SYMPOSIUM

Late to the Table: Diversification of Tetrapod Mandibular Biomechanics Lagged Behind the Evolution of Terrestriality

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Synopsis The origin of digit-bearing tetrapods in the Middle to Late Devonian (ca. 393–360 Mya) and their subsequent invasion of land represent a classic case of a major evolutionary radiation driven by new ecological opportunities. In this and other examples, exploration of new eco-space is hypothesized to correlate with functional innovation and adaptive divergence of phenotypes. Simultaneous changes in all morphofunctional systems are rare in major evolutionary transitions and may be non-existent. Here, we focus on the mandibles of early tetrapods and their kin as a model system to test whether shifts in functional innovation were coeval with some major events in tetrapod history. To this end, we quantified mechanical variation in the mandibles of tetrapodomorphs ranging in age from Early Devonian to earliest Permian. Biomechanical disparity is stable from the Devonian to the Early Pennsylvanian, even though the origin of weight-bearing, digited limbs, and the initial phases of the colonization of land occurred during this interval. An appreciable increase in functional variation is detected in the latest Pennsylvanian and earliest Permian, when stem and crown amniotes began to explore new regions of mechanical morphospace, a pattern partly attributable to the origin of herbivory. We find no difference in the rate of functional change between tetrapodomorph "fish" and early digited tetrapods, although two independent shifts are detected among Devonian stem tetrapods more crownward than *Acanthostega*. Instead, the most profound shifts in evolutionary rate are nested well within the tetrapod crown and are associated with amniotes (particularly diadectomorphs and some synapsids). The substantial temporal gap between the origin of postcranial features associated with terrestriality, such as limbs with functional elbow/knee and wrist/ankle joints capable of weight-bearing, and the onset of divergence in jaw biomechanics provides a compelling example of "functional modularity" during a major adaptive radiation.

Introduction

The evolutionary transition from fishes to limbed vertebrates (tetrapods) was accompanied by remarkable structural and functional modifications. Exceptional fossil discoveries and new analytical protocols have recast our notions of anatomical, physiological, ecological, and developmental aspects of the invasion of land (e.g., Coates and Clack 1991; Coates 1996; Clack 2002a, 2012; Clack et al. 2003; Ahlberg et al. 2005a; Brazeau and Ahlberg 2006; Shubin et al. 2006; Coates et al. 2008; Anderson et al. 2011; Pierce et al. 2012). However, patterns and processes of vertebrate terrestrialization still remain poorly understood. Here, we investigate biomechanical changes in the mandibular system both across the fish–tetrapod transition and during the initial radiation of limbed vertebrates. Knowledge of these changes is key to understanding the ecology of feeding during a major evolutionary radiation as well as the assembly of late Paleozoic terrestrial food webs (Roopnarine and Angielczyk 2011).

A question of broad interest to comparative biologists centers on the timing and correlation of changes in different morphofunctional systems during an "adaptive radiation" (e.g., Osborn 1902; Schluter 2000; Slater et al. 2010; Jetz et al. 2012; Wagner et al. 2013), and on the impact of innovation (structural, functional, or otherwise) on the diversification of

clades. In this work, we are concerned with major shifts in feeding modes, such as may be inferred from the biomechanical construction of the mandible. In particular, we are interested in the chronology of these shifts relative to the acquisition of major structural/functional innovations and the origin of key features during the evolution of tetrapods.

Changes in mandibular function in tetrapods may have been delayed relative to the origin or modification of other traits (e.g., acquisition of digits, enlargement/elaboration of girdles, loss of opercular bones, and loss of median fins). A recent survey of mandibular mechanics in Silurian–Devonian gnathostomes (Anderson et al. 2011) showed that the lower jaws of the earliest known digited tetrapods (e.g., *Acanthostega* and *Ichthyostega*) were comparable with those of tetrapodomorph fishes. The findings of Anderson et al. (2011) contradict informal arguments for functional experimentation in the mandibles of early tetrapods from the Devonian (Ahlberg et al. 2005b, 723), and inferences that *Acanthostega* fed through biting rather than fish-like suction based on the fact that the suture patterns of its skull roof (Clack 2002b) are similar to those of terrestrial vertebrates (Markey et al. 2006; Markey and Marshall 2007). However, Anderson et al.'s (2011) results are consistent with the observation that the most profound changes in tetrapod jaws (e.g., loss of Meckelian cartilage, reduction/loss of coronoid dentition, and heterodonty) did not occur until later in the Paleozoic, and even then only in some groups (Clack 2012).

These observations imply a lag in functional diversification of mandibles relative to the acquisition of postcranial traits associated with terrestriality among early tetrapods, particularly the acquisition of elaborate girdles and limbs capable of bearing weight. These traits occur in representatives of various stem amniote groups, including gephyrostegids, seymouriamorphs, and diadectomorphs (Clack 2012), in the crownward part of the tetrapod stem as well as early in the amphibian stem. Indeed, the ability to support the body on land may have preceded the separation between total-group amphibians and total-group amniotes (Ruta and Clack 2006). This lag would support previous hypotheses concerning the diversification of craniomandibular versatility in tetrapods (Olson 1966; Janis and Keller 2001; Clack 2012). However, more definitive inferences require quantitative data of broader taxonomic and stratigraphic scope. We therefore surveyed biomechanical traits in the lower jaws of early tetrapods and their fish-like relatives from the Early Devonian to the earliest Permian in order to explore three issues:

(1) variation in jaw mechanics in early tetrapod history, particularly the timing of key changes in functional disparity, (2) diversification of the lower jaws in mechanical morphospace, including the distribution of major taxonomic assemblages, and (3) rates of mechanical evolution across phylogeny, with emphasis on large shifts in the tempo of change.

