculated from the four individuals measured per species) to assess the effect of intraspecific variation on estimated nodal reconstruction values. Using the DOS-based computer program PD_SE

(Garland et al., 2004), we altered the lengths of terminal (tip) branches to reflect relative certainty of the species' mean values in relation to their standard errors. These trees with altered branch lengths were then entered back into PDTREE for computing ancestral values.

Results

Descriptive statistics for body, limb and gross muscle dimensions for each species are presented in Table 1. Species' mean body masses for this sample range from 3.5 g [*Uta* (us) and *Urosaurus* (uo)] to 65 g [*Dipsosaurus* (dd)] and snout–vent lengths (SVL) range from 50 mm [*Holbrookia* (hm)] to 122 mm [*Dipsosaurus* (dd)]. Hindlimb spans (HLS) range from 62 mm [*Elgaria* (gk)] to 194 mm [*Crotaphytus* (cc)] and forelimb spans (FLS) range from 51 mm [*Carlia* (Cf)] to 138 mm [*Laudakia* (ls)]. The cross-sectional area of the thigh muscle ranges from 6.5 mm^2 [*Carlia* (Cf)] to 71 mm^2 [*Laudakia* (ls)]; however, we do not have muscle size data for *Dipsosaurus* (dd; see Table 1), our heaviest species. The cross-sectional area of the iliofibularis (IF) ranges from 0.5 mm^2 [*Carlia* (Cf)] to 7.3 mm^2 [*Laudakia* (ls)]. *Carlia* (Cf) and *Laudakia* (ls) often defined the ends of morphometric ranges for our sample of 24 species.

Within each IF muscle, we found an oxidative core and a fast twitch-glycolytic perimeter, as has also been found in all other species of lizards that have been examined (see references in table 1 of Bonine et al., 2001). 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. Descriptive statistics for IF morphometric and histochemical data are presented in Table 2. The percentage of the thigh that is IF ranges from 5.3% [*Gambelia* (gw)] to 12% [*Aspidoscelis* (ct), formerly *Cnemidophorus*; Crother et al. (2003)]. Within the IF, the percentage of white muscle ranges from 43% [*Phrynosoma modestum* (pm)] to 82% [*Aspidoscelis* (ct)], whereas the percentage of red-oxidative muscle ranges from 18% to 57% (the same two species defining the ends of the spectrum). FG and FOG fibers made up the largest percentage of the IF muscle cross-sectional area for all 92 individuals. The percentage of FOG and FG fibers was also the most variable. The total cross-sectional area of FG fibers in the IF ranges from 0.2 mm^2 [*P. modestum* (pm)] to 2.7 mm^2 [*Laudakia* (ls)], whereas the percentage of the IF that is FG fibers ranges from 22% [*P. platyrhinos* (pp)] to 76% [*Aspidoscelis* (ct)]. Members of the sand lizard subclade had percentages of FG between 64% and 70%; *Dipsosaurus* (dd) had 71% FG. For FOG fibers, the total area in the IF ranges from 0.2 mm^2 [*Carlia* (Cf)] to 3.3 mm^2 [*Laudakia* (ls)] with the percentage of the IF that is FOG fibers ranging from 22% [*Aspidoscelis* (ct)] to 72% [*P. platyrhinos* (pp)]. Among subclades, FOG fiber percentage was highest in the horned lizards (56%–72%). The SO fiber total area in the IF ranges from 0.02 mm^2 [*Callisaurus* (cd) and *Holbrookia* (hm)] to 1.27 mm^2 [*Laudakia* (ls)] and the percentage of the IF that is SO fibers ranges from <1% [*Callisaurus* (cd)] to 17% [*Sceloporus magister* (sm) and *Laudakia* (ls)]. The maximum and minimum species' mean fiber cross-sectional areas for each of the three fiber types range from FG: 2128–9111 μm^2 [*Podarcis* (Ps) and *Dipsosaurus* (dd), respectively]; FOG: 1318–6013 μm^2 [*Aspidoscelis* (ct) and *Laudakia* (ls), respectively]; SO: 516–4983 μm^2 [*Gambelia* (gw) and *Laudakia* (ls), respectively]. Note that one of our largest species, *Gambelia* (gw), had the smallest SO fibers. Mean values of morphometric and histochemical variables for each clade are also presented in Tables 1 and 2.

Table 3 contains additional details of individual muscle-fibers, including mean sample sizes examined per species. We have published some of these data previously (for 11 phrynosomatid species; Bonine et al., 2001). We present the data again here for completeness, and with minor adjustments as noted in the footnotes of Table 3.

Occasionally, we found fibers that did not stain darkly for either mATPase or SDH [10 of 92 individuals; less than 1% in one individual each of *Uta* (us), *Cophosaurus* (cx), *Phrynosoma cornutum* (pc), *Laudakia* (ls); 3% in one individual *P. modestum* (pm); a second individual of *P. cornutum* (pc) was 6% tonic, but this may be a result of unusually light staining overall for that individual]. Fibers with these staining characteristics have been termed 'tonic' by previous researchers. Because we only rarely found these fibers, because their diameter was consistent with SO fiber size, and because their twitch properties are undetermined, we grouped these fibers in with the SO category for analyses. The *Acanthodactylus* are an exception.

All four individual *Acanthodactylus* (As) examined had a large proportion of different fibers that should be classified as tonic fibers. These fibers are located medially, in the oxidative region of the muscle, but were very large and did not stain darkly for either mATPase or SDH. On average, 12.0% of the IF cross-sectional area was tonic in *Acanthodactylus* (As) and 3.2% was SO fiber type. The oxidative proportion of the IF, the SO proportion of the IF, and the total SO cross-sectional area reported (Table 2) include both tonic and SO fibers for the *Acanthodactylus*, as these two fiber types should have similar contractile characteristics as compared to the fast contracting FG or FOG fibers. However, these tonic fibers are not included in calculation of the mean individual SO fiber cross-sectional area (Tables 2 and 3). For each individual, we measured a mean of 39.5 of these tonic fibers and report a mean size (±S.D.) of 4404±544 μm^2. Almost all fibers (of all types) in the oxidative region were measured in these four individuals.

Refer to Fig. 2 for bivariate scatterplots of morphometric variables with body mass. Clear relationships with body mass are evident for SVL, HLS and FLS. However, species such as *Callisaurus* (cd; long limbs), *Eumeces* (ef; short limbs), and *Elgaria* (gk; elongate body, short limbs) are consistently different from the other species examined. The ratio of HLS:FLS does not have a clear relationship with body mass. However, the total range of variation of this metric is bounded by non-phrynosomatid species. Among phrynosomatids, the

Table 1. *Mean (± S.D.) linear body and limb dimensions, and cross-sectional muscle areas for 14 species of Phrynosomatidae and 10 from other lineages*

