RESEARCH ARTICLE

The evolution of asymmetrical gaits in gnathostome vertebrates

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ABSTRACT

The difficulty of quantifying asymmetrical limb movements, compared with symmetrical gaits, has resulted in a dearth of information concerning the mechanics and adaptive benefits of these locomotor patterns. Further, no study has explored the evolutionary history of asymmetrical gaits using phylogenetic comparative techniques. Most foundational work suggests that symmetrical gaits are an ancestral feature and asymmetrical gaits are a more derived feature of mammals, some crocodilians, some turtles, anurans and some fish species. In this study, we searched the literature for evidence of the use of asymmetrical gaits across extant gnathostomes, and from this sample (*n*=308 species) modeled the evolution of asymmetrical gaits assuming four different scenarios. Our analysis shows strongest support for an evolutionary model where asymmetrical gaits are ancestral for gnathostomes during benthic walking and could be both lost and gained during subsequent gnathostome evolution. We were unable to reconstruct the presence/absence of asymmetrical gaits at the tetrapod, amniote, turtle and crocodilian nodes with certainty. The ability to adopt asymmetrical gaits was likely ancestral for Mammalia but was probably not ancestral for Amphibia and Lepidosauria. The absence of asymmetrical gaits in certain lineages may be attributable to neuromuscular and/or anatomical constraints and/or generally slow movement not associated with these gaits. This finding adds to the growing body of work showing the early gnathostomes and tetrapods may have used a diversity of gaits, including asymmetrical patterns of limb cycling.

KEY WORDS: Gallop, Gait, Tetrapod, Locomotion, Evolution, Comparative methods

INTRODUCTION

A key aspect of how animals move is locomotor gait. Gait is defined as the cyclical pattern of limb movements used during locomotion (Goiffon and Vincent, 1779; Muybridge, 1887; Hildebrand, 1976). Substrate-based gaits are typically divided into symmetrical gaits and asymmetrical gaits. Symmetrical gaits are those where limbs move in regular, evenly timed and alternating sequence with symmetry of left and right footfall times. Trots, singlefoots, lateral-sequence, diagonal-sequence and paces are examples of symmetrical gaits (Fig. 1A). Asymmetrical gaits are those where left and right footfall timings are not evenly spaced and include gallops, half-bounds, bounds, pronks, crutching and punting (Hildebrand, 1977; Koester and Spirito, 2003). Gallops are

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defined as all four footfalls occurring at different and unevenly spaced times, with the timing of the forefoot lead and the hindfoot lead defining the type of gallop (Fig. 1E). Half-bounds occur when the hind-footfalls are simultaneous, but the fore-footfalls are not synchronized. Bounds occur when the hind-footfalls are simultaneous, and the fore-footfalls are simultaneous but the forelimbs and hindlimbs are not synchronized (Fig. 1D). Pronks occur when all four footfalls are simultaneous. While these four types of asymmetrical gaits have historically been the only ones considered, it is important to note that from a neuromuscular perspective any motor program that involves asynchronous, cyclical movements of the limb pairs is an asymmetrical gait. As such, it is appropriate to classify crutching and punting as asymmetrical gaits even though each involves only one limb pair (forelimbs for crutching, hindlimbs for punting). Crutching is like the half-bound, but with the forelimb footfalls synchronized and the hindlimb footfalls unevenly temporally spaced and/or completely absent as seen in some fishes, some sea turtles and pinnipeds (Fig. 1C). In contrast, punting occurs during benthic locomotion in some fishes and involves the simultaneous use of the pelvic fins against the substrate (Fig. 1, Table 1).