Methods

Data on the lower jaw

We examined mandibles in 89 fossil members of Tetrapodomorpha, the total group of tetrapods (a total group consists of a monophyletic group of extant species plus all fossil taxa more closely related to those living species than to any other living group) (see Fig. 1 for examples, and also Supplementary Information) from the Early Devonian (Pragian; 410.8–407.6 Mya) to the earliest Permian (Asselian; 298.9–295.5 Mya). This interval spans over 100 Myr and encompasses such major events as the origin of limbs and digits in the Devonian, the origin of crown tetrapods in the Mississippian, and the origin of crown amniotes in the Pennsylvanian (Ruta and Coates 2007; Friedman and Brazeau 2011; Clack 2012). We use individual species as proxies for their containing genera, because many genera span stratigraphic intervals during which some constituent species preserve no, or insufficient, jaw material. In cases where there is compelling evidence that genera are not monophyletic, we have treated their constituent species separately (e.g., *Pholiderpeton* and *Platycephalichthys*; Ruta and Coates 2007; Anderson et al. 2011). A full taxon list with sources for images of mandibles is included in Supplementary Information.

Stratigraphic assignments are based on primary literature. Estimates of absolute ages for stratigraphic stages and for period boundaries derive from Gradstein et al. (2012). For analyses of disparity, we assigned taxa to six composite intervals of time: (1) Early–Middle Devonian (Lochkovian–Givetian; 419.2–382.7 Mya), (2) Late Devonian (Frasnian–Famennian; 382.7–358.9 Mya), (3) Mississippian (Tournaisian–Serpukhovian; 358.9–323.2 Mya), (4) Early–Middle Pennsylvanian (Bashkirian–Moscovian; 323.2–307.0 Mya), (5) Late Pennsylvanian (Kasimovian–Gzhelian; 307.0–298.9 Mya), and (6) earliest Permian (Asselian; 298.9–295.5 Mya). Assignments to these intervals are based on stratigraphic ranges of genera, not just the locality of individual taxa. As the analysis of evolutionary rates requires point ages rather than ranges, we used the first-appearance dates (FADs) of genera. Midpoints
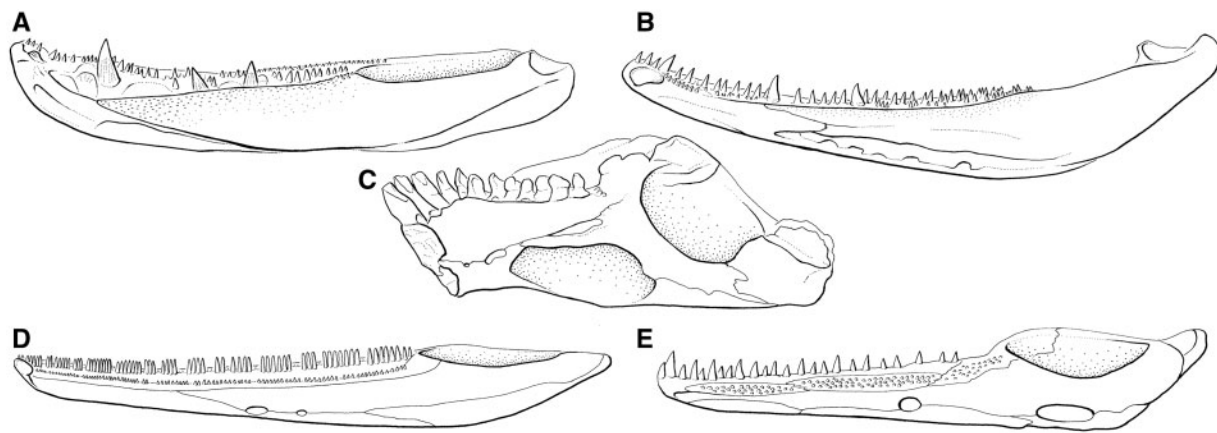
Fig. 1 Mandibles of selected Paleozoic tetrapodomorphs showing examples of structural diversity. (**A**) *Platycephalichthys*, a Late Devonian stem tetrapod fish (based on specimen PIN 54/162, Paleontological Institute of the Russian Academy of Sciences, Moscow). (**B**) The Late Devonian stem tetrapod *Ventastega*, branching crownward of "elpistostegalians" (modified from Ahlberg et al. 2008). (**C**) *Diadectes*, a Permian stem amniote crown tetrapod (modified from Welles 1941). (**D**) The Mississippian stem tetrapod *Doragnathus* (modified from Smithson 1980). (**E**) The temnospondyl *Sclerocephalus*, a Pennsylvanian-Permian stem lissamphibian crown tetrapod (modified from Schoch and Witzmann 2009). Images not to scale.

of stages provided estimates of FAD in many instances, but we determined more precise estimates when geochronological data permitted.

We used 10 unit-less and scale-independent metrics to capture biomechanical disparity of lower jaws. Biomechanical disparity is analogous to morphological disparity (Foote 1992; Wills et al. 1994), but is based on functionally and/or biomechanically significant variables. One example is mechanical advantage, which measures bite-force efficiency (Barel 1983; Wainwright and Richard 1995). Our metrics rely on established mechanical principles that are applicable to different groups, give insights into the relationship between form and function in extinct organisms, and are more relevant to functional analyses than is overall morphological disparity (Anderson 2009; Anderson and Friedman 2012).

The 10 metrics describe the basic geometry of the jaw, lever mechanics, and tooth mechanics (see Supplementary Information) and were measured from images of jaws in both lateral and dorsoventral views. The images consist of published photographs (or photographs taken by the first two authors), drawings of specimens, or reconstructions (see Supplementary Information). Measurements were standardized using Z-transformation before carrying out subsequent analyses.