Species	Body mass (g)	Snout–vent length (mm)	Hindlimb span (mm)	Forelimb span (mm)	Total thigh muscle (mm^2)	Iliofibularis muscle (mm^2)
1 us *Uta stansburiana*	3.5±0.6	52±4	83±3	57±2	11.8±1.9	0.7±0.2
2 uo *Urosaurus ornatus*	3.5±0.4	52±2	72±2	56±1	11.7±1.9	0.9±0.1
3 sg *Sceloporus graciosus*	7.5±2.0	66±4	100±8	73±7	23.1±6.6	1.8±0.5
4 sm *Sceloporus magister*	34.0±11.9	105±11	151±11	120±9	53.3±16.2	4.1±0.8
5 su *Sceloporus undulatus*	4.5±0.3	59±2	87±1	64±1	15.8±4.5	1.3±0.5
6 sv *Sceloporus virgatus*	5.5±0.9	60±2	90±2	67±2	19.2±2.5	1.6±0.4
Sceloporus group subclade mean ± S.D. (*N*=6)	9.8±12.0	66±20	97±28	73±24	22.5±15.7	1.7±1.2
7 un *Uma notata*	31.5±3.1	110±6	166±7	123±5	56.3±7.6	3.6±1.2
8 cd *Callisaurus draconoides*	10.6±6.2	76±11	146±18	98±12	25.9±9.0	2.5±1.1
9 cx *Cophosaurus texanus*	11.3±3.1	76±7	144±12	96±7	29.3±12.2	2.1±1.0
10 hm *Holbrookia maculata*	3.9±1.0	50±3	82±2	59±2	13.1±3.2	0.8±0.2
Sand lizard subclade mean ± S.D. (*N*=4)	14.3±11.9	78±25	134±36	94±26	31.2±18.2	2.3±1.2
11 pc *Phrynosoma cornutum*	38.5±7.9	97±9	136±8	118±5	37.0±5.5	3.1±0.4
12 pp *Phrynosoma platyrhinos*	17.4±6.0	77±8	109±7	94±8	27.2±5.8	2.8±0.7
13 pM *Phrynosoma mcallii*	12.7±2.5	73±4	109±4	92±3	19.6±10.4	1.9±0.7
14 pm *Phrynosoma modestum*	5.3±0.9	51±4	76±5	66±4	6.7±1.1	0.7±0.2
Horned lizard subclade mean ± S.D. (*N*=4)	18.5±14.2	74±19	108±24	93±21	22.6±12.8	2.1±1.1
Phrynosomatidae family mean ± S.D. (*N*=14)	13.6±12.2	72±20	111±32	84±24	25±15	2.0±1.1
15 gw *Gambelia wislizenii*	16.7±4.9	89±7	147±6	90±4	35.7±9.3	1.9±0.3
16 cc *Crotaphytus collaris*	39.2±5.6	108±3	194±4	116±3	68.3±11.5	3.8±0.8
17 dd *Dipsosaurus dorsalis*	65±9.4	122±5	188	117	–	–
18 ls *Laudakia stellio*	51.4±15.4	110±9	187±11	138±6	71±14	7.3±2.1
Non-phrynosomatid iguania mean ± S.D. (*N*=4*)	43.1±20.5	107±14	179±22	115±20	58.3±19.6	4.3±2.7
Iguania clade mean ± S.D. (*N*=18†)	20.1±18.7	80±24	126±41	91±26	30.9±20.1	2.4±1.7
19 ct *Aspidoscelis tigris*	10.4±5.7	82±13	125±17	75±10	24.6±9.6	3.0±1.2
20 Ps *Podarcis sicula*	3.8±1.3	59±5	73±6	52±5	8.9±3.1	0.7±0.2
21 As *Acanthodactylus scutellatus*	7.6±0.8	73±1	98±1	63±1	18.4±2.1	1.7±0.2
22 Cf *Carlia fusca*	3.5±0.4	59±2	65±1	51±0	6.5±0.3	0.5±0.1
23 ef *Eumeces fasciatus*	5.9±1.8	70±6	64±4	53±3	10.7±2.6	0.7±0.2
24 gk *Elgaria kingii*	10.0±4.0	93±10	62±6	55±6	7.2±1.5	0.6±0.1
Autarchoglossa clade mean ± S.D. (*N*=6)	6.9±3.0	73±13	81±25	58±9	12.7±7.2	1.2±1.0

*N=3, †N=17 where *Dipsosaurus* data are absent.

The two-letter code preceding each species' name allows for easy reference to species' mean values in other figures and tables. Of the first 14 species listed (representatives of Phrynosomatidae), *Urosaurus ornatus* (uo), *Sceloporus graciosus* (sg) and *Phrynosoma platyrhinos* (pp) are new data; the other 11 phrynosomatids were first reported in Bonine et al. (2001).

For all species, except *Dipsosaurus dorsalis*, N=4 individuals per species for all variables, except N=3 for forelimb span of *Sceloporus virgatus* and for forelimb and hindlimb span of *Eumeces fasciatus*. Muscle cross-sectional area data for *Dipsosaurus* were not collected. The body mass and SVL reported here are from Gleeson and Harrison, 1988 (N=20). The *Dipsosaurus* hindlimb and forelimb span values are antilogs of values calculated from regressions of $\log_{10}$ limb span on $\log_{10}$ body mass for nine individuals (body mass range 17.7–83.7 g) measured by T. Garland (unpublished); $\log_{10}$ HLS=1.90+0.21($\log_{10}$ body mass), r^2=0.91; $\log_{10}$ FLS=1.62+0.25($\log_{10}$ body mass), r^2=0.92.

horned lizards have low HLS:FLS ratios and the sand lizards have relatively high ratios. Fig. 3 shows the range of variation in the proportion of FG fibers in the IF cross-sectional area across the phylogenetic sample we studied. The range of variation across all 24 species is almost the same as that found just within Phrynosomatidae; only *Dipsosaurus* (dd) and *Aspidoscelis* (ct) exceed the FG fiber proportion found in the sand-lizard subclade of Phrynosomatidae. Fig. 4 shows the relationship between body mass and the size and proportion of each of the three fiber types. The proportion of the iliofibularis composed of a given fiber type does not vary with body mass; however, the size of individual fibers does increase with body mass. Note that the horned lizards have a large proportion of FOG fibers in the IF (Fig. 4C). The proportion of IF composed

Table 2. *Mean (± S.D.) iliofibularis muscle data for 14 species of Phrynosomatidae and 10 more distantly related species*

Species code	IFprop thigh	wtprop	oxprop	Fast glycolytic		Fast-oxidative glycolytic		Slow oxidative		Cross-sectional area of individual fibers		
				area (mm^2)	propIF	area (mm^2)	propIF	area (mm^2)	propIF	FG (μm^2)	FOG (μm^2)	SO (μm^2)
1 us	0.06±0.01	0.61±0.09	0.39±0.09	0.34±0.12	0.48±0.07	0.31±0.07	0.46±0.10	0.05±0.03	0.07±0.04	3166±397	1789±220	642±206
2 uo	0.08±0.02	0.51±0.06	0.49±0.06	0.41±0.06	0.45±0.02	0.37±0.06	0.41±0.03	0.13±0.03	0.14±0.02	4182±412	2351±353	1214±238
3 sg	0.08±0.01	0.59±0.05	0.41±0.05	0.85±0.21	0.49±0.06	0.73±0.24	0.41±0.06	0.19±0.07	0.11±0.02	4449±1266	2840±707	1417±330
4 sm	0.08±0.02	0.50±0.09	0.50±0.09	1.68±0.44	0.41±0.07	1.72±0.47	0.42±0.06	0.69±0.24	0.17±0.07	5263±855	3349±629	2441±680
5 su	0.08±0.02	0.60±0.13	0.40±0.12	0.58±0.27	0.43±0.06	0.55±0.14	0.43±0.06	0.19±0.07	0.15±0.02	3973±1240	2450±723	1150±352
6 sv	0.08±0.01	0.55±0.05	0.45±0.05	0.68±0.11	0.44±0.03	0.70±0.23	0.45±0.04	0.18±0.03	0.11±0.01	4015±305	2871±348	1508±110
Sceloporus group subclade mean ± S.D. (N=6)												
	0.08±0.01	0.56±0.05	0.44±0.05	0.76±0.49	0.45±0.03	0.73±0.51	0.43±0.02	0.24±0.23	0.12±0.04	4175±685	2608±535	1395±594
7 un	0.06±0.02	0.67±0.08	0.33±0.08	2.37±1.11	0.64±0.09	0.88±0.16	0.25±0.05	0.35±0.06	0.11±0.04	5598±1306	3198±500	2464±302
8 cd	0.09±0.01	0.81±0.05	0.19±0.05	1.77±0.90	0.70±0.10	0.70±0.26	0.29±0.10	0.02±0.02	0.01±0.004	4298±1759	2537±1164	703±281
9 cx	0.07±0.01	0.69±0.05	0.31±0.05	1.39±0.77	0.65±0.05	0.68±0.27	0.33±0.06	0.06±0.03	0.03±0.02	4569±1853	2779±831	792±132
10 hm	0.06±0.01	0.70±0.05	0.30±0.05	0.52±0.12	0.65±0.03	0.26±0.08	0.32±0.03	0.02±0.01	0.03±0.01	2661±817	1612±364	702±232
Sand lizard subclade mean ± S.D. (N=4)												
	0.07±0.02	0.72±0.06	0.28±0.06	1.51±0.78	0.66±0.03	0.63±0.26	0.30±0.03	0.11±0.16	0.04±0.04	4281±1217	2531±671	1165±867
11 pc	0.08±0.01	0.64±0.08	0.36±0.08	0.77±0.36	0.25±0.12	2.03±0.45	0.66±0.10	0.25±0.09	0.08±0.04	5385±491	3510±406	2327±421
12 pp	0.10±0.01	0.54±0.11	0.46±0.11	0.62±0.12	0.23±0.04	2.05±0.61	0.72±0.03	0.16±0.07	0.06±0.02	4817±749	3218±832	1427±702
13 pM	0.10±0.02	0.57±0.03	0.44±0.03	0.59±0.31	0.31±0.10	1.00±0.27	0.56±0.14	0.26±0.19	0.13±0.05	4305±1977	2706±898	1678±714
14 pm	0.11±0.02	0.43±0.09	0.57±0.09	0.21±0.09	0.30±0.07	0.45±0.11	0.64±0.07	0.05±0.02	0.06±0.02	2635±491	1845±306	1027±378
Horned lizard subclade mean ± S.D. (N=4)												
	0.10±0.01	0.55±0.09	0.46±0.09	0.55±0.24	0.27±0.04	1.38±0.79	0.65±0.06	0.18±0.10	0.08±0.03	4285±1186	2820±730	1615±545
Phrynosomatidae mean ± S.D. (N=14)												
	0.08±0.02	0.60±0.10	0.40±0.10	0.91±0.64	0.46±0.16	0.89±0.61	0.45±0.14	0.19±0.18	0.09±0.05	4237±922	2647±592	1392±640
15 gw	0.05±0.01	0.69±0.07	0.31±0.07	1.07±0.16	0.58±0.06	0.73±0.23	0.38±0.06	0.07±0.01	0.04±0.004	2273±643	1426±388	516±132
16 cc	0.06±0.01	0.70±0.05	0.31±0.05	2.18±0.43	0.58±0.08	1.43±0.45	0.37±0.07	0.22±0.07	0.06±0.02	4931±1391	3707±1056	1767±400
17 dd	–	–	–	–	0.71±0.05	–	0.24±0.04	–	0.05±0.02	9111±3627	4056±1494	2644
18 ls	0.10±0.01	0.57±0.03	0.43±0.03	2.66±0.70	0.37±0.05	3.33±1.02	0.46±0.08	1.27±0.57	0.17±0.05	7695±2652	6013±1866	4983±2414
Non-phrynosomatid iguania mean ± S.D. (N=4*)												
	0.07±0.03	0.65±0.07	0.35±0.07	1.97±0.82	0.56±0.14	1.83±1.35	0.36±0.09	0.52±0.66	0.08±0.06	6003±3032	3801±1880	2477±1885
Iguania mean ± S.D. (N=18[†])												
	0.08±0.02	0.61±0.09	0.39±0.09	1.10±0.77	0.48±0.15	1.05±0.82	0.43±0.14	0.24±0.31	0.09±0.05	4629±1686	2903±1066	1633±1075
19 ct	0.12±0.01	0.82±0.04	0.18±0.04	2.20±0.70	0.76±0.07	0.70±0.46	0.22±0.07	0.08±0.04	0.03±0.002	2509±1035	1318±651	653±294
20 Ps	0.08±0.01	0.75±0.04	0.25±0.04	0.35±0.10	0.53±0.05	0.27±0.12	0.39±0.05	0.05±0.02	0.07±0.005	2128±601	1333±387	821±251
21 As	0.10±0.004	0.66±0.05	0.34±0.05	1.05±0.18	0.60±0.03	0.43±0.02	0.25±0.03	0.27±0.05	0.15±0.02	3926±219	2461±384	2544±278[‡]
22 Cf	0.08±0.01	0.66±0.03	0.35±0.03	0.27±0.02	0.54±0.05	0.20±0.04	0.39±0.04	0.04±0.01	0.07±0.01	2706±391	1689±227	1026±225
23 ef	0.07±0.003	0.62±0.06	0.38±0.06	0.36±0.11	0.49±0.04	0.28±0.07	0.38±0.04	0.10±0.03	0.13±0.01	3367±902	2044±476	1197±391
24 gk	0.08±0.02	0.53±0.05	0.47±0.05	0.24±0.07	0.43±0.04	0.23±0.06	0.41±0.03	0.09±0.02	0.16±0.01	3033±892	1564±442	883±299