Much of the foundational work on substrate-based gaits suggests that symmetrical gaits are ancestral and asymmetrical gaits are a more derived feature of mammals (de la Croix, 1936; Gambaryan, 1974; Edwards, 1977; Wimberly et al., 2021). Yet, mounting evidence suggests that asymmetrical gaits may not be a recent or uniquely mammalian innovation. Several species of crocodilians have long been known to gallop (Zug, 1974; Hutchinson et al., 2019), studies of anuran locomotion have highlighted the use of bounding gaits by some species (Gans and Parsons, 1966; Reilly et al., 2015) and at least one pleurodire turtle species bounds when underwater (Mayerl and Blob, 2017). In fact, Hildebrand (1977) hypothesized that asymmetrical gaits evolved repeatedly in tetrapods, including among amphibians, reptiles and mammals. Additionally, some sea turtle species use a crutching gait when moving on land to deposit eggs (Wyneken, 2017). The fossil record has evidence that some ancient tetrapods may have used asymmetrical gaits (Clack, 1997; Niedźwiedzki et al., 2010) and application of computer modeling to fossils suggests that asymmetrical forelimb movements were likely the only possibility in some groups (e.g. *Ichthyostega*; Pierce et al., 2012). Some extant fish species during benthic and/or terrestrial locomotion will use asymmetrical gaits (King et al., 2011; Kawano and Blob, 2013).
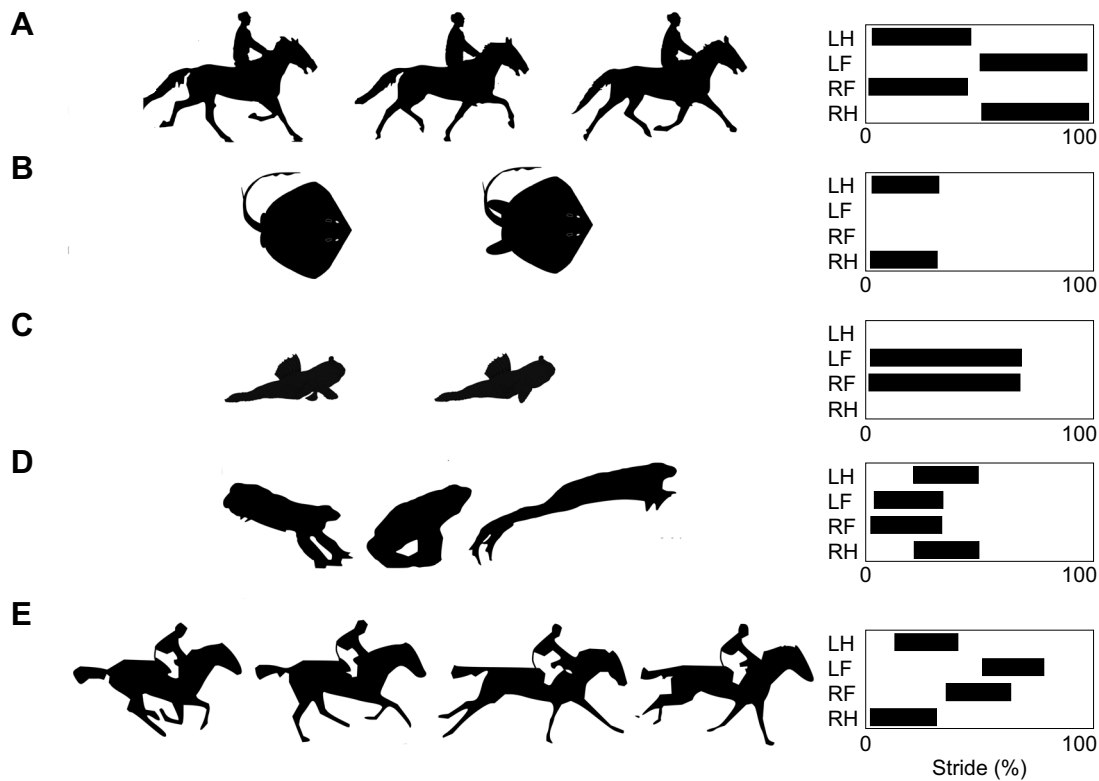
However, asymmetrical gaits are conspicuously absent from entire groups, such as lizards, monotremes and salamanders (Table 1). Why these groups seemingly cannot or do not use asymmetrical gaits is unknown, yet the disparate positions of these groups on the gnathostome phylogeny coupled with the fact that many other groups can use asymmetrical gaits suggests three possibilities for the sequence of asymmetrical gait evolution. First, the absence of asymmetrical gaits could be the ancestral character state for gnathostomes that has been retained in lizards, monotremes and salamanders. Under this hypothesis, asymmetrical gaits are a

Fig. 1. Examples of various gaits. (A) A symmetrical trotting gait. (B–E) Examples of asymmetrical gaits, including punting (B), crutching (C), bounding (D) and galloping (E). Plots to the right of each image sequence represent footfall patterns associated with each gait. RH, right hindlimb; RF, right forelimb; LF, left forelimb; LH, left hindlimb.

derived feature that has convergently evolved in therian mammals, several crocodilians, some anurans, some turtles, African lungfish (*Protopterus annectens*) and several actinopterygian species. Second, an asymmetrical gait could be an ancient feature of gnathostomes that was present at the tetrapod and amniote nodes but has been lost in lizards, monotremes, salamanders, some anurans, most turtles, some therian mammals and some fishes. Third, asymmetrical gaits are highly labile and have been lost and gained repeatedly during gnathostome evolution.