Concerning the use of reconstructions, we confined our selection of mandibles to those taxa for which reliable jaw renditions are available in the literature. Of course, it is impossible to assess whether such reconstructions approximate mean shapes, as in the majority of cases the authors of the original works used only one or very few jaws as a basis for their descriptions. Deviations of these reconstructions from real mandibles are equally difficult to estimate. However, in agreement with other fossil-based analyses of biological shape (e.g., Angielczyk and Ruta 2012), we regard such reconstructions as being adequate for this type of study in that they are all illustrated in standard anatomical orientation and show correct relative proportions of their individual parts.

Calculation of disparity

Biomechanical metrics were subjected to a phylogenetic principal components analysis (Revell 2009) of a correlation matrix in R (R Core Team 2012) using the package phytools (Revell 2011). The tree used for this analysis is described below. The scores (coordinates) of taxa on PC axes 1 and 2 were used to construct a mechanical morphospace (Fig. 2A). The same axes were also used for analyses of rates of biomechanical evolution (Fig. 2B). For analyses of disparity using the MATLAB package MDA (Navarro 2003), we employed scores from all 10 PC axes (Figs. 3 and 4).

We calculated 10 disparity metrics (see Supplementary Information) and associated 95% confidence intervals based on 10,000 taxonomic bootstrapping pseudoreplicates. We also calculated partial disparity (Foote 1993) for a series of taxonomic assemblages in order to compare their relative contributions to overall biomechanical variation. These assemblages, representing monophyletic, paraphyletic, and (in the case of lepospondyls only) polyphyletic groups (Ruta
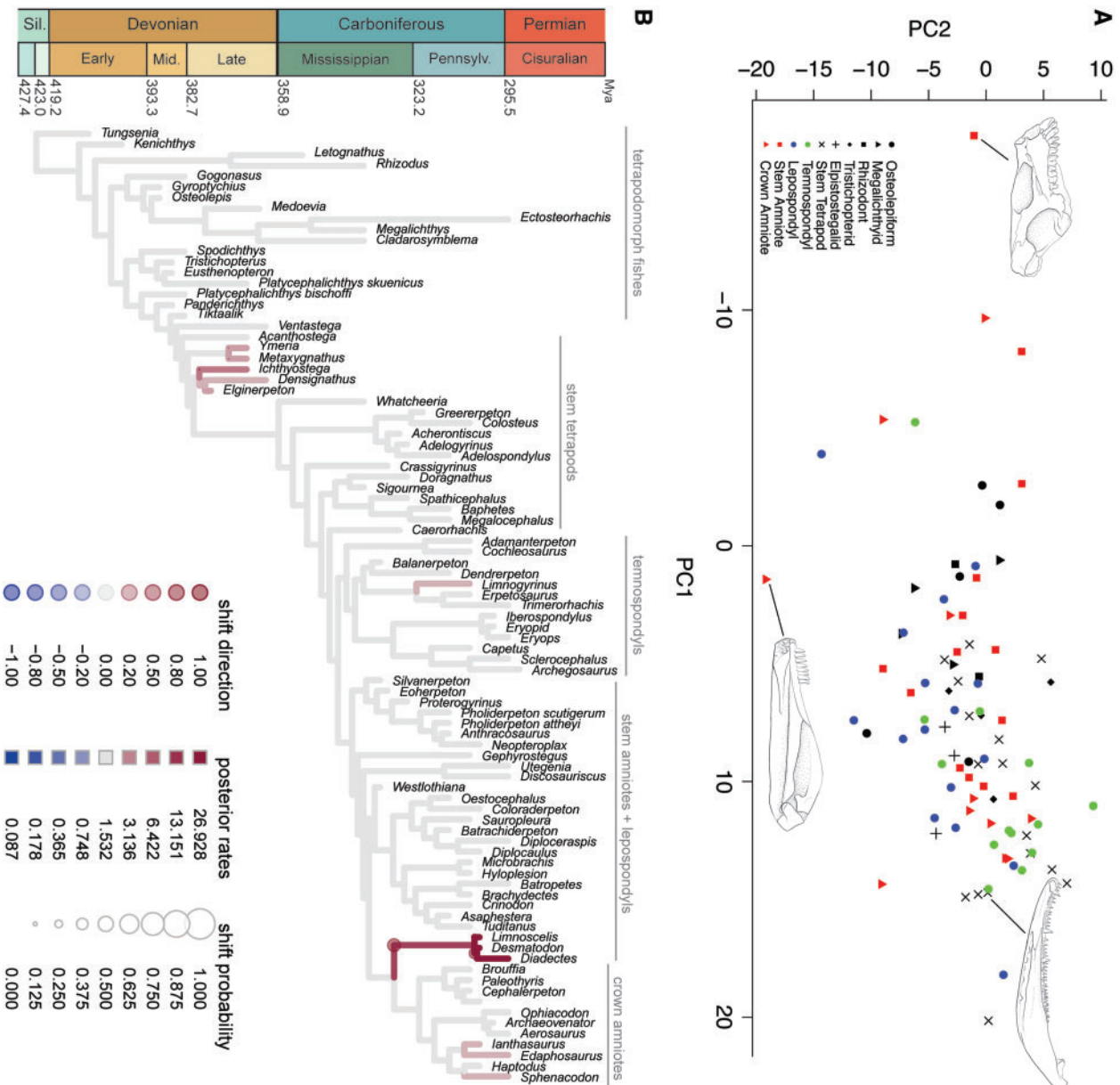
A

PC2

Legend:
- ► Osteolepiform
- ■ Megalichthyid
- ● Rhizodont
- × Tristichopterid
- + Elpistostegalid
- • Stem Tetrapod
- ▶ Temnospondyl
- ● Lepospondyl
- Stem Amniote
- Crown Amniote

