The importance of muscle architecture in biomechanical reconstructions of extinct animals: a case study using Tyrannosaurus rex

Karl T. Bates1 and Peter L. Falkingham2

1Department of Musculoskeletal Biology, Institute of Aging and Chronic Disease, University of Liverpool, Liverpool, UK

2School of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool, UK

Abstract

Functional reconstructions of extinct animals represent a crucial step towards understanding palaeocological interactions, selective pressures and macroevolutionary patterns in the fossil record. In recent years, computational approaches have revolutionised the field of 'evolutionary biomechanics' and have, in general, resulted in convergence of quantitative estimates of performance on increasingly narrow ranges for well studied taxa. Studies of body mass and locomotor performance of Tyrannosaurus rex – arguably the most intensively studied extinct animal – typify this pattern, with numerous independent studies predicting similar body masses and maximum locomotor speeds for this animal. In stark contrast to this trend, recent estimates of maximum bite force in T. rex vary considerably (> 50%) despite use of similar quantitative methodologies. Herein we demonstrate that the mechanistic causes of these disparate predictions are indicative of important and underappreciated limiting factors in biomechanical reconstructions of extinct organisms. Detailed comparison of previous models of T. rex bite force reveals that estimations of muscle fibre lengths and architecture are the principal source of disagreement between studies, and therefore that these parameters represents the greatest source of uncertainty in these reconstructions, and potentially therefore extinct animals generally. To address the issue of fibre length and architecture estimation in extinct animals we present data tabulated from the literature of muscle architecture from over 1100 muscles measured in extant terrestrial animals. Application of this dataset in a reanalysis of T. rex bite force emphasises the need for more data on jaw musculature from living carnivorous animals, alongside increased sophistication of modelling approaches. In the latter respect we predict that implementing limits on skeletal loading into musculoskeletal models will narrow predictions for T. rex bite force by excluding higher-end estimates. Key words: biomechanics; bite performance; evolution; modelling; Tyrannosaurus.

Introduction

Palaeontologists and evolutionary biologists strive to understand how extinct organisms lived and interacted with each other and their environment. Subsequently, they attempt to identify how these interactions changed during earth history to shape the evolution of major animal groups. Achieving this 'higher level' understanding of past life requires reconstructing how individual extinct organisms functioned: for example, how they ate, manipulated food and locomoted. Witmer (1995) conceptualised this scientific process as a hierarchy of sequential steps in the form of an 'inverted pyramid of inference', in which progression from actual fossilised hard tissues to 'higher level' ecological and macroevoluationary conclusions requires extrapolation and inference in the form of soft tissue and functional reconstructions (Fig. 1a). Biomechanical assessments of extinct organisms therefore represent a crucial stepping-stone to the understanding of ecological and macroevolutionary patterns in the fossil record. It is therefore implicit that uncertainty and error in functional reconstructions translate directly to imprecision and potentially inaccuracy in higher tiers in the pyramid of inference (Fig. 1a). In recent years, functional analyses of extinct organisms have been revolutionised by the widespread adoption of quantitative computational approaches, many adapted from the field of biomedical engineering (e.g. Hutchinson & Allen, 2009; Hutchinson, 2011; Bates, 2013; Maidment et al. 2014). These approaches have many advantages, but one challenging aspect in their use on extinct animals is that they require precise specification of numerical values for soft tissue parameters that are rarely, or never, preserved in fossils. One approach to gauging progress and success in biomechanical reconstructions of extinct organisms is to examine how predictions for extensively studied 'exemplar' taxa have changed over time and with increased research effort. Conceptually, one might predict that with continued research effort and methodological refinements predictions of biomechanical performance of well studied taxa would begin to converge on an increasingly narrower range, which hopefully lies close to the actual functional capabilities of that organism (Fig. 1b).

Tyrannosaurus rex is a model organism for studies of many aspects of palaeobiology (Brusatte et al. 2010). Its statusasanexemplarorganismderives from anumberoffactors, most notably extremely large body size, seemingly highly adapted and 'unusual' morphological features (e.g. large skull, long hind limbs, short forelimbs, bipedal) and the fact that the organism is known from a large number of near-complete specimens (Larson, 2008; Brusatte et al. 2010). These features make *T. rex* an ideal system for examining questions related to physiology and growth (e.g. Erickson et al. 2004; Hutchinson et al. 2011), body size and anatomical scaling (e.g. Currie, 2003; Hutchinson et al. 2007; Bates et al. 2009), and various aspects of functional morphology and biomechanics (e.g. Farlow et al. 1995; Hutchinson & Garcia, 2002; Hutchinson, 2004; Rayfield, 2004; Hutchinson et al. 2005; Sellers & Manning, 2007; Lipkin & Carpenter, 2008; Gatesy et al. 2009; Bates & Falkingham, 2012; Gignac & Erickson, 2017; Sellers et al. 2017). Collectively, these studies underpin our understanding of the role of multi-ton carnivorous animals within dinosaurian ecosystems of the Mesozoic.