We compiled a large dataset from the literature of substrate-based asymmetrical gait presence/absence in extant species and used phylogenetic comparative methods to fit evolutionary models and infer the ancestral states for the presence/absence of asymmetrical gaits for several key gnathostome clades. It should be

Table 1. Asymmetrical gait use by different groups of gnathostomes

	Gallop	Half-bound	Bound	Pronk	Crutch	Punt
Chondrichthyes						✓
Actinopterygii	✓				✓	
Dipnoi			✓			
Caudata						
Anura			✓			
Testudines			✓		✓	
Lepidosauria						
Crocodylia	✓	✓	✓			
Monotremata						
Marsupialia	✓	✓	✓			
Eutheria	✓	✓	✓	✓	✓	

The presence of a gait is indicated by a tick; absence of a tick indicates absence of a gait. See Introduction for a definition of different gaits.

noted that our inclusion of taxa in our sample only involved species that make fin/limb contact with benthic, terrestrial or arboreal substrates. As such, throughout the paper, the use of 'gnathostome', 'chondrichthyan', 'actinopterygian' or 'sarcopterygian' is limited to species that move on benthic or terrestrial substrates. As such, the reader should not apply any of the forthcoming results or discussion to the neural circuitry involved in swimming. We modeled the evolution of asymmetrical gaits assuming four different evolutionary scenarios: (1) gains and losses of asymmetrical gaits occur at different rates (all rates different, ARD model), (2) gains and losses of asymmetrical gaits occur at equal rates (equal rates, EQUAL model), (3) asymmetrical gaits occurred at the root of gnathostome phylogeny and then could only be lost and not regained (LOSS model) and (4) asymmetrical gaits did not occur at the root of gnathostome phylogeny and could only be gained but could not be lost once gained (GAIN model). We note that the ARD and EQUAL models allow free changes between asymmetrical and symmetrical gaits anywhere on the phylogeny and only differ in that the EQUAL model forces the evolutionary rate of losses and gains to be equal. The LOSS model specifically tests the hypothesis that asymmetrical gaits are ancestral for gnathostomes while the GAIN model tests the hypothesis that asymmetrical gaits have convergently evolved in several crown clades.

MATERIALS AND METHODS

We extensively searched the literature for evidence for the use of asymmetrical gaits across extant gnathostomes. We treated studies of locomotor gait that did not observe asymmetrical gaits as evidence that the observed species did not use asymmetrical gaits. This choice may result in an underestimation of the number of species that use asymmetrical gaits in our sample, as some studies

may not have collected data on fast running speeds or other situations where asymmetrical gaits were more likely to occur. We only included studies that involved a limb–substrate interaction, even when underwater. Thus, swimming gaits in which the limb/fin did not contact the substrate were not considered in this analysis. Species that exhibited symmetrical gaits but lacked asymmetrical gaits were coded as 0. Single synchronous limb movements (i.e. a jump) were not considered a gait because they are not rhythmic and thus species that only jump (i.e. many anurans) were not included in our dataset. For example, walking frogs (*Kassina*) were coded as 0 because they use a symmetrical walking gait, can jump, but do not exhibit a rhythmic asymmetrical gait (Ahn et al., 2004), whereas some toad species use a bounding gait and were coded as 1 (Reilly et al., 2015). Several hopping anurans may be capable of a bounding gait (Zug, 1978; Emerson, 1979); however, specific kinematic criteria were used from high-speed video to determine that toad hopping was actually a bounding gait and so we chose to not include hopping anurans that lacked these specific kinematic data in our dataset. We lumped all subtypes of asymmetrical gaits into a single

category and coded that category as presence of asymmetrical gaits=1. Lumping of different types of asymmetrical gaits was done because many subtypes are used by a single or only a few clades, making analysis of the subtypes using phylogenetic comparative methods not possible.