Table 2. *Continued*

Species code	IFprop thigh	wtprop	oxprop	Fast glycolytic		Fast-oxidative glycolytic		Slow oxidative		Cross-sectional area of individual fibers		
				area (mm^2)	propIF	area (mm^2)	propIF	area (mm^2)	propIF	FG (μm^2)	FOG (μm^2)	SO (μm^2)
Autarchoglossa mean ± S.D. (*N*=6)												
	0.09±0.02	0.67±0.10	0.33±0.10	0.75±0.77	0.56±0.11	0.35±0.19	0.34±0.09	0.10±0.08	0.10±0.05	2945±642	1735±445	1187±690
All species measured in this study; mean ± S.D. (*N*=24)												
	0.08±0.02	0.63±0.10	0.37±0.10	1.01±0.77	0.50±0.15	0.87±0.77	0.41±0.13	0.21±0.28	0.09±0.05	4208±1657	2611±1072	1522±998

*N=3, †N=17 where *Dipsosaurus* data are absent.

Data reported include the proportion of total thigh muscle that is iliofibularis (IFprop thigh), the proportion of the iliofibularis that is predominantly white fibers (wtprop) or oxidative/red fibers (oxprop), cross sectional area (area) and proportional area (propIF) of the iliofibularis cross section for each of the three fiber types (FG, fast glycolytic; FOG, fast-oxidative glycolytic; SO, slow oxidative), and cross-sectional area of individual fibers of each type. For all species, N=4 individuals per species, except *Dipsosaurus dorsalis* (dd) where N=20 (Gleeson and Harrison, 1988). Furthermore, the S.D. value for *D. dorsalis* FOG propIF is an estimate because the actual value was not reported in Gleeson and Harrison (1988). Mean values for species were calculated using mean values for each of 92 individual lizards (four per species, except for *Dipsosaurus dorsalis*); mean number of fibers measured (excluding *D. dorsalis*) per individual lizard was 439.

‡On average, 12.0% of the IF was tonic in *Acanthodactylus scutellatus* (As), and only 3.2% was SO (the mean size value reported in the above table). Tonic fiber cross-sectional areas in *A. scutellatus* were (mean ±S.D.) 4404±544 μm^2 (see Results).

of SO fibers is consistently low across all the species we examined (Fig. 4E). Also apparent is the small size of fibers in *Gambelia* (gw; Fig. 4B,D,F); *Aspidoscelis* (ct) and *Elgaria* (gk) also had consistently small fiber cross-sectional areas for their body masses. *Acanthodactylus* (As), the only species to clearly contain large tonic fibers, also had large SO fibers (Fig. 4F).

Fig. 5 shows the relationships between thigh and muscle-specific variables with body mass. The proportion of the thigh that is IF does not show a clear relationship with body mass (Fig. 5A). However, the total thigh muscle cross-sectional area is related to body mass, with *Phrynosoma modestum* (pm) and *Elgaria* (gk) having thinner than expected thighs (Fig. 5B). Fig. 5C and D indicate that the proportion of white fibers in the IF is almost always higher than the proportion of red fibers; however, the proportions are roughly equal for many of the horned lizards and members of the *Sceloporus* group. No clear relationship with body mass is apparent.

Phylogenetically informed correlation analyses

As would be expected, contrasts in log body mass were strongly positively correlated with contrasts in snout-vent length and with contrasts in log hindlimb span (Table 4A). In addition, however, log SO individual fiber size was correlated with log body mass, log hindlimb span, and proportion of SO fibers in the IF (all are contrasts). Log FOG individual fiber size was correlated with log body mass and log hindlimb span, as well as with log SO individual fiber size – both SO and FOG fibers were most often found together in the oxidative region of the muscle. The log of individual FG fiber cross-sectional areas was correlated with log body mass, log hindlimb span, and the logs of SO and FOG individual fiber sizes (all are contrasts). Overall, individual fiber size is positively correlated with body size and hindlimb span, even after taking into account the influence of phylogenetic relatedness. As might be expected, the size of individual fibers appears to have changed in concert during the evolutionary divergence of this group of 24 lizard species. Contrasts of proportion of FG and FOG fiber composition were strongly negatively correlated, both using a star phylogeny (phylogenetically uninformed analysis, r=–0.940, two-tailed P<0.001; Fig. 6A) and when phylogeny was taken into account (r=–0.886, two-tailed P<0.001; Fig. 6B). [Deleting the influential sand *vs* horned contrast (Fig. 6B) lowers the correlation coefficient to –0.794 (P<0.001).] This finding is consistent with earlier results for 11 species of Phrynosomatidae, perhaps driven by the fairly consistent low level of SO cross-sectional area in the IF across species.