The breadth and depth of this research means that aspects of *T. rex* palaeobiology have been investigated using a wide variety of methods, thereby providing a natural case study to evaluate progress and highlight challenges in biomechanical reconstructions (Fig. 1). In many instances, notably body mass and locomotor performance, increased use of quantitative mathematical approaches has resulted in clear convergence in terms of quantitative estimates of biomechanical parameters for *T. rex*. For example, most computational estimates of body mass for near-complete adult specimens of *T. rex* lie within the range of 6500– 7500 kg (e.g. Henderson, 1999; Hutchinson et al. 2007, 2011; Allen et al. 2009; Bates et al. 2009; Sellers et al. 2017), and various biomechanical models suggest *T. rex* was most likely limited to maximum locomotor speeds below 8 m/s (e.g.

Hutchinson & Garcia, 2002; Hutchinson, 2004; Hutchinson et al. 2005, 2007, 2011; Sellers & Manning, 2007; Gatesy et al. 2009; Sellers et al. 2017). This suggests either that different workers have converged on similar approaches to soft tissue reconstruction and mechanical assessment or that different methods have been used with similar levels of confidence in precision and accuracy. In stark contrast to this trend, recent quantitative estimates of maximum bite force in *T. rex* vary considerably despite the use of very similar methodological approaches across studies (Fig. 2). Bates & Falkingham (2012, 2018) presented an estimated maximum static or sustained bite force of 44 940 N at posterior bite positions using a multibody dynamics (or rigid-body) model (Fig. 2). These authors conducted a sensitivity analysis on their model in acknowledgement of potential inaccuracies in their estimates of unpreserved soft tissue parameters for *T. rex* (Fig. 2b), which yielded a range of predictions for maximum static bite force between 29 510 and 53 735 N at posterior bite positions (Bates & Falkingham, 2012, 2018). By contrast, Gignac & Erickson (2017) used a very similar 3D rigid body model of the same adult *T. rex* specimen (BHI 3033) and produced a considerably lower estimate of 24 272 N at posterior bite positions (Fig. 2b). This equates to a nearly 50% difference between studies, with the lower value of Gignac & Erickson (2017) falling comfortably outside the range of predictions that Bates & Falkingham (2012, 2018) presented as a measure of potential error in their model construction (Fig. 2b).

Where quantitative predictions of biomechanical performance vary across studies using identical methods it is likely that this reflects a limited ability to constrain input values for soft tissue parameters. Confronting and overcoming this uncertainty surrounding soft tissue parameters has repeatedly been cited as the major challenge currently facing studies of evolutionary biomechanics in recent years (e.g. Hutchinson & Allen, 2009; Bates et al. 2010; Hutchinson, 2011; Bates, 2013; Maidment et al. 2014). The magnitude of disparity in recent estimates of T. rex bite force (Fig. 2b) emphasises this point and suggests that it may represent an important methodological case study. Specifically, understanding the mechanistic causes of differences in model predictions for T. rex bite force may identify important limiting factors in biomechanical reconstructions of extinct organisms more generally.

The aim of this study is therefore to investigate systematically the underlying causes of highly disparate bite force predictions for T. rex (Bates & Falkingham, 2012; Gignac & Erickson, 2017). In summary, a detailed comparison of all model inputs revealed that muscle fibre length and architecture are the principal source of disagreement between studies, and therefore that this parameter currently represents the greatest source of uncertainty in reconstructions of bite performance in T. rex, and indeed extinct animals generally. As a starting point to addressing this major issue we subsequently present data tabulated from the literature of muscle architecture from over 1100 muscles measured in extant terrestrial animals. We carry out a preliminary analysis of this data in the context of T. rex jaw musculature to illustrate the importance of better constraining muscle fibre lengths in future biomechanical analyses of extinct animals.