We used a variety of sources to compile a phylogeny for the gnathostome species in our sample. We first built the trunk of the tree to include the most recent common ancestor (mrca) of each of the following crown groups: Chondrichthyes, Actinopterygii, Dipnoi, Amphibia, Lepidosauria, Testudines, Crocodylia, Aves, Mammalia. Tree topology was fixed to widely accepted relationships among these major groups (Fig. 2) and the date of each mrca node was fixed to the mean value reported at TIMETREE (www.timetree.org) (Hedges et al., 2006, 2015; Kumar and Hedges, 2011; Kumar et al., 2017). Next, we grafted a tree for each crown group onto this trunk. To do this, we retrieved 1000 posterior samples of trees from VertLife.org (www.vertlife.org/phylosubsets) that were generated from phylogenetic analyses of Lepidosauria (Tonini et al., 2016), Amphibia (Jetz and Pyron, 2018), Mammalia
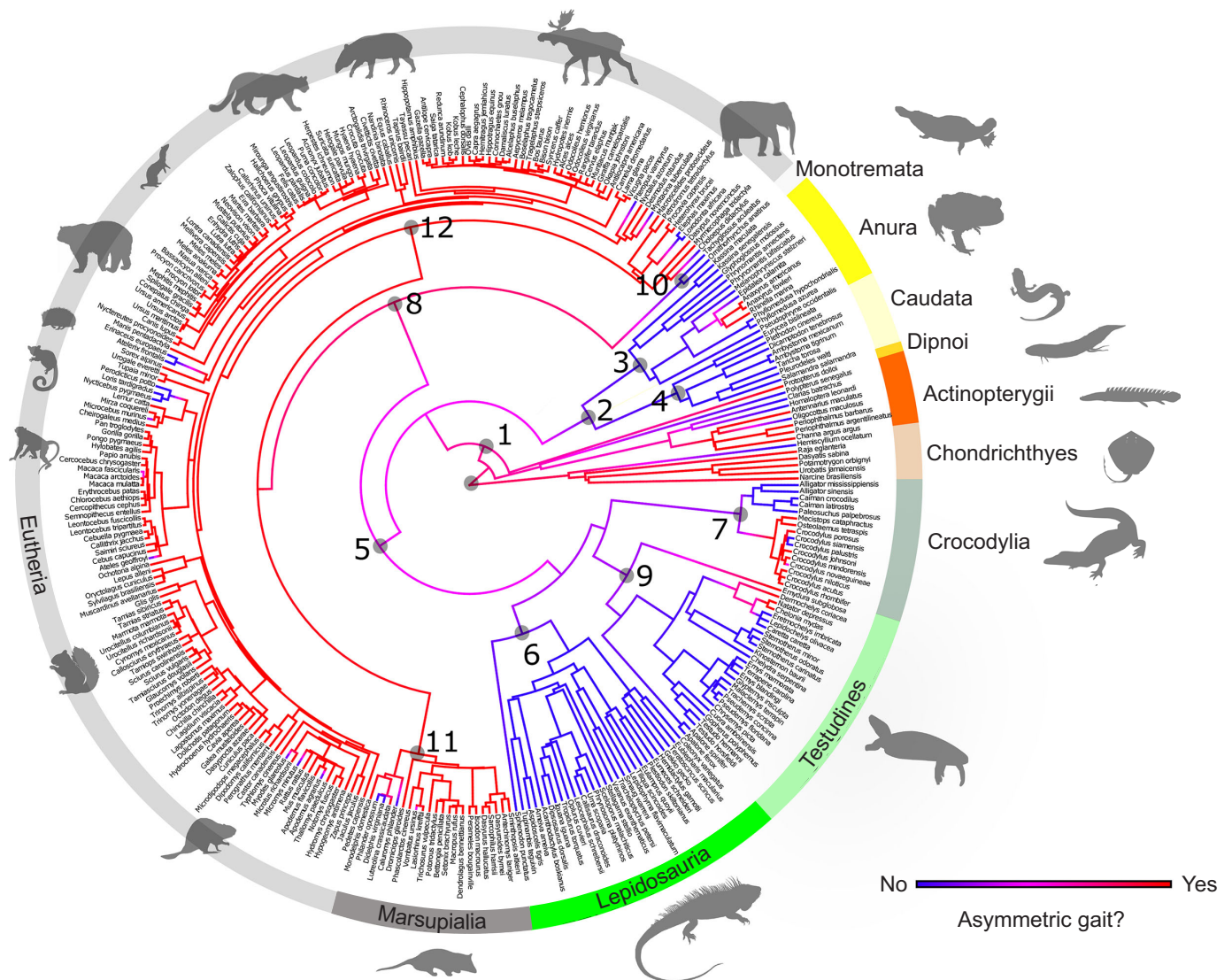
Fig. 2. Stochastic character mapping for the best fit evolutionary model (ARD) showing the probability of having an asymmetrical gait. Branch color is the probability of having an asymmetrical gait (red) or not (blue) or mixed support (pink/purple). Key nodes are numbered as follows: (1) Tetrapoda, (2) Amphibia, (3) Anura, (4) Caudata, (5) Amniota, (6) Lepidosauria, (7) Crocodylia, (8) Mammalia, (9) Testudines, (10) Monotremata, (11) Marsupialia, (12) Eutheria. Data derived from 308 gnathostome species. Silhouettes are from phylopic.org (available for reuse under a Public Domain license).