Table 4B contains results for 23 species analyses (data unavailable for *Dipsosaurus*). The square root of total thigh muscle area was correlated with log IF cross-sectional area as well as with the cross-sectional area of the IF consisting of each of the three fiber types. The log of IF cross-sectional area was also highly correlated with the cross-sectional area of each of the three IF fiber types (Table 4B). Other significant correlations were among the IF cross-sectional areas for each

Table 3. *Mean individual muscle fiber cross-sectional areas*

Number	Species code	Body mass (g)	Fast glycolytic (FG)	*N*	Fast oxidative-glycolytic (FOG)	*N*	Slow oxidative (SO)	*N*
1	us	3.5	3166±397	65	1789±220	79	642±206	54
2	uo	3.5	4182±412	72	2351±353	133	1214±238	95
3	sg	7.5	4449±1266	107	2840±707	176	1417±330	97
4	sm	34.0	5263±855	184	3349±629	290	2441±680	139
5	su	4.5	3973±1240	60	2450±723	105	1150±352	107
6	sv	5.5	4015±305	58	2871±348	152	1508±110	81
7	un	31.5	5598±1306	188	3198±500	203	2464±302	113
8	cd	10.6	4298±1759	236	2537±1164	198	703±281	24
9	cx	11.3	4569±1853	188	2779±831	149	792±132	43
10	hm	3.9	2661±817	142	1612±364	153	702±232	33
11	pc	38.5	5385±491	72	3510±406	310	2327±421	66
12	pp	17.4	4817±749	59	3218±832	337	1427±702	74
13	pM	12.7	4305±1977	78	2706±898	243	1678±714	73
14	pm	5.3	2635±491	68	1845±306	215	1027±378	39
15	gw	16.7	2273±643	264	1426±388	327	516±132	104
16	cc	39.2	4931±1391	259	3707±1056	255	1767±400	87
17	dd	65	9111±3627	⩾50	4056±1494	⩾50	2644	⩾50
18	ls	51.4	7695±2652	122	6013±1866	193	4983±2414	103
19	ct	10.4	2509±1035	450	1318±651	330	653±294	99
20	Ps	3.8	2128±601	125	1333±387	166	821±251	61
21	As	7.6	3926±219	184	2461±384	137	2544±278	18
22	Cf	3.5	2706±391	93	1689±227	114	1026±225	35
23	ef	5.9	3367±902	88	2044±476	115	1197±391	70
24	gk	10.0	3033±892	64	1564±442	126	883±299	91

Values are means ± S.D. (μm^2) calculated using mean values for each of 92 individual lizards [four per species, except *Dipsosaurus dorsalis* (dd)] and then calculating mean values for each species. Mean number of individual fibers measured per individual lizard is 439 (excepting *D. dorsalis*).

Note: In Bonine et al. (2001) we reported the SO individual area data for 11 of the above phrynosomatid species without any of the controversial 'tonics' included. However, in the table above, the SO values include many fibers that initially seemed to be tonics, but upon more discriminating examination, were lightly-staining SO fibers. We made this change in the spirit of thoroughness and in an effort to get a more complete picture of the muscle composition of the iliofibularis muscle. Some fibers (especially prevalent in *Acanthodactylus*; discussed in the text) were definitely tonic, and these still are not included in the above table, nor in subsequent analyses of individual SO fiber cross-sectional area. Earlier (Bonine et al., 2001) SO size values for *Uta stansburiana*, us (636 μm^2), *Phrynosoma cornutum*, pc (2061 μm^2), and *Phrynosoma modestum*, pm (973 μm^2) have changed slightly in the above table. *Dipsosaurus dorsalis* (dd) data are from Gleeson and Harrison (1988); standard deviation for SO fiber size was not reported.

of the three fiber types. Overall, these results once again emphasize the important influence of body size on hindlimb muscle morphology. It is important to note that the proportional representation of the IF or the individual fibers is not related to body size; a relationship that would have complicated our efforts to separate the effects of body size and phylogenetic relatedness (see Fig. 5).

Ancestor reconstruction

Estimated ancestral values for FG and FOG fiber proportions, and their 95% confidence intervals, are reported in Table 5 and depicted in Fig. 8. Note that the extant range of variation, especially as manifest in the horned lizards (*Phrynosoma*), in the proportion of either FG or FOG fibers falls outside the 95% confidence interval of the estimated ancestral values for this sample of 24 species. Inclusion of the standard errors of species' mean values (calculated from the four individual measurements taken per species) changed terminal branch lengths in a noticeable way (Fig. 7), but had virtually no effect on the estimated nodal values (Table 5). However, confidence intervals around the estimates were narrowed in every case with inclusion of standard errors (Table 5).

Discussion

Within the iliofibularis (IF) muscle of lizards, the proportions of fast-twitch glycolytic (FG) and fast-twitch oxidative-glycolytic (FOG) fibers were negatively related among the 24 species studied. Thus, the variation in observed FG and FOG proportions within Phrynosomatidae, evident with traditional statistical analyses, as well as when using phylogenetically informed independent contrast methodologies (Fig. 6; see Bonine et al., 2001), is a characteristic of 24 lizard species from eight different families

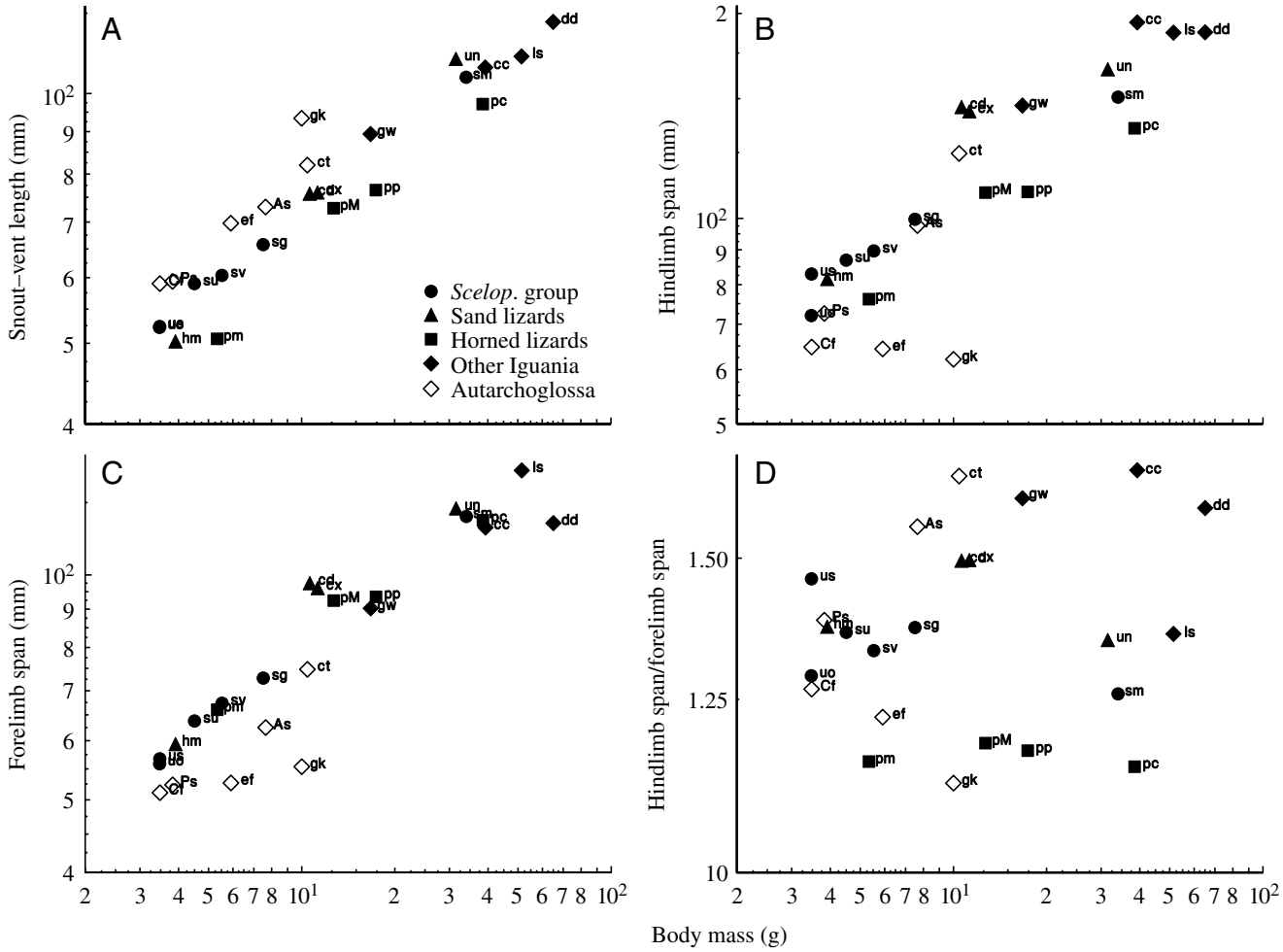

Fig. 2. Bivariate scatterplots of body and limb linear dimensions in relation to body mass for 24 species of lizard (see Fig. 1 for species codes), using mean values reported in Table 1.