PC1

B

Time scale: Sil. | Devonian (Early, Mid., Late) | Carboniferous (Mississippian, Pennsylv.) | Permian (Cisuralian)
Mya: 427.4, 423.0, 419.2, 393.3, 382.7, 358.9, 323.2, 295.5

Group labels: tetrapodomorph fishes; stem tetrapods; temnospondyls; stem amniotes + lepospondyls; crown amniotes

shift direction: 1.00, 0.80, 0.50, 0.20, 0.00, −0.20, −0.50, −0.80, −1.00
posterior rates: 26.928, 13.151, 6.422, 3.136, 1.532, 0.748, 0.365, 0.178, 0.087
shift probability: 1.000, 0.875, 0.750, 0.625, 0.500, 0.375, 0.250, 0.125, 0.000

Fig. 2 (A) Biomechanical morphospace showing the distribution of all taxa along PCs 1 and 2. Line drawings illustrate divergent jaw shapes of selected taxa. Some amniotes occupy a unique position relative to other groups, with strongly negative scores on PC 1. This region is dominated by taxa interpreted as early herbivores. Taxa represented (clockwise from bottom) are: the pelycosaur *Ianthasaurus*, the diadectid *Diadectes*, and the stem tetrapod *Ventastega*. (B) Rates of biomechanical evolution on PC 1. Branch color indicates estimated rates of evolutionary change. The size and color of discs at the internal nodes indicate the posterior probability and magnitude of rate shifts, respectively. Amniotes are unique in showing consistently high rates of functional change relative to background rates. A similar pattern is apparent for PC 2. A full version of this diagram and a comparable figure for PC 2 are given in Supplementary Information.

and Coates 2007), are as follows: (1) 18 tetrapodomorph fishes (*Tungsenia* to *Tiktaalik*), (2) 17 post-"elpistostegalian" stem tetrapods (*Ventastega* to *Colosteus*, plus *Crassigyrinus* to *Caerorhachis*), (3) 16 lepospondyls (*Acherontiscus* to *Adelospondylus*, plus *Oestocephalus* to *Tuditanus*), (4) 13 temnospondyls (*Adamanterpeton* to *Archegosaurus*), (5) 25 total-

group amniotes inclusive of 11 "reptiliomorph" stem amniotes (*Silvanerpeton* to *Westlothiana*, plus *Limnoscelis* to *Diadectes*), and 14 crown amniotes (*Brouffia* to *Sphenacodon*) (see Fig. 2B and Supplementary Information).

In order to test for significant shifts in jaw disparity between the six time bins, we used paired-sample
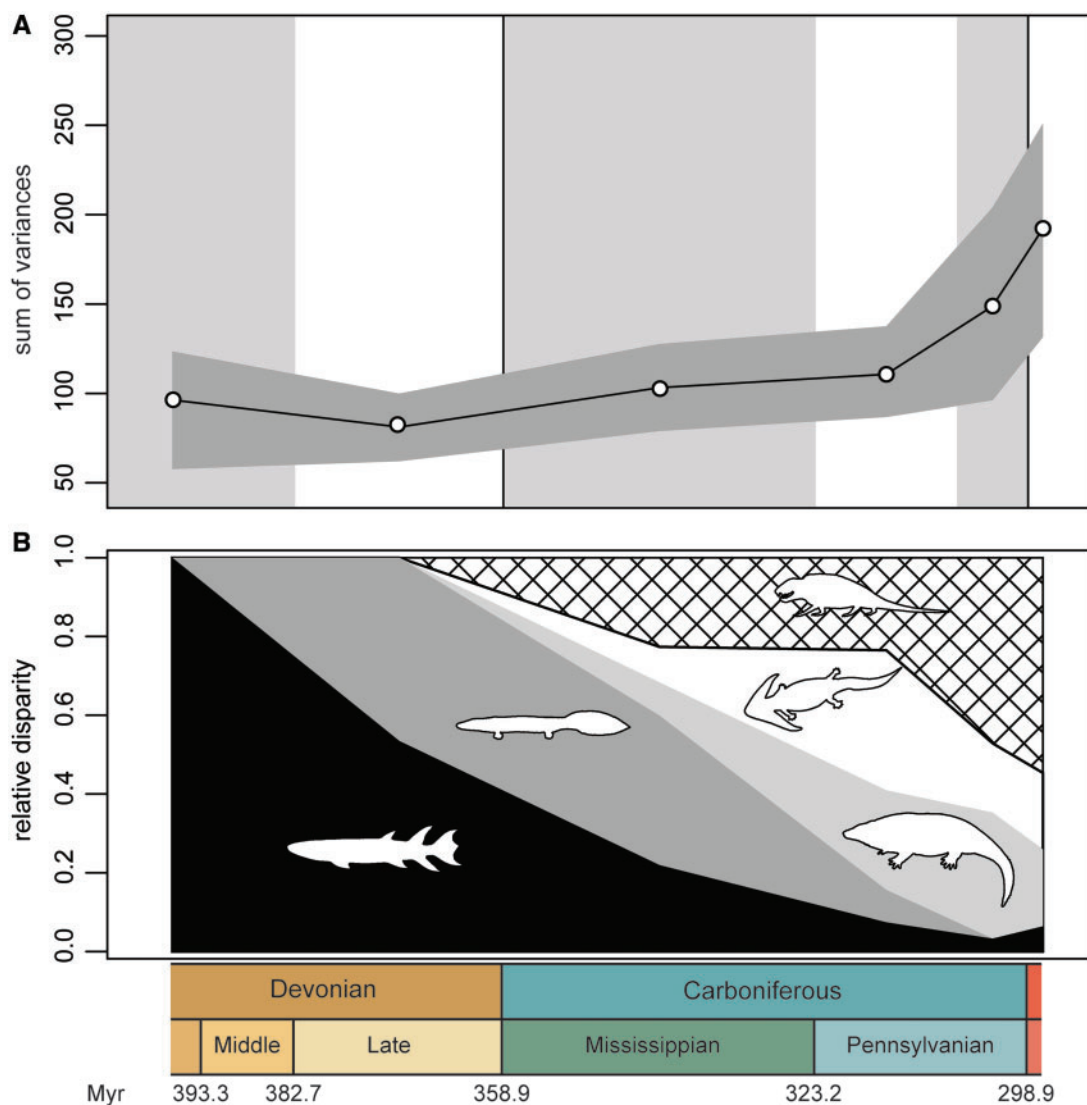
Fig. 3 (**A**) Biomechanical disparity (measured as sum of variances) through time. Mean disparity values (white circles) and associated 95% confidence envelope (gray region) are based on 10,000 bootstrap replicates. Disparity remains constant through most of the Devonian and Carboniferous until an almost exponential increase characterizes the latest Carboniferous and earliest Permian. (**B**) Partial disparity (*sensu* Foote 1993) graph. This shows the relative contributions of five groups to overall disparity: tetrapodomorph fishes (black), digited stem tetrapods (dark gray), temnospondyls (stem amphibians; light gray), lepospondyls (herewith assigned to stem amniotes; white), and amniotes (cross hatched). The overall increase in disparity reflects the dominant contribution of amniotes.