Table 2. Statistical results from maximum likelihood (ML) and Markov chain Monte Carlo (MCMC) fitting of the four models for the evolution of asymmetrical gaits in gnathostomes

Model	ML						MCMC		
	q_{gain}	q_{loss}	Max. lnL	AIC	AIC_w	ΔAIC	Marg. lnL	q_{gain}	q_{loss}
ARD	0.011	0.036	−101.9	207.9	0.91	0	−110.1	0.013±0.006	0.038±0.008
LOSS	0	0.038	−105.8	213.5	0.05	5.6	−109.9	0	0.039±0.006
EQUAL	0.024	0.024	−106.0	214.0	0.04	6.1	−110.3	0.026±0.006	0.026±0.006
GAIN	0.048	0	−173.7	349.3	0.00	141.4	−177.8	0.049±0.007	0

MCMC results are means±s.d. ARD, evolutionary gains and losses of asymmetrical gaits occur at different rates; EQUAL, evolutionary gains and losses of asymmetrical gaits occur at equal rates; LOSS, asymmetrical gaits occurred at the root of gnathostome phylogeny and then could only be lost and not regained; GAIN, asymmetrical gaits did not occur at the root of gnathostome phylogeny and could only be gained but not lost once gained; q_{gain}, evolutionary rate for gaining asymmetrical gaits; q_{loss}, evolutionary rate for losing asymmetrical gaits; Max. lnL, maximum log likelihood; AIC, Akaike information criteria; AIC_w, Akaike information criteria weight; Marg. lnL, marginal log likelihood.

(Upham et al., 2019) and Chondrichthyes (Stein et al., 2018). From these posterior samples, we computed the maximum clade credibility tree for each clade. The tree for Actinopterygii was downloaded from the Fish Tree of Life database (https://fishtreeoflife.org/) using the package *fishbase* (Chang et al., 2019; Rabosky et al., 2018). We used a pruned maximum clade credibility tree for Testudines from a recent study (Thomson et al., 2021). The tree for Crocodylia was downloaded from TIMETREE (www.timetree.org) (Hedges et al., 2006, 2015; Kumar and Hedges, 2011; Kumar et al., 2017). The final tree was not ultrametric because of the decimal precision of the branch length estimates in the grafted trees; therefore, we forced the final tree to be ultrametric by adding small amounts of branch lengths as needed. R-packages used to construct the trees included *ape* (Paradis et al., 2004) and *phangorn* (Schliep, 2011).

Using the final constructed phylogeny and tip data on the use of asymmetrical gaits, we tested the four different models of evolution noted above. First, we assumed that gains and losses of asymmetrical gaits evolved freely and thus had separately estimated rate parameters (ARD model). Second, we assumed that gains and losses of asymmetrical gaits evolved at equal rates (EQUAL model). Third, we assumed that asymmetrical gaits could only be gained (GAIN model), which assumes that the rate parameter for losses of asymmetrical gaits is zero and so the rate parameter for gains is estimated. Fourth, we assumed that asymmetrical gaits could only be lost (LOSS model), which assumes that the rate parameter for gains of asymmetrical gaits is zero and then estimates the rate parameter for losses. Note that the GAIN model assumes that the root of the tree does not have an asymmetrical gait while the LOSS model assumes the root has an asymmetrical gait.

We assessed relative support for these models using two different methods: maximum likelihood (ML) and Bayesian inference using Markov chain Monte Carlo (MCMC) analysis. ML and MCMC analyses were done in BayesTraits V3.0 (Pagel et al., 2004) using a wrapper function from the *btw* package (https://rdrr.io/github/rgriff23/btw/) in R studio version 1.3.1093 (http://www.rstudio.com/). For these analyses we assumed a continuous time Markov model of trait evolution and scaled branch lengths=0.1. First, we computed the maximum likelihood estimate for each model and compared models using Akaike information criteria (AIC), delta AIC and Akaike weights. Second, we used a Bayesian framework with MCMC analysis to estimate the posterior distributions of log-likelihoods, rate parameters and ancestral character states for each model. All MCMC analyses had these settings: 1 million iteration burn-in followed by 4 million iterations which were sampled every 1000 iterations, uniform prior distribution with a range of 0 to 1, stepping stone sampler to estimate the marginal likelihood with 100 stones each run for 10,000 interactions. For this analysis, we

computed log Bayes factors (BF) between each pair of evolutionary models; negative log BF suggests support for the alternative model, log BF between 0 and 2 is weak evidence supporting the alterative model and BF>5 is strong evidence of one model versus another model (Kass and Raftery, 1995). We examined trace and autocorrelation plots and found no issues with the MCMC analyses. Effective sample sizes were always large (>1000). For each evolutionary model, we ran MCMC analysis 3 times with different starting points and we found that the MCMC converged on qualitatively similar results. We estimated ancestral character states using the methods above as well as stochastic character mapping (SCM) using the *phytools* package (Revell, 2012).