(Fig. 1). Together, the FG + FOG account for 83% to >99% of the total fiber area, which begs the question of why the proportion of slow-oxidative (SO) fibers is 'constrained' to no more than 17%. In principle, any of the three fiber types could constitute the vast majority, but that was never observed for SO in the present sample of 24 species. Two basic processes, not mutually exclusive, can account for such apparent 'constraints' on the distribution of phenotypes among species. First, the additive genetic correlations, arising from pleiotropic gene actions (caused by shared developmental and biochemical pathways), might preclude (or at least severely impede) the evolution of certain fiber-type combinations in this group of small (≤65 g) terrestrial lizards. Second, natural selection might not 'allow' species with very high proportions of SO fibers in their IF muscles to evolve. For example, if natural selection places a lower limit on speed, through its relationship to prey capture and predator avoidance (e.g. Miles, 2004), then terrestrial lizards with a high proportion of SO fibers may be quickly removed from the population. This would suggest that terrestrial lizards with a high SO proportion would experience a substantial decrement in terms of muscular performance. Indeed, that might be the case if SO fibers, because of their slower twitch kinetics, are just 'in the way' during burst locomotion. Previous studies on *D. dorsalis* suggest that twitch kinetics may limit limb-cycling frequency and thereby limit maximal sprint speed (Johnson et al., 1993).

Whatever the origin of the apparent 'constraint' on fiber-type distribution in lizards, it is potentially an important mechanistic explanation for hypothesized trade-offs in whole-animal performance abilities. Indeed, we predict, based on the FG and FOG proportions, that our sample of 24 species will exhibit a trade-off in speed and stamina (see Bonine, 2001; Bonine, in press). Furthermore, we hypothesize that examination of the 12 lacertids studied by Vanhooydonck et al. (2001; see also Huey et al., 1984) will reveal variation in fiber-type composition that explains the negative relationship between speed and endurance observed in those species (we have muscle data for only two of the species they measured). Contractile velocities at 40°C for each of the three fiber types in *Dipsosaurus* support the above predictions: SO, ~4 lengths s^{-1}; FOG and FG, ~8–9 lengths s^{-1} and 16 lengths s^{-1}, respectively (data from table 1, fig. 2, and

related text of Johnston and Gleeson, 1984). If FG fibers also contract about twice as fast as FOG fibers in other small lizard species, then the observed negative relation between proportion of FOG and FG fiber types across species may be a main cause of the negative relation between locomotor speed and endurance (Vanhooydonck et al., 2001).

Reconstructed ancestral values (Fig. 8) suggest that rather extreme divergence of FG and FOG fiber proportions have occurred in both the sand- and horned-lizard lineages, and especially in the latter. Although not formally tested (see equation A10 of Garland et al., 1999), the 95% confidence intervals around the nodal values do not include the values found in the living horned lizard species we measured, indicating a tremendous shift in the IF composition toward FOG fibers, and away from FG fibers, during the evolution of this subclade. Their sister group, the sand lizards, have diverged in the opposite direction, with more FG than FOG fibers, but not to the same extent. Evolution of fiber-type proportions has also resulted in a dramatic shift in muscle composition of *Aspidoscelis* (ct) toward many more FG fibers and fewer FOG fibers (although this apparent trend must be interpreted with caution because we have data only for four individuals of one species). Indeed, *Aspidoscelis* has the highest proportion of FG fibers, and the lowest proportion of FOG fibers, of any species measured, and was well outside the 95% confidence interval values for the root of all 24 species. Based on Fig. 8, we hypothesize that natural selection has shaped the IF muscle composition of sand lizards, horned lizards, and *Aspidoscelis* in accordance with their differences in ecology, behavior and morphology. Horned lizards are relatively slow (Bonine and Garland, 1999) and often rely on crypsis to avoid predation (Sherbrooke, 1981). Sand lizards live in open desert areas (Stebbins, 1985) and rely on speed to escape predators (Bulova, 1994). The *Sceloporus* group, sister to the sand and horned lizards, is often considered intermediate in many aspects of its biology (e.g. Bonine and Garland, 1999). *Aspidoscelis* is the archetypal active, widely foraging lizard and has exceptionally high stamina in addition to being a fast sprinter (Garland, 1994; Dohm et al., 1998; Bonine and Garland, 1999; Bonine, 2001).

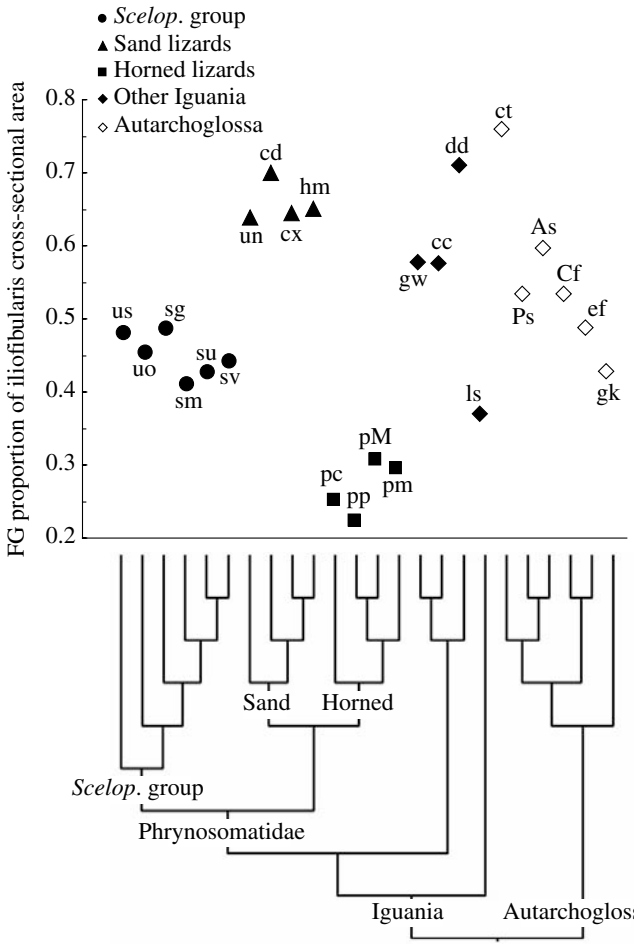

Fig. 3. Proportion of fast-glycolytic fibers in the iliofibularis muscle (Table 2) in relation to hypothesized phylogenetic relationships among 24 lizard species (see Fig. 1 for species codes).

Inclusion of standard errors (Fig. 7), based on the values from the four individuals measured per species, did not change the node estimates except in the third decimal place (Table 5). However, confidence intervals around the estimates did narrow slightly, which will enhance inferential power. It is also important to note that these confidence intervals are relatively narrow as compared with some published examples, which involved fewer species (e.g. see fig. 8 in Schluter et al., 1997; fig. 2 in Garland et al., 1999; fig. 3 in Losos, 1999). Thus, our study illustrates that reconstructions of ancestral states for continuous-valued characters can indeed be useful for drawing evolutionary inferences (see also Johnston et al., 2003; Espinoza et al., 2004). However, it should be kept in mind that these techniques assume no phylogeny-wide directional trends have occurred in past character evolution. If such trends have occurred, then even rather narrow confidence intervals may be wildly misleading (e.g. see fig. 3 in Garland et al., 1999). In the present study, it is hoped that the inclusion of several outgroup taxa has countered this possibility with respect to inferring ancestral values at the base of the Phrynosomatidae (see also Garland et al., 1997; Schultz and Churchill, 1999; Polly, 2001).

Although we did not determine twitch kinetics or analyze enzyme activity levels in these species (but for this information from *Dipsosaurus dorsalis* see Gleeson et al., 1980; Gleeson and Johnston, 1987), we usually found three types of fibers that were relatively easy to characterize, based on histochemical assays: FG, FOG and SO (Guth and Samaha, 1969). However, examination of the *Acanthodactylus* (As) IF revealed many large fibers in the oxidative, medial area that did not appear to stain for either SDH or mATPase activity. These tonic fibers, not commonly found in mammalian muscle (Saltin and Gollnick, 1983), are apparently common in some other species that we did not measure. For example, some chameleon species have 50% tonic fibers in the IF, and each tonic fiber is twice the size (cross-sectional area) of the other twitch fibers in the muscle (Abu-Ghalyun et al., 1988). In contrast to the

chameleon muscle, tonic fibers are only about 10% larger than FG fibers in our *Acanthodactylus*. Therefore, it seems that lizard IF fiber-type composition may not be restricted to FG, FOG, and SO fiber types. Our sampling of small (⩽65 g) lizards that are primarily terrestrial was intentional, to facilitate meaningful comparisons among species, but larger species and/or other locomotor modes, such as arboreality or fossoriality, may reveal more diverse muscle-fiber type properties. Currently, comparisons with values from other studies of lizard IF in the literature are problematic because of the different methods used by other researchers and the different data reported (see table 1 of Bonine et al., 2001).