Models of bite performance in T. rex

Bates & Falkingham (2012) and Gignac & Erickson (2017) used rigid body models to estimate maximum bite force in T. rex. These models require the geometry of the musculoskeletal system under study to be defined in mathematical terms, along with the physical and contractile properties of the relevant musculotendon units. This basic mathematical framework allows estimation of the contractile force of muscle-tendon units, and subsequently the impact of muscle contraction on joints (e.g. joint torques) and the external environment (e.g. bite force at a tooth–object contact) to be predicted. Rigid body models typically use high-resolution digital skeletal scans (e.g. CT or laser scans) as a basis to define the 3D positions of joint centres, bite points and muscle positions. A

number of calculations are then required to estimate the force-generating capacity of muscles, based on their physical and architectural properties. First and foremost, muscle force estimates require the physiological crosssectional area of the muscles to be calculated. In non-pennate muscles this is typically calculated as:

Muscle physiological cross-sectional area = muscle volume/muscle fibre length (1)

and in pennate muscles:

Muscle physiological cross-sectional area = (muscle volume/muscle fibre length) * COS(pennation angle) (2)

These equations dictate that larger muscle volumes will yield larger physiological cross-sectional areas for any constant fibre length, while longer fibre lengths will result in smaller physiological cross-sectional areas for any constant muscle volume. These basic physical and architectural properties thus interact to determine muscle force output, which is directly proportional to physiological cross-sectional area. For example, under isometric contraction, such as that broadly encountered in a sustained static bite (i.e. at a constant gape angle) muscle force is typically calculated according to:

Muscle force = physiological cross-sectional area * maximum isometric stress (3)

Gignac & Erickson (2017) carried out a static analysis of bite performance in T. rex using the model of muscle contraction shown in Eq. 3. However, having constructed a similar anatomical model, Bates & Falkingham (2012) used a dynamic simulation approach to simulate jaw-closure under maximal muscle contraction because their goal was to investigate bite speed (hypothesised to be functionally and ecological disparate in theropod dinosaurs (e.g. Mazzetta et al. 2009; Sakamoto, 2010)), in addition to bite force. This dynamic analysis used a previously published model of muscle contraction (Minetti & Alexander, 1997; Sellers et al. 2003) that includes Hill-style velocity-dependent force generation. This model specifies that for a muscle with a maximum tension of T0 (in N), maximum rate of shortening of vmax (in m/s), current rate of shortening of v (in m/s), and activation of a, the force (T) is given by:

$$\text{If } v \leq 0 \text{ then } T = \alpha T_0 (1.8 - 0.8(v_{max} + v/v_{max} - 7.56v/k)),$$
$$\text{or}$$
$$\text{If } 0 < v < v_{max} \text{ then } T = \alpha T_0 (v_{max} - v + v/k). \quad (4)$$

Bates & Falkingham set the constant k to 0.17, in accordance with Minetti & Alexander (1997), while the effects of using values between 4–12 m s−1 for Vmax were tested in their sensitivity analysis, although the value of 300 000 N m−2 for maximum isometric stress was used throughout (see further details below). In the models of both Bates & Falkingham (2012) and Gignac & Erickson (2017) maximal activation of muscles (i.e. a = 1inEq. 4) was assumed and the resulting linear muscle forces converted to torques by multiplying a muscle's force by its moment arm (Bates & Falkingham, 2012; Gignac & Erickson, 2017). Bite force was then calculated by summing muscle torques about the jaw joint and dividing this value by the distance between the jaw joint and the bite point. While the dynamic model of Bates & Falkingham (2012) simulated jaw closure and tooth-object impact forces, they presented predicted equilibrium bite forces, or in other words, static bite forces with the jaw in a sustained biting position, which are directly comparable to the scenario modelled by Gignac & Erickson (2017).

Previous explanations for disparate predictions of bite force in *T. rex*

Gignac & Erickson (2017) put forward potential explanations for their considerably lower bite force estimates for adult T. rex relative to the earlier study of Bates & Falkingham (2012). Specifically they state: 'We suspect the differences stem primarily from previous models not implementing archosaurian-specific, jaw-closing musculature and force generation as well as not utilizing experimentally validated neontological models'. They carry out no further analysis or present any data to confirm or reject these suggested causative factors. Figure 3 demonstrates that none of the factors proposed by Gignac & Erickson (2017) explains why their model generated lower bite force predictions for T. rex than that of Bates & Falkingham (2012) (see Supporting Information Appendix S1 for more detailed discussion of the data presented in Fig. 3). For these suppositions to be correct, the model of Bates & Falkingham (2012) would have had to use higher input values for muscle mass (Fig. 3a), muscle moment arms (Fig. 3b) and/or muscle isometric stress (Fig. 3c). However, direct comparison of these input parameters (Fig 3a–c) reveals that in fact the values used by Bates & Falkingham (2012) were universally lower (in some cases nearly 50% lower; Fig 3a) than those of Gignac & Erickson (2017) for all these parameters. This demonstrates not only that the potential explanations offered by Gignac & Erickson (2017) are incorrect, but that other factors are influencing the relative bite force predictions to such an extent that they are reversing the differences one would expect to recover from the two studies given the disparities highlighted in Fig. 3. In other words, despite reconstructing smaller muscle volumes (Fig. 3a) and moment arms (Fig. 3b), and choosing slightly lower values for muscle isometric stress (Fig. 3c), Bates & Falkingham (2012) predict considerably higher bite forces for T. rex than Gignac & Erickson (2017).