t-tests (Zelditch et al. 2004, 222) with significance levels corrected for multiple comparisons (Holm 1979). We also calculated marginal likelihoods for variance (Edwards 1992; Royall 1997) as an additional test for significant changes in disparity across adjacent time bins (Finarelli and Flynn 2007; Friedman 2010; Anderson et al. 2011).

We evaluated differences among groups (both temporal and taxonomic) in the mechanical morphospace with a non-parametric multivariate analysis of variance (npMANOVA; Anderson 2001) and an analysis of similarity (ANOSIM; Clarke 1993) in PAST (Hammer and Harper 2006), using PC scores on all axes. For both

methods, we applied a Bonferroni correction to *P*-values. Significance levels were based on 10,000 permutations.

Analysis of evolutionary rates

Plots of disparity through time record temporal changes in morphological variation, but provide no direct indication of shifts in rates of phenotypic evolution; thus, different evolutionary processes can yield remarkably similar trajectories of disparity (Foote 1996). Estimates of evolutionary rates require a hypothesis of phylogeny. We assembled an informal supertree by combining published
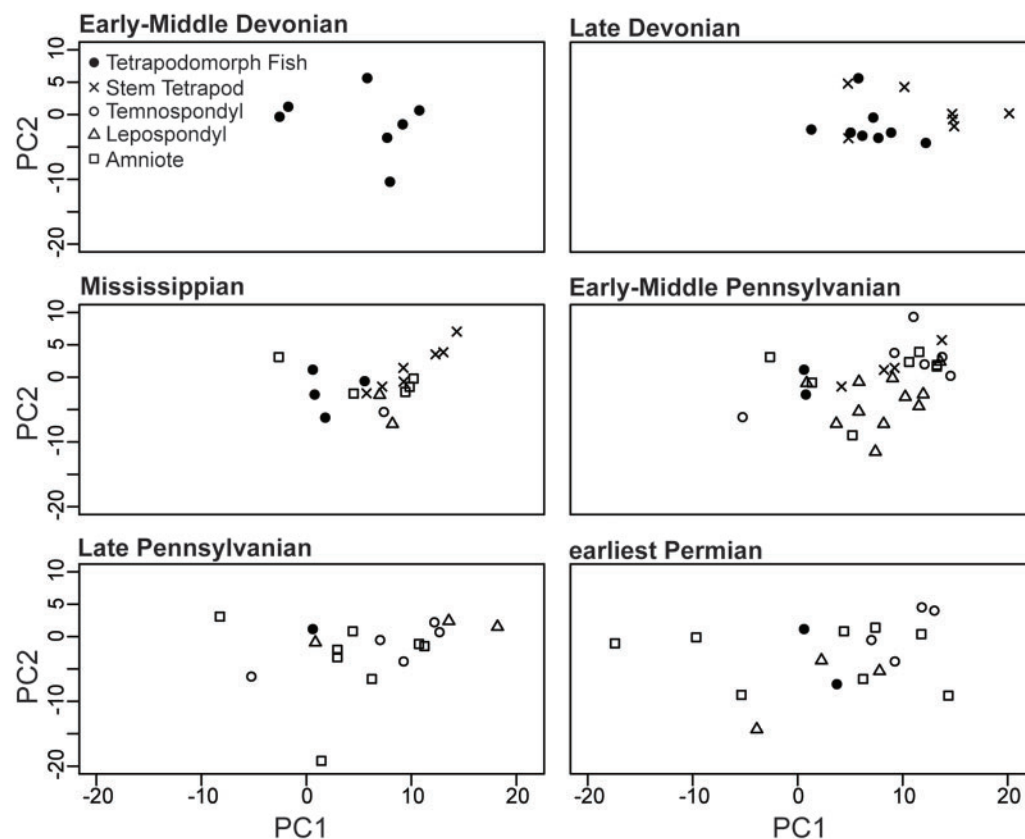
Fig. 4 Occupation of mechanical morphospace through time. For each time bin, we show plots of relevant taxa along PCs 1 and 2, using PC scores from the multivariate analysis. The Devonian sample consists of fishes and stem tetrapods, restricted to the upper right quadrant of the morphospace. By the Early–Mid Carboniferous, crown tetrapods show a minor excursion into the upper left and lower right quadrants of the morphospace. However, the left half of the morphospace remains mostly empty until the Late Pennsylvanian and Early Permian, when crown amniotes and their closest relatives began to expand.

topologies for different components of the tetrapod total group (Fig. 2B). The interrelationships of tetrapodomorph fishes derive from Swartz (2012), with *Tungsenia* placed as the deepest diverging branch within Tetrapodomorpha (Lu et al. 2012).