Ideally for the generality and reliability of our results, the properties of the IF would be indicative of the physiology and function of many of the muscles involved in lizard locomotion. We chose to focus on the IF for several reasons, including its variable fiber-type composition, its parallel-fibered structure, and its consistent location across species. Published

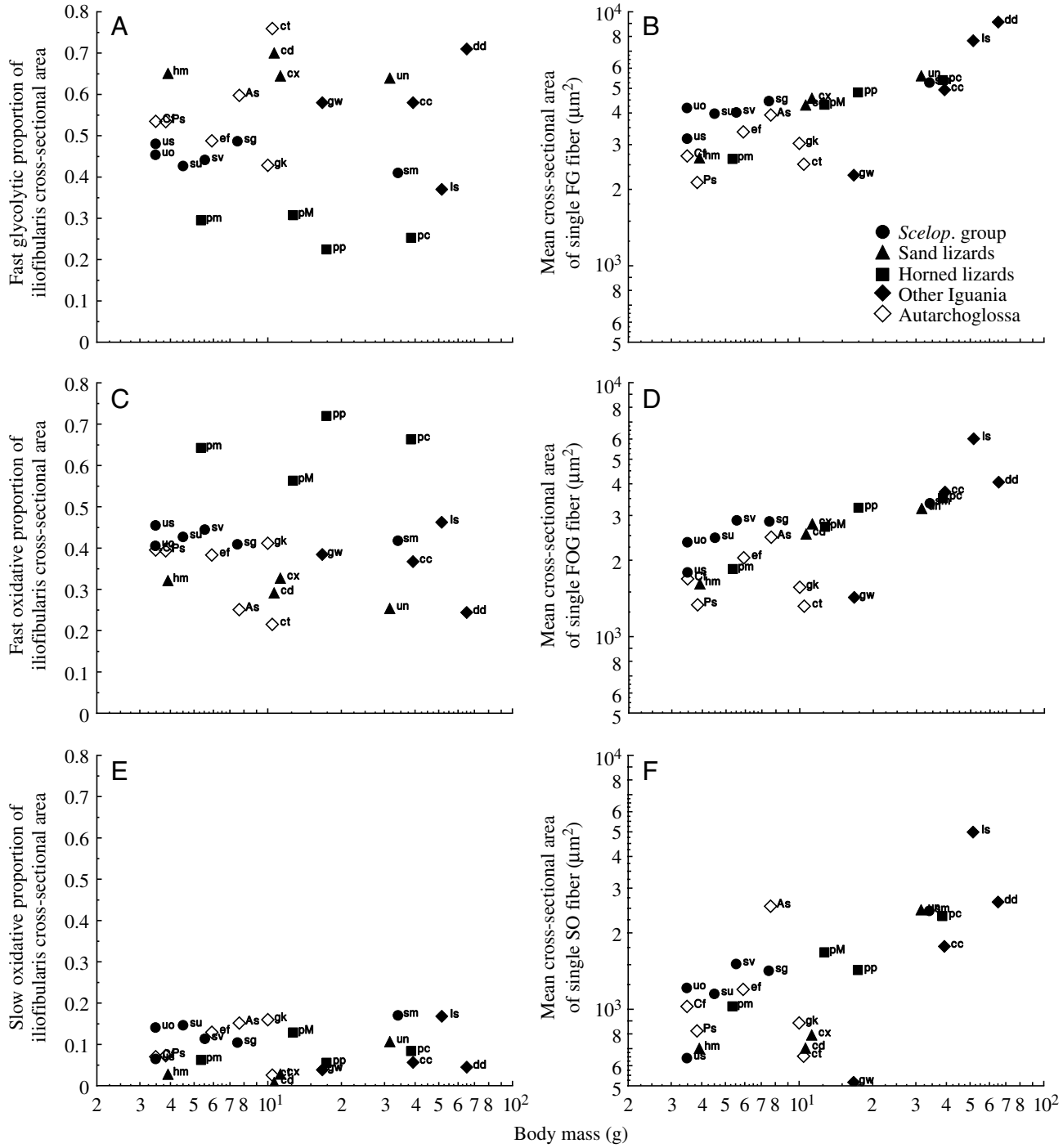

Fig. 4. Bivariate scatterplots of fiber-type proportion of the iliofibularis and cross-sectional area of individual fibers in relation to body mass for 24 species of lizard (see Fig. 1 for species codes), using mean values reported in Tables 2 and 3.

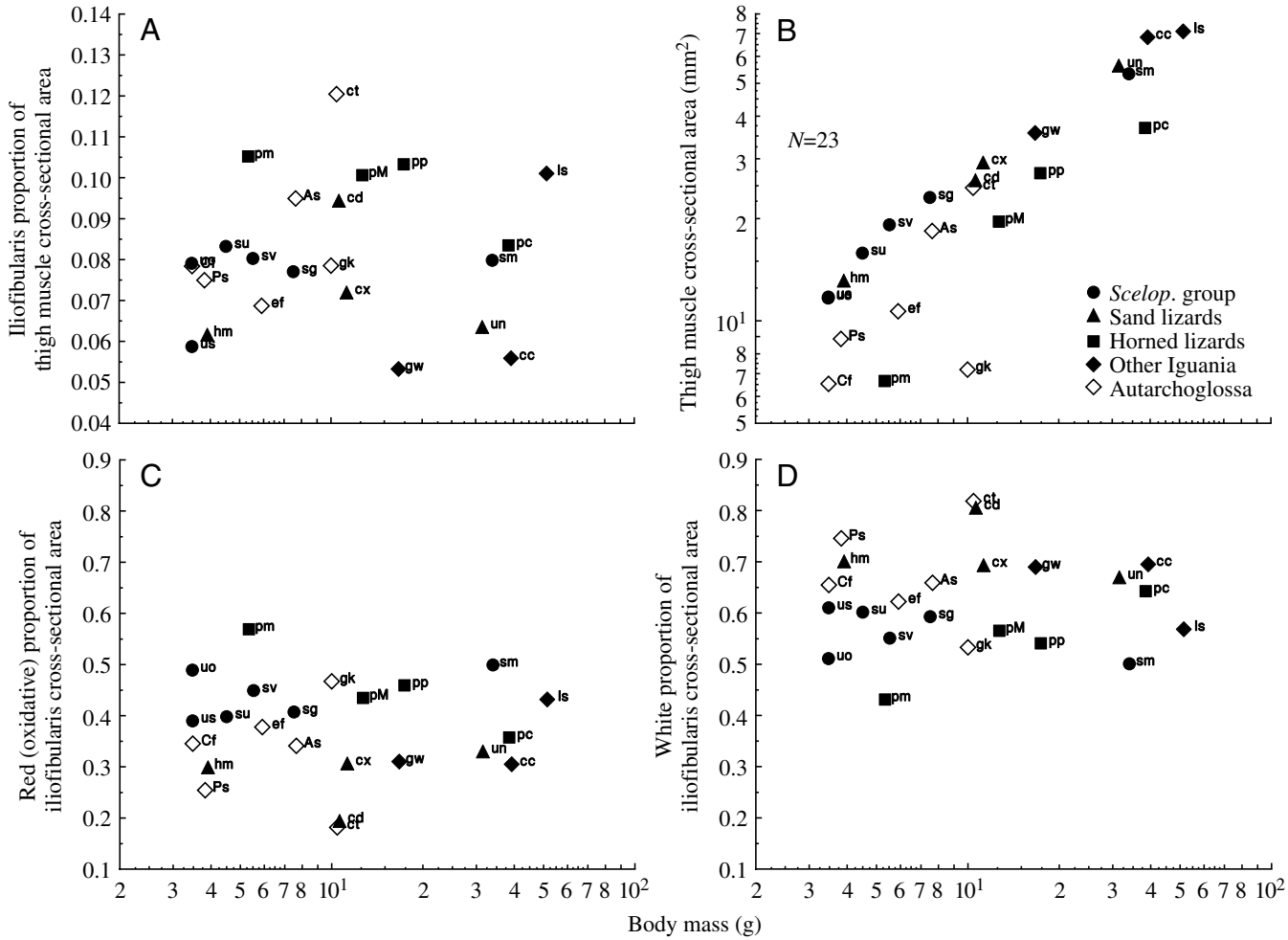

Fig. 5. Bivariate scatterplots of thigh and iliofibularis muscle properties in relation to body mass (see Fig. 1 for species codes) for 23 species of lizard (*Dipsosaurus* data not available for these variables), using mean values from Tables 1 and 2.

examination of other lizard hindlimb muscles indicates important properties in common with the IF. Putnam et al. (1980) examined 12 muscles, including the IF, involved in *D. dorsalis* locomotion, and they all contain similar fiber-type proportions. However, only two of the twelve muscles were parallel-fibered (IF and extensor digitorum longus). In another study of *D. dorsalis*, muscle fiber cross-sectional areas were similar across different locomotory muscles within individuals, but individuals varied in mean fiber size (Gleeson and Harrison, 1988). In four distantly related species of lizard, Putnam and Bennett (1982) reported that the IF and gastrocnemius have similar contractile properties within each species.