New explanations for discrepancies in bite force predictions

Thorough examination of the reconstructive methods used by the two studies in fact reveals that disparate approaches to deriving values for muscle fibre lengths are primarily responsible for the difference in bite force estimates. Muscle fibre length influences estimates of static bite force because it is used in the calculation of muscle physiological cross-sectional area (Haxton, 1944; Carlow & Alexander, 1973) in both studies, as described in Eqs 1 and 2 above. A longer fibre length will result in a smaller physiological cross-sectional area than a shorter fibre length for a given volume of muscle (Eqs 1 and 2). This is turn will result in lower muscle forces (Eqs 3 and 4) and ultimately lower bite forces because both these outputs are proportional to muscle physiological cross-sectional area. The two studies of T. rex bite force approached their estimation of muscle fibre length and physiological cross-sectional area in different ways, reflecting differences in their chosen models of muscle contraction and subjective considerations of likely jaw adductor muscle architecture in carnivorous archosaurs. Expanding on their sensitivity analysis of Vmax and muscle mass (see above), Bates & Falkingham (2012) estimated physiological cross-sectional area according to both Eqs 1 and 2. Initially, Bates & Falkingham (2012) seteachmusclefibrelengthtobe0.25of the maximum length of the muscle in their simulations (i.e. 0.25 of the length at the maximum gape angle used; Fig. 2b), and calculated physiological cross-sectional area according to Eq. 1. They chose this value based on human values stating: 'FLs of jaw-closing muscles in our human model ranged between 10 and 40 per cent of maximum muscle length across the range of joint motion investigated'. These authors subsequently investigated the impact of fibre length and pennation angle on bite force and velocity predictions by conducting both 'one-at-a-time' (OAAT) and Monte Carlo-style sensitivity analyses (Bates & Falkingham, 2012, 2018). In the OAAT sensitivity analysis, fibre lengths were altered without any compensatory changes to other associated parameters, including muscle physiological cross sectional area. As a result, bite velocity predictions were shown to vary considerably, but there was relatively little impact on static or sustained bite force (Bates &

Falkingham, 2012). Pennation effects were tested in both the OAAT and Monte Carlo-style analyses. In the former case, Bates & Falkingham (2012, 2018) considered pennation effects (of up to 20°) on muscle force output by recalculating muscle physiological cross-sectional area according to Eq. 2, with all other parameters constant at the initial model values (including fibre length). The value of 20° was chosen as an upper bound or maximal value based on direct measurements of jaw-closing muscles in Alligator (Porro et al. 2011), which indicate that most are not strictly parallel-fibred. In the Monte Carlo analysis, Bates & Falkingham (2012, 2018) attempted to account for the functional consequences expected of pennate muscle architecture by simultaneously altering physiological cross-sectional areas (again calculated according to Eq. 2) and reducing fibre lengths. However, they manipulated cross-sectional area in their model by altering muscle volume (using their minimum and maximum volumes estimated for T. rex,Figs 1 and 2), and used constant fibre lengths throughout in their calculation of physiological cross-sectional areas.