The relationships of early-digited stem tetrapods are mostly from Clack et al. (2012), while those of more crownward taxa derive largely from Ruta and Coates (2007). We have used additional trees to provide further details of internal relationships for some groups: Schoch (2013) for temnospondyls, Anderson (2007) and Maddin and Anderson (2012) for lepospondyls, Benson (2012) for synapsids, and Müller and Reisz (2006) for diapsids. We have time-scaled this tree using the method described by Brusatte et al. (2008) with R scripts developed by G. T. Lloyd (available at: http://graemetlloyd.com/methdpf.html). This approach requires tip ages (specifically, first-appearance data, or FADs) plus a constraint for the age of the root. We used generic FADs

for the former and 423.0 Mya for the latter, reflecting the age of the earliest known sarcopterygian (Zhu et al. 2009).

We adopted a Bayesian approach to detecting rate-shifts on a phylogeny, as implemented in the R package auteur (Eastman et al. 2011). PCs 1 and 2 collectively summarize over 43% of total variation in our dataset. A total of 10,000,000 generations were run for each of these axes, with the chain sampled every 1000 generations. We discarded the first 2500 samples as "burn-in." Color-coded shifts in rates were visualized using the appropriate functions in auteur (Eastman et al. 2011). We used the bootstrap test implemented in this same package in order to test for significantly different rates between different divisions of tetrapod phylogeny. Specifically, we considered the following comparisons: crown amniotes plus diadectids versus all remaining taxa, total-group amniotes versus all remaining taxa, and digit-bearing tetrapods versus tetrapodomorph fishes.

Results

Mechanical morphospace

The mechanical morphospace (Fig. 2A) shows the distribution of all sampled taxa based on biomechanical data from the jaw. Loadings of each of our mechanical characters along PCs 1 and 2 are provided in the Supplementary Information. PC 1 is dominated by mandibular aspect ratio, relative length of the adductor fossa, and the size of the symphysis. Jaws with strongly negative scores on this axis are dorsoventrally deeper and anteroposteriorly shorter than are most other sampled mandibles, with a longer adductor fossa and a larger symphysis. By contrast, jaws with strongly positive scores on PC 1 are long and narrow, with a relatively short adductor fossa. PC 2 is strongly influenced by the two mechanical advantage characters (MA hereafter), length of the dental row, and size of the adductor fossa. Jaws with strongly positive scores on PC 2 tend to show low MA values (particularly at the anterior bite point), combined with shorter dental rows, and smaller adductor fossae. Negative scores along PC 2 indicate long dental rows, high MAs, and longer adductor fossae.

Tetrapodomorph fishes show a restricted range of mechanical profiles and are generally characterized by positive scores on PC 1 (long, narrow jaws with short adductor fossae). The jaws of early digit-bearing tetrapods that fall outside the modern radiation are similar to those of "fish," but some taxa do show mechanical novelty as indicated by higher scores on PC 1. Extinct tetrapod assemblages such as lepospondyls and temnospondyls are more varied, with representatives distributed through most regions of mechanical morphospace. However, it is crown and near-crown amniotes that most fully explore mechanical morphospace, with several taxa characterized by strongly negative scores on PC 1. The taxa occupying this region include the stem amniote *Diadectes* and the crown amniote, pelycosaur-grade synapsids *Edaphosaurus* and *Ianthasaurus*.

Disparity through time

Stability in mandibular disparity is apparent from the Devonian until the Late Carboniferous (Fig. 3A). This is followed by a marked increase in variance, with a peak in the earliest Permian at the end of our data series. Statistical analysis (*t*-tests and marginal likelihoods) shows no significant shifts in disparity between any successive time bins when results are corrected for multiple comparisons. However, *t*-tests do indicate a significant overall shift in disparity from the Early Carboniferous to the Early Permian ($P < 0.006$).

Partial disparities reveal the relative contribution of five higher order taxonomic groups to overall variation over our sampling interval (Fig. 3B). The general pattern is one of introduction of new groups that slowly supersede older assemblages in terms of contribution to mechanical disparity. Stem tetrapods (both fishes and digited forms) dominate through the Mississippian, but groups such as lepospondyls, temnospondyls, and amniotes contribute overwhelmingly to overall disparity of tetrapods by the Pennsylvanian. The rapid rise in overall variation in the Late Carboniferous/Early Permian coincides with an increased contribution from amniotes.

Similar conclusions can be drawn from investigating patterns in the occupation of mechanical morphospace through time (Fig. 4). The Devonian sample of finned and digited stem tetrapods is restricted in terms of jaw mechanics and populates a relatively small area centered on the upper right quadrant of this plot (corresponding to positive scores on PCs 1 and 2). These taxa broadly overlap in space with tetrapodomorph fishes, but there is some evidence of functional innovation associated with the origin of extremely slender mandibles like that of *Elginerpeton*. The appearance of crown-group tetrapods in the Mississippian is not associated with a major change in occupancy of morphospace, with only a minor excursion into the upper left quadrant (corresponding to negative scores on PC 1 and positive scores on PC 2). It is not until the Late Carboniferous that tetrapods begin to more fully explore mechanical morphospace, with a major expansion into regions of space characterized by strongly negative scores on PCs 1 and 2. This shift is driven by amniotes and coincides with a clear increase in disparity.