Some of the variables reported here were not formally analyzed [for example, using ANCOVA; but see Bonine et al., (2001) for more in-depth analysis of data for 11 phrynosomatids] as our sample size in families other than Phrynosomatidae was so small (1 or 2 species). However, we can point out a few interesting observations. *Gambelia* (gw), a large crotaphytid that often lives in sympatry with many of these other species and also feeds on them, has surprisingly small iliofibularis muscle fibers for its body size (Tables 2, 3; Fig. 4). The reasons for this are unclear, and the other crotaphytid measured, *Crotaphytus* (cc), does not display this pattern. However, both of these species have a relatively small proportion of iliofibularis muscle in their thigh cross-sectional area (Fig. 5A). The autarchoglossa species all have longer snout-vent lengths for a given body mass than phrynosomatids and other iguanians (Fig. 2A). Other potentially important differences in body plan between iguanians and non-iguanians include the short hindlimb span of *Elgaria* [gk; Anguidae; see also Wiens and Slingluff (2001)] and *Eumeces* (ef; Scincidae; Fig. 2B). The forelimbs of all autarchoglossa are relatively short as compared with iguanians (Fig. 2C). Within autarchoglossa, the relatively longer hindlimbs of *Aspidoscelis* (ct), *Acanthodactylus* (As) and *Podarcis* (Ps) may be related to their increased ecological reliance on speed. Indeed, of the species measured by Bonine and Garland (1999), including some of the phrynosomatids represented here, members of the genus *Aspidoscelis* were the fastest [up to 6 m s^{-1}; *Elgaria* (gk) were the slowest (1 m s^{-1})]. When comparing the ratios of hindlimb span to forelimb span, *Aspidoscelis* (ct) and *Crotaphytus* (cc) have the highest ratios, and *Elgaria* (gk) has

Table 4. *Pearson product-moment correlations*

A. *Pearson product-moment correlations* (*through the origin*) *between independent contrasts of morphometric and fiber-type variables for 24 species* (*23 contrasts*)

Variable*	Body mass	Hindlimb span	SO proportion	FOG proportion	FG proportion	SO fiber size	FOG fiber size	FG fiber size
Log body mass (SDLMASS)	1.0	**0.873**	0.218	–0.145	0.036	**0.713**	**0.748**	**0.783**
Log hindlimb span (SDLHLS)		1.0	0.045	–0.357	0.324	**0.578**	**0.681**	**0.687**
SO proportion of IF c-s area (SDSOP)			1.0	–0.172	–0.304	**0.549**	0.360	0.352
FOG proportion of IF c-s area (SDFOGP)				1.0	**–0.886**	–0.172	–0.089	–0.246
FG proportion of IF c-s area (SDFGP)					1.0	–0.091	–0.083	0.073
Log indiv SO fiber c-s area (SDLSOF)						1.0	**0.905**	**0.863**
Log indiv FOG fiber c-s area (SDLFOGF)							1.0	**0.945**
Log indiv FG fiber c-s area (SDLFGF)								1.0

Variables listed as column headings are shortened names for the same variables listed with more explanatory names for each row.
c-s area, cross section area.
Significant correlations are shown in bold.
For 22 degrees of freedom, the two-tailed critical value of r is 0.404 for α=0.05 (not corrected for multiple comparisons).
Independent contrasts of log body mass and log snout–vent length are highly positively correlated, r=0.967.

B. *Pearson product-moment correlations (through the origin) between independent contrasts of thigh, iliofibularis and fiber-type variables for 23 species*†

Variable*	Thigh area	IF area	IF proportion	Oxidative proportion	FG area	FOG area	SO area
Square root of thigh muscle c-s area (SDTMA)	1.0	**0.946**	0.114	–0.180	**0.859**	**0.853**	**0.694**
Log IF c-s area (SDLIFA)		1.0	0.379	–0.243	**0.919**	**0.915**	**0.673**
IF proportion of thigh muscle c-s area (SDIFPM)			1.0	–0.073	0.385	0.402	0.128
Oxidative (red) proportion of IF (SDOXP)				1.0	–0.385	–0.093	0.263
Log total FG c-s area (SDLFGA)					1.0	**0.786**	**0.613**
Log total FOG c-s area (SDLFOA)						1.0	**0.690**
Log total SO c-s area (SDLSOA)							1.0

†Twenty-two contrasts; data not available for *Dipsosaurus dorsalis* (dd). For 21 degrees of freedom, the two-tailed critical value of r is 0.413 for α=0.05 (not corrected for multiple comparisons).
c-s area, cross section area.
Significant correlations are shown in bold.
*In both A and B, the SPSS (Statistical Package for the Social Sciences, SPSS, Inc., Chicago, II, USA) variable names in brackets were our shorthand for the full names. We keep them here for future reference.

Table 5. *Utilizing standard errors of data measurements of 24 species* (*four individuals per species*) *to alter branch tip lengths*

	Standard errors not included		Standard errors included	
Node reconstruction	Fast-oxidative glycolytic	Fast glycolytic	Fast-oxidative glycolytic	Fast glycolytic
root for 24 species	0.401±0.1635	0.495±0.1691	0.401±0.1538	0.495±0.1614
Phrynosomatid origin (node PH)	0.427±0.1153	0.483±0.1192	0.425±0.1093	0.483±0.1145
Sceloporus group origin (node SG)	0.426±0.1147	0.474±0.1186	0.426±0.1097	0.474±0.1142

Estimates of ancestral reconstructions and 95% confidence intervals are presented for independent contrasts of the proportion of the iliofibularis area consisting of fast-twitch oxidative-glycolytic (FOG) or fast-twitch glycolytic (FG) fibers. See Fig. 7 for tip length changes. In all cases, incorporating within-species variation reduced standard errors of nodal estimates. Hypothesized ancestral trait values in Fig. 8 reflect inclusion of standard errors.

the lowest (Fig. 2D). Most of the fast species (Bonine and Garland, 1999) have a relatively high ratio of hindlimb span to forelimb span, consistent with the importance of limb length, and therefore stride length (e.g. Irschick and Jayne, 1999; Vanhooydonck et al., 2001), especially for these species that tend to run bipedally (all measured except for horned lizards, scincids and anguids; personal observation).