RESULTS

In total, we collected data from 308 gnathostome species. The ML analysis clearly favored the ARD evolutionary model over all other models (Table 2). The rate parameter for losing asymmetrical gaits (q_{loss}) was ~3 times larger than the rate parameter for gaining asymmetrical gaits (q_{gain}). The MCMC analysis was less decisive, with log BF suggesting equal support for the ARD, LOSS and EQUAL models (Table 3). The ARD and LOSS models had similar rate parameters (i.e. $q_{loss} \gg q_{gain}$). Both the ML and MCMC analysis clearly rank the GAIN model as the least likely evolutionary scenario compared with all other models.

Ancestral reconstructions of asymmetrical gait use for the ARD model are found in Table 4. The ARD model favors the reconstruction of asymmetrical gaits at the gnathostome root: Mammalia, Eutheria and Marsupialia (Fig. 2, red). Lack of asymmetrical gaits are reconstructed at Amphibia, Anura, Caudata, Lepidosauria and Monotremata (Fig. 2, blue). Reconstructions at the tetrapod, amniote, crocodilian and turtle nodes are equivocal (Fig. 2, pink/purple). The ML, MCMC and SCM analyses all reached similar conclusions for most nodes.

Table 3. log Bayes factor (BF) between pairwise comparisons of the different evolutionary models fitted using MCMC

log BF	ARD	EQUAL	GAIN
LOSS	0.4	0.9	135.8
ARD		0.5	135.5
EQUAL			134.9

log BF was computed as 2×(row log marginal likelihood−column log marginal likelihood). ARD, evolutionary gains and losses of asymmetrical gaits occur at different rates; EQUAL, evolutionary gains and losses of asymmetrical gaits occur at equal rates; LOSS, asymmetrical gaits occurred at the root of gnathostome phylogeny and then could only be lost and not regained; GAIN, asymmetrical gaits did not occur at the root of gnathostome phylogeny and could only be gained but not lost once gained.

Table 4. Probability of having an asymmetrical gait (P_{asym}) at various key nodes in the Gnathostome phylogeny for the ARD model

Node no.	Node name	P_{asym}		
		ML	MCMC	SCM
	Vertebrata (root)	0.791	0.747±0.161	0.774
1	Tetrapoda	0.489	0.520±0.175	0.649
2	Amphibia	0.126	0.145±0.050	0.166
3	Anura	0.048	0.071±0.054	0.026
4	Caudata	0.024	0.031±0.017	0.008
5	Amniota	0.546	0.557±0.186	0.421
6	Lepidosauria	0.107	0.118±0.035	0.130
7	Crocodylia	0.508	0.513±0.127	0.237
8	Mammalia	0.926	0.912±0.047	0.751
9	Testudines	0.468	0.487±0.143	0.255
10	Monotremata	0.027	0.031±0.012	0.048
11	Marsupialia	0.987	0.988±0.013	0.997
12	Eutheria	1.000	1.000±0.001	1.000

Values for MCMC P_{asym} are presented as means±1 s.d. ARD, evolutionary gains and losses of asymmetrical gaits occur at different rates; SCM, stochastic character mapping.

DISCUSSION

Explaining patterns of asymmetrical gait evolution

We provide evidence that the ancestor of gnathostomes could have used asymmetrical gaits during benthic locomotion but we were unable to equivocally determine gait use for the ancestor of tetrapods and amniotes, even though our analysis clearly reconstructs the presence or absence of asymmetrical gaits as ancestral for most other clades (crocodylians and turtles being notable exceptions). A possible explanation for this pattern is that the neural circuitry required for asymmetrical gaits could be a feature of all gnathostomes that is only expressed in certain species or clades that have accompanying musculoskeletal systems that allow for asynchronous limb movements. Under such a scenario, the equivocal reconstruction at the tetrapod and amniote nodes may reflect the diversity of stem tetrapod and amniote bauplans and locomotor modes (Pierce et al., 2013), with groups that could and could not use asymmetrical gaits being present during early tetrapod and amniote evolution (but see 'Limitations', below). This result is in concordance with recent analyses that show that the earliest tetrapods (e.g. *Ichthyostega*) could have moved with a crutching, mudskipper-like asymmetrical gait (Pierce et al., 2012; Molnar et al., 2021) and a modern view that early tetrapods may have used a variety of locomotor modes, including both asymmetrical gaits (Pierce et al., 2013) and symmetrical gaits (Nyakatura et al., 2014; Nyakatura et al., 2019; Wimberly et al., 2021).