Global tests of separation among groups of taxa in the six time bins returned significant results for both npMANOVA ($F = 2.594$; $P = 0.0001$) and ANOSIM ($R = 0.09053$; $P = 0.0023$). The following pairwise comparisons among time bins are significant (see Supplementary Information): in npMANOVA, Late Devonian taxa differ significantly from Mississippian, Early–Middle Pennsylvanian, Late Pennsylvanian, and earliest Permian taxa; in addition, Early–Middle Devonian and Early–Middle Pennsylvanian taxa also differ significantly; in ANOSIM, the only significant difference is between Late Devonian and earliest Permian taxa.

Disparity by groups

Overall, the five groups of taxa are significantly separate in morphospace (npMANOVA: $F = 3.63$,

$P = 0.0001$ and ANOSIM: $R = 0.1107$, $P = 0.0001$). In both npMANOVA and ANOSIM, the following three pairwise comparisons are significant: tetrapodomorph fishes versus post-"elpistostegalian" stem tetrapods and temnospondyls, and post-"elpistostegalian" stem tetrapods versus lepospondyls. Additional significant comparisons are between tetrapodomorph fishes and lepospondyls (in ANOSIM only), and between post-"elpistostegalian" stem tetrapods and amniotes (in npMANOVA only) (see Supplementary Information).

Evolutionary rates

Bayesian inference of evolutionary rates provides support for broadly congruent patterns of shifts for PC 1 and PC 2 (Fig. 2B). For both axes, our analyses highlight major increases in the rate of change within amniotes. On PC 1, this elevated rate characterizes the diadectids and some pelycosaurs. In particular, diadectids show the highest evolutionary rates relative to the mean value across our taxon sample. The only other concentration of consistently elevated rates of change is found within some early digit-bearing tetrapods. Shifts in rate estimated for PC 2 provide a complement to results for PC 1, with elevated rates found again within amniotes, especially members of the crown radiation (see Supplementary Information). Unlike the case for PC 1, there are clear examples of elevated rates of change for early diverging radiations along the amniote stem, with increases inferred within lepospondyls and anthracosaurs. Bootstrapping tests provide a means of assessing whether different regions of the tetrapod tree are characterized by significantly different rates of anatomical change. For PC1, we find that crown amniotes plus diadectids are characterized by significantly higher rates than all other taxa considered in our analysis ($P = 0.0166$), but that differences in rates between total-group amniotes and all remaining taxa ($P = 0.156$), and between tetrapods and tetrapodomorph fishes ($P = 0.305$), are not significant. The same three sets of comparisons do not provide support for significant differences in evolutionary rates on PC2 ($P = 0.223$–0.442).

Discussion

Delay in the diversification of jaw mechanics

The earliest body-fossil evidence for digit-bearing limbs is Famennian (372.2–358.9 Mya; Coates et al. 2008; Clack 2012), although the discovery of older putative trackways (Niedźwiedzki et al. 2010) and quantitative modeling of diversification (Friedman and Brazeau 2011) suggest that the origin of digits

might lie further back in time. Other tetrapod features, such as the absence of dorsal and anal fins, must have arisen even earlier, based on conditions in outgroups. It may then seem surprising that the feeding mechanics of tetrapods appear to change relatively little over the ~70–80 Myr interval following these profound postcranial transformations. The jaws of digited tetrapods are easily distinguished from those of their closest fish-like relatives by a series of specific features (Ahlberg and Clack 1998; Ruta and Bolt 2008), and there is some evidence for increased "experimentation" in some early digited tetrapods in the form of elevated rates of change (cf. Ahlberg et al. 2005b). However, these modifications do not appear to result in profound functional shifts, as gauged by our biomechanical measures. Our quantitative results therefore support previous hypotheses for a later diversification of mandibular function in tetrapods (Olson 1966; Clack 2012).

This result contradicts theoretical expectations that invasion of new habitats or acquisition of key novelties should lead to rapid initial diversification both in morphology and function. A partial solution to this problem lies with the issue of terrestrialization itself. The possession of digits is not unambiguous evidence for terrestriality. The earliest digited tetrapods were either mostly or obligately aquatic (Coates and Clack 1991), and qualitative arguments for terrestrial locomotion in some taxa (Ahlberg et al. 2005a) have not survived quantitative biomechanical analyses (Pierce et al. 2012). Added to this, it is apparent that many Carboniferous stem (e.g., *Crassigyrinus*, colosteids) and crown tetrapods (e.g., some embolomeres) were largely aquatic. Such patterns suggest that terrestrialization was a protracted process that cannot be neatly isolated to a particular phylogenetic node or a specific geological interval (Coates et al. 2008). The East Kirkton site records a diverse terrestrial ecosystem including tetrapods and provides a conservative minimum estimate for the origin of terrestriality in at least some lineages (Clack 2012). However, East Kirkton is Viséan (346.7–330.9 Mya) in age, implying a gap of tens of millions of years between the conquest of land and appreciable diversification of tetrapod feeding mechanics.

The most substantial innovations in tetrapod mandibular function are restricted both stratigraphically and phylogenetically. Late Pennsylvanian or younger taxa that can be placed within the amniote crown, or form immediate outgroups to this clade, drive major shifts in patterns of morphospace occupancy. These taxa tend to have deep, robust mandibles with high closing mechanical advantages, large

symphyses, and a jaw joint that is highly offset from the level of the tooth row. These traits are commonly observed in modern mammals that utilize a grinding action to break down food and to process tough materials (Ungar 2010). The exploration of new regions of morphospace by amniotes is also accompanied by a statistically significant increase in rates of mandibular functional evolution relative to other tetrapods along PC1.