Greater research emphasis on muscle variation, and related traits, across lizard species should improve our ability to explain differences in performance abilities. For example, calculations of the power input required to reach burst sprint speed in one lacertid species (*Acanthodactylus boskianus*, not tested by us here) indicate that the force produced by the muscle fibers is sufficient; no elastic enhancement from tendons is required (Curtin et al., 2005). This finding suggests that the links between muscle traits and whole-animal performance may be relatively direct. Examination of sprint speed and iliofibularis muscle composition in *D. dorsalis* revealed significant negative relationships between fiber cross-sectional area and both sprint speed and enzyme activities (Gleeson and Harrison, 1988). Attempts to include

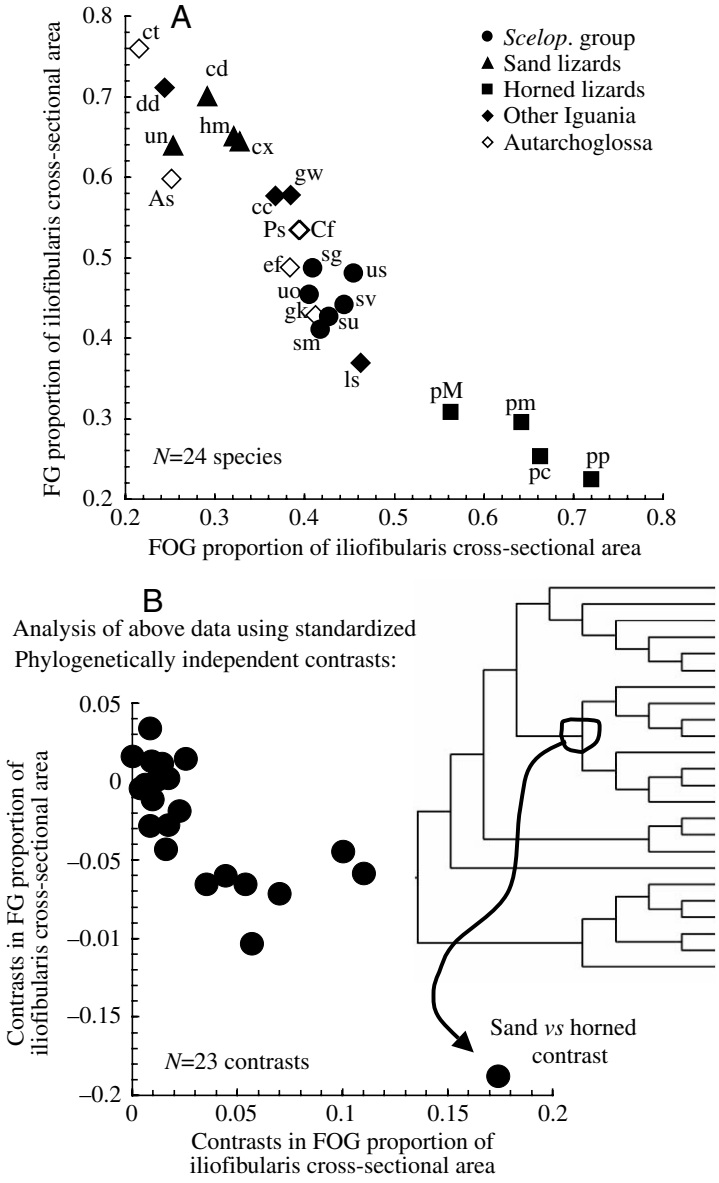

Fig. 6. (A) Proportion of fast twitch-glycolytic (FG) and fast twitch-oxidative glycolytic (FOG) muscle fiber types in the iliofibularis in 24 species of lizard (see Fig. 1 for species codes). Across all 24 species, the correlation is r=–0.940 (2-tailed, P<0.001). (B) Phylogenetically independent contrasts of data presented in A, using topology and branch lengths shown in Fig. 1 (number of independent contrasts is always one less than the number of species). Correlation (computed through origin)=–0.886 (P<0.001). Deleting the sand *versus* horned contrast reduces the magnitude of the correlation to –0.794 (P<0.001).

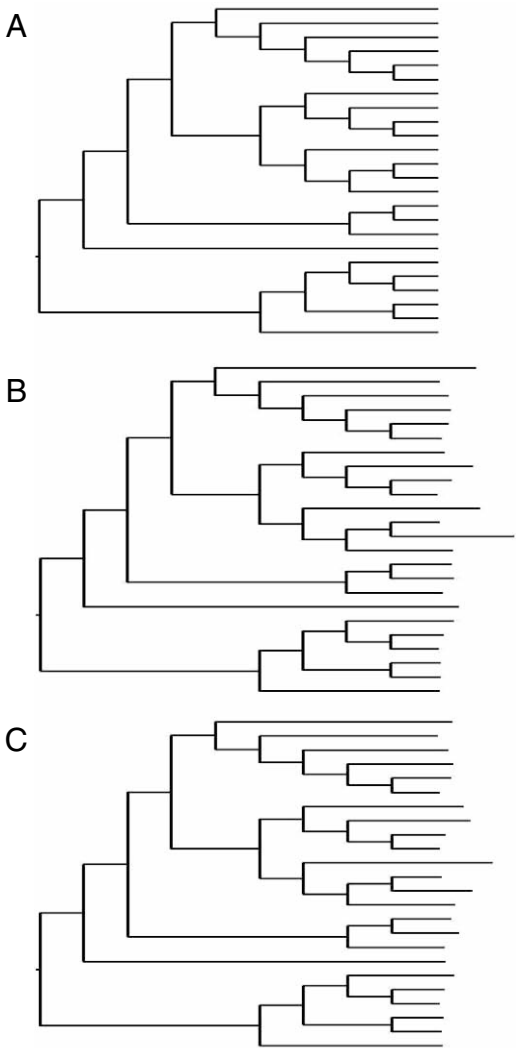

Fig. 7. Arbitrary branch lengths used for initial estimates of ancestral values (A; following Pagel., 1992) and after alteration based on standard errors for the proportional area of fast twitch-oxidative glycolytic (FOG) fibers (B) or the proportional area of fast twitch-glycolytic (FG) fibers in the iliofibularis (C). Topology is the same as in Fig. 1. Refer to text and Table 5 for numerical values of reconstructed ancestral nodes and demonstration of how inclusion of standard errors alters both node estimates and confidence intervals.

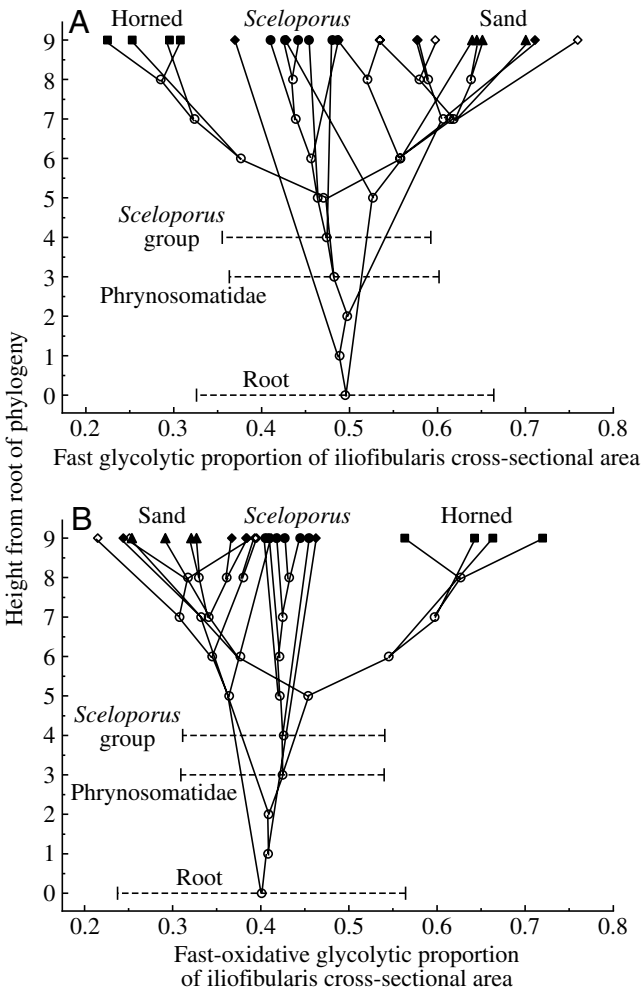

Fig. 8. (A) Graphical depiction of reconstructed nodal values of fast-twitch glycolytic (FG) fiber proportion during the evolution of the 24 lizard species included in this study (see Fig. 1 for topology and branch lengths used). Confidence intervals (95%) were calculated for selected nodes; root of all 24 species, origin of Phrynosomatidae, and origin of *Sceloporus* group within Phrynosomatidae. (B) Same as A, but for fast-twitch oxidative-glycolytic (FOG) fiber proportion. The reconstructed ancestral nodes and confidence intervals in each panel incorporate the standard errors for proportion measurements (see Fig. 7; refer to text and Table 5 for numerical values).

performance breadth across a wide range of temperatures may further enrich our understanding of the mechanistic drivers of variation in these well-studied ectotherms (e.g. Johnston and Gleeson, 1984; Marsh and Bennett, 1985). We predict that analysis using multiple morphological and physiological variables, including those pertaining specifically to muscle such as fiber-type composition and individual fiber cross-sectional area, will provide a more complete understanding of the observed variation in locomotor performance abilities across lizard species. Moreover, these kinds of synthetic analyses should allow us to infer what sorts of selective regimes, active in the past, gave rise to the existing diversity of species and phenotypes.

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