The clearer reconstruction of several clades (Eutheria, Marsupialia, Monotremata, Lepidosauria, Anura, Caudata) may reflect how these groups have coalesced around clade-specific bauplans and locomotor modes. But clades are not completely 'locked': asymmetrical gaits evolve or are lost in a scattering of species in these extant clades; for example, sea turtles and toads evolved asymmetrical gaits from ancestors that probably did not use asymmetrical gaits while several eutherian mammals, monotremes, some crocodilians and some fishes lost asymmetrical gaits from ancestors that probably used asymmetrical gaits.

So why do some clades and/or terminal species not use asymmetrical gaits? The absence of any 'gait' could occur because species or clades either (1) do not move at the fast speeds that are often associated with asymmetrical gaits or (2) lack the neural circuitry and/or the musculoskeletal anatomy required to generate specific limb coordination patterns. Some species may lack the athletic ability that is often associated with asymmetrical gaits.

For example, hedgehogs, *Philander*, lorisids, monotremes, many salamanders and many turtles are relatively slow moving and thus may never reach the speeds typically associated with asymmetrical galloping and bounding in many mammals and some crocodilians.

A second possibility is that the lack of asymmetrical gaits in several clades could be due to the lack of the required neural circuits or a change in musculoskeletal anatomy. Gigantism is thought to limit the use of galloping gaits as a result of biomechanical challenges of extreme bone loading (Hutchinson, 2021). In this case, selection for very large body size results in myriad phenotypic changes that make galloping mechanically impossible or at least very dangerous, which may explain why elephants do not gallop. Yet, very large sea turtles use a crutching gait and several artiodacytls and perissodactyls are large and gallop. What is harder to explain is why two diverse clades, lizards and salamanders, are unable to use asymmetrical gaits. If salamanders move rapidly on land, they do so using a lateral undulatory land-swimming gait in which the limbs are pressed against the body (Edwards, 1977) and thus do not cycle the limbs at all when moving at high speeds. It is most difficult to explain why lizards have lost the ability to use asymmetrical gaits. Many species can move at very fast speeds and routinely negotiate variable substrates, both factors that are thought to favor the use of asymmetrical gaits in other groups (Gambaryan, 1974; Webb and Gans, 1982). Lizards do show flexibility in symmetrical gait, with species spanning a portion of the symmetrical gait landscape (McElroy et al., 2008) and many species routinely using symmetrical bipedal gaits (Clemente, 2014). Yet, lizards have never been reported to gallop or bound at high speeds. The lack of asymmetrical gaits in lizards and salamanders may suggest the existence of a musculoskeletal or neuromuscular constraint that prevents asymmetrical footfall patterns.

Perhaps specialization for lateral undulation with a sprawling posture precludes the use of asymmetrical gaits. Lizards and salamanders (and crocodilians) have vertebral shapes characterized by wide, flat zygapophyses and narrow centra (Jones et al., 2021). This vertebral morphology is related to lateral mobility of the vertebral column and has pushed lizards, salamanders and crocodilians to a different functional adaptive peak aligned with lateral bending compared with the mammalian adaptive peak that is more aligned with sagittal bending (Molnar et al., 2014; Jones et al., 2021). Thus, the specialization for lateral undulation and symmetrical gaits may have made asymmetrical gaits functionally impossible in lizards and salamanders. That crocodilians combine a lateral mobility-type vertebral morphology with the dorsoventral oscillations observed in their gallops may represent evolution from a terrestrial and cursorial ancestral crocodilian towards the aquatic lifestyles and large body size observed in many extant species (Zug, 1974; Webb and Gans, 1982; Hutchinson et al., 2019). While the functional underpinnings of crocodilian gait use have received some recent attention (Hutchinson et al., 2019), future functional studies would be useful for illuminating how crocodilians circumvent some of the constraints that may preclude lizards and salamanders from using asymmetrical gaits.