This Late Pennsylvanian–Early Permian interval of innovation corresponds to the inferred onset of tetrapod herbivory (Olson 1966; King 1996; Hotton et al. 1997; Sues and Reisz 1998; Reisz and Sues 2000; Sues 2000; Pearson et al. 2013). Taxa of this age that conspicuously expand the limits of previously realized tetrapod morphospace include diadectid stem amniotes (*Diadectes*, *Desmatodon*, and *Limnoscelis*) and edaphosaurid synapsids (*Ianthasaurus* and *Edaphosaurus*), both previously interpreted as the earliest herbivorous tetrapods (Reisz and Sues 2000). Our analysis therefore provides quantitative corroboration for trophic innovation in these clades relative to other tetrapods (cf. Olson 1966; Sahney et al. 2010) and adds further detail to our understanding of the origin of tetrapod herbivory by showing that this breakthrough was associated with an increase in rates of functional evolution of the lower jaw. Such claims must be tempered with the caveat that exploitation of plant resources could have preceded the origin of mandibular traits associated with dedicated herbivory. Most vertebrates are dietary generalists despite their functional specializations ("Liem's paradox"; Liem 1980; Robinson and Wilson 1998). Deeper roots for herbivory cannot be dismissed out of hand, given direct evidence for a plant diet in Paleozoic tetrapods that lacked obvious specializations for this feeding strategy (Munk and Sues 1992).

Biomechanical shifts in evolution of the jaw and in the fish–tetrapod transition

Delayed functional innovation in tetrapod jaws relative to the emergence of key postcranial transformations for terrestrial life might be unexpected, but this offset pattern is not unprecedented. Complexes of anatomical traits that contribute to a biomechanical function and covary independently of anatomical traits from other mechanical systems can be considered functional modules (Klingenberg 2010). Potential functional modularity at an evolutionary scale is illustrated in other major evolutionary transitions such as the origin of primates, horses, and birds (MacFadden 1994; Clarke and Middleton

2008; Jurmain et al. 2011). However, testing for functional modularity in early tetrapods is beyond the scope of this study. Rather, our analyses of rates have allowed us to map biomechanical shifts in one mechanical system and note whether these shifts coincide topologically with acquisition of apomorphic traits that mark major structural modifications in tetrapod evolution. Several studies suggest that offsets between intervals of locomotor and trophic diversification might be a relatively general feature of vertebrate adaptive radiations (e.g., Streelman and Danley 2003; Gavrilets and Losos 2009; Young et al. 2009; Glor 2010; Sallan and Friedman 2012), and our results lend partial support to this.

Although early terrestrial vertebrates were confronted by new dietary resources on land, our results suggest that terrestriality alone was insufficient to permit radiation into highly divergent trophic roles. The delayed ascent of tetrapod herbivory in particular has long been appreciated (reviewed by Reisz and Sues 2000), with several hypotheses proposed to account for this macroevolutionary lag. Apart from the modifications to jaws and teeth that we have considered, effective processing of vegetable matter demands changes in physiology and soft-tissue anatomy that are not preserved in fossils. These requirements include an enlarged or lengthened digestive tract, coupled with a gut flora capable of efficiently breaking down high-fiber plant material. Circumstantial evidence for the former can be drawn from bodily proportions, but there is no obvious means of detecting the latter in fossil species (Reisz and Sues 2000).

Independent appearances of herbivory in stem (diadectids) and crown amniotes (edaphosaurids) in the Late Pennsylvanian were followed by at least four other origins of this feeding strategy in Permian crown amniotes, and dozens more in subsequent intervals (Sues and Reisz 1998; Reisz and Sues 2000). The proliferation of herbivores within amniotes is even more striking when contrasted with patterns in lissamphibians, their extinct temnospondyl relatives, and stem tetrapods. Despite being characterized by impressive morphological (e.g., Stayton and Ruta 2006; Angielczyk and Ruta 2012), taxonomic (e.g., Ruta et al. 2007; Clack 2012), and presumably ecological diversity (Fortuny et al. 2011), none of these non-amniote assemblages contain radiations of herbivorous forms. In fact, only a single folivorous example is known among extant amphibians (the frog *Euphlyctis hexadactylus*; Das 1996).

The diversity of herbivorous lineages within amniotes compared with their effective absence in other tetrapods demands explanation. It may be argued

that non-amniote groups have been excluded from herbivorous roles by incumbent amniote groups. However, this hypothesis cannot explain the failure of non-amniotes to exploit this trophic strategy before the origin of amniotes, and the numerous independent origins of herbivory within amniotes questions whether incumbency represents a major evolutionary barrier. Instead, it seems more probable that some aspects of amniote biology or anatomy have allowed this group to adopt herbivory, while the feeding repertoires of non-amniotes are constrained by the absence of these features. Several amniote traits have been proposed as the "key innovation" permitting herbivory (e.g., Hotton et al. 1997), but the hypothesis outlined by Janis and Keller (2001) is particularly intriguing. These authors propose that the shift from buccal pumping to costal breathing in the amniote stem lineage freed the skull from constraints associated with ventilation, thereby permitting structural innovations necessary for consuming plant matter. A more general prediction that could be drawn from this hypothesis is that the relaxation of constraints may lead to increase in disparity (whether structural or functional), although this may or may not be underpinned by higher rates of evolutionary change in amniote cranial structure in general. However, our discovery that crown amniotes and their closest extinct relatives are characterized by higher rates of mandibular functional evolution than are non-amniotes is consistent with this prediction and the expected patterns associated with adaptive radiations.

Circumstances leading to the origin of herbivory are still debated (e.g., Janis and Keller 2001), but it is clear that the appearance of this feeding strategy marks the beginning of recognizably modern community structure in tetrapod assemblages (Reisz and Sues 2000). On this basis, it could be argued that the evolution of herbivory in the Late Mississippian—rather than the colonization of land—forms a more natural bookend to the story of vertebrate terrestrialization that began in the Late Devonian or earlier.

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Supplementary data

Supplementary Data available at *ICB* online.

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