Our results also suggest the evolution of asymmetrical gaits in several species that are nested with clades that lack asymmetrical gaits. The basal node for cryptodire turtles (Fig. 1) is reconstructed as lacking an asymmetrical gait. However, three sea turtle species use a crutching gait when moving on the beach during nesting. Our analysis shows that this is a derived condition within some sea turtles and may be a consequence of moving on land in large species with morphological specializations (e.g. large body mass, flippers,

streamlining) ill-suited for terrestrial locomotion (Wyneken, 2017). A similar set of specializations are observed in pinnipeds and some of those species also move with a crutching gait on land (Tennett et al., 2018). The base of Anura reconstructs as lacking an asymmetrical gait; yet, three toad species use a bounding gait. This may be a derived feature of toad locomotion that is linked to several other specializations (Reilly et al., 2015). But such an evolutionary scenario depends upon the true sequence of gait evolution in Anura. Many anurans routinely hop, but previous definitions of 'hopping' rely on relative jump length as opposed to a rhythmic gait (Zug, 1978; Emerson, 1979) and thus without detailed kinematic analysis of other 'hopping' anurans we are unable to determine whether they are just short jumpers or are using a rhythmic bounding gait. Further complicating this matter is the debate over the locomotor mode of the earliest fossil anurans based on fossil evidence; it is unclear whether basal anurans were more likely rhythmic walker/hoppers or more saltational jumpers (Shubin and Jenkins, 1995; Jenkins and Shubin, 1998; Reilly and Jorgensen, 2011; Sigurdsen et al., 2012; Herrel et al., 2016; Lires et al., 2016).

Limitations

Various methodological limitations are important to consider when interpreting our results. First, although our sample is large and diverse, we recognize that this sample is incomplete. Related to this, ancestral character reconstructions may be biased by a lack of data on extinct forms and/or poor taxon sampling, especially amongst the oldest clades (fishes, amphibians). Additional sampling, especially in fishes and amphibians, could help reduce this bias, including the addition of swimming gaits in future studies. Second, we treated studies that did not observe asymmetrical gaits as evidence that the observed species did not use asymmetrical gaits. This choice may result in an underestimation of the number of species that use asymmetrical gaits in our sample, as some studies may not have collected data on fast running speeds or other situations where asymmetrical gaits were more likely to occur. Third, no extant species truly represents the ancestral state for gnathostomes at the water–land transition (Molnar et al., 2021; Coates et al., 2002). Stem taxa represented by fossils might be widely divergent from extant morphologies and as such inferring states at internal nodes based on tip data might result in the reconstruction of states that are not supported by the fossil record. Using modeling techniques with more fossil species that are at/near key nodes could help to verify whether the patterns observed here were possible. While no method can unequivocally show that asymmetrical gaits (or any locomotor pattern) existed in a fossil, phylogenetic comparative statistical methods and computer modeling can help determine whether certain possibilities are not likely or functionally impossible.

We note that all asymmetrical gaits are not equivalent and thus our coding of asymmetrical gaits as present or absent is a simplification. The galloping gaits of highly cursorial mammals are used in a wider variety of behavior contexts (traveling, escape, foraging) and clearly have additional neural and anatomical underpinnings that more ancient gnathostomes lack. Crocodilians indeed gallop, but never more than a few tens of meters and typically only during escape. Likewise, while toads, some sea turtles and some fish use an intermittent bounding/crutching/punting asymmetrical gait, they do not exhibit the athletic ability of hares, mice and kangaroos, which is achieved by additional specializations of both anatomy and the nervous system. Notwithstanding, data within this study demonstrate that limb coordination patterns that define asymmetrical gaits are an ancient neuromuscular pattern that

may have been present by the origin of the gnathostome crown group.

Conclusions

While we will never know the locomotor patterns of ancient gnathostomes, our comparative statistical analysis suggests that asymmetrical gaits were likely present in the most ancient gnathostomes. This finding adds to the growing body of work showing that early gnathostomes and tetrapods may have used a diversity of gaits, including an asymmetrical pattern of limb cycling. While additional data on extant species will improve future analyses like ours, another fruitful path forward would be to use modeling, simulation and robotics approaches (Karakasiliotis et al., 2016; Nyakatura et al., 2019) to put bounds on the locomotor capabilities of various extinct species and then dovetail such analyses with phylogenetic comparative statistics to infer patterns of locomotor evolution.

Author contributions

Conceptualization: E.J.M., M.C.G.; Methodology: E.J.M., M.C.G.; Software: E.J.M.; Validation: E.J.M., M.C.G.; Formal analysis: E.J.M., M.C.G.; Investigation: E.J.M., M.C.G.; Resources: E.J.M.; Data curation: E.J.M.; Writing - original draft: E.J.M., M.C.G.; Visualization: E.J.M.; Funding acquisition: M.C.G.

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