In contrast, Gignac & Erickson (2017) chose to make their fibre length equal to muscle length, and to reconstruct all muscles as parallel-fibred. As such they calculated physiological cross-sectional area using only Eq. 1. As a result of using this approach, the muscle fibre lengths used by Gignac & Erickson (2017) are considerably longer than those of Bates & Falkingham (2012). Subsequently, the longer fibre lengths chosen by Gignac & Erickson (2017) result in smaller muscle physiological cross-sectional areas (Fig. 3d) and ultimately lower muscle and bite force estimates for T. rex (Figs 2 and 3c,d), despite this study reconstructing larger muscle volumes (Fig. 3a). For example, summing the muscle physiological cross-sectional areas from Gignac & Erickson (2017) yields a value of 0.23 m2 vs. 0.46 m2 (Fig. 3d) in Bates & Falkingham (2012), despite muscle volumes being approximately 41% lower in the latter study (Fig. 3a). If fibre lengths are set to muscle length in the model of Bates & Falkingham (2012) then the summed physiological cross-sectional area of this reconstructions drops to around half that of Gignac & Erickson (2017) (Fig. 3d). To support their assumption of universally parallel fibred architecture, and thus muscle fibre lengths equal to muscle length, Gignac & Erickson (2017) cite their own previous work on Alligator jaw mechanics (Gignac & Erickson, 2016). Specifically, they state: ‘As demonstrated by Gignac & Erickson (2016) muscle length can serve as a proxy for fascicle length in parallel-fibered muscles when statically modeled’. The terminology used here inherently suggests that this previous work (Gignac & Erickson, 2016) quantitatively ‘demonstrated’ that Alligator jaw closing muscles are universally composed of long parallel fibres, with no tendinous component. However, in this previous study of Alligator, Gignac & Erickson (2016) state that seven of the eight jaw-closing muscles ‘are tightly bound by the bony adductor chamber and by one another, which precluded making direct measurements of fascicle lengths. Each has parallel-arranged fibers; therefore, muscle length (Ml) served as a proxy for mean fascicle length in each of these cases’. Thus fibre lengths and architecture were not actually directly measured in seven of the eight jaw-closing muscles in Alligator. However, Porro et al. (2011) did carry out a full quantitative dissection of the jaw-closing musculature of a juvenile Alligator, in which a full suite of architectural properties were directly measured for all muscles. Porro et al. (2011) suggest that five of the seven muscles are not strictly parallel-fibred, and subsequently that the ratio of fibre length to muscle length varies considerably between muscles in the jaw of Alligator, with values ranging from 0.28 to 0.9 (mean 0.47, SD 0.25).

Three important points arise from this discussion. First,

muscle fibre length and architecture are responsible for the highly disparate bite force predictions for T. rex (Fig. 2), and the effect of these interlinked parameters can completely drown out the impact of other differences in anatomical reconstructions (e.g. disparate muscle volume [Fig. 3a] and muscle force production estimates [Fig. 3c]). To our knowledge this is the first time that muscle fibre

length has been identified as having such a dramatic impact on biomechanical predictions for an extinct animal: previous studies have suggested that muscle mass and physiology (isometric stress and maximum contraction velocity) are likely to be the most limiting parameters in quantitative estimates of biomechanical performance (e.g. Hutchinson & Garcia, 2002; Hutchinson, 2004; Sellers & Manning, 2007; Gatesy et al. 2009; Bates et al. 2010; Bates & Falkingham, 2012). Therefore the potential for muscle fibre length to impact on biomechanical assessments, and subsequently on our ability to make 'higher-level' inferences (Fig. 1), of extinct animals has been considerably underestimated. This finding that fibre length can exert a major influence on quantitative bite force predictions is consistent with conceptual discussions (e.g. Curtis et al. 2010) and recent sensitivity studies carried out on models of extant taxa (e.g. Groning et al. 2013). Secondly, the differing approaches to fibre length estimation between these studies originate from subjective choices related to differences in the methodological (e.g. dynamic vs. static models) and philosophical approaches used (e.g. consideration of a range of parallel and pennate architectures vs. only parallel-fibred architecture). Thirdly, there has been relatively little research effort directed at deriving objective approaches to deliver fibre length estimates for extinct taxa based on available quantitative data from living animals. Therefore this parameter represents a major, and under-appreciated, source of uncertainty in biomechanical predictions for extinct animals generally. Herein, we attempt to address these interlinked problems by collating and analysing a large dataset of muscle architectural values from the published literature. We make this dataset freely available (Supporting Information Appendix S2) so that it can be further interrogated, added to and analysed in future biomechanical studies of extant and extinct taxa.

Muscle fibre length data from extant terrestrial vertebrates

We amassed published data on the architectural properties of over 1100 muscles from terrestrial (i.e. non-marine) vertebrates (Fig. 4, Appendix S2). Specifically, we tabulated data where values for muscle fibre length, pennation angle, muscle belly length and overall muscle-tendon unit length were measured directly from dissections (Appendix S2). This allowed us to calculate the ratio of fibre length to muscle length and subsequently assess what, if any, ratio is empirically supported for the estimation of fibre length in extinct animals (Fig. 4). We assessed fibre length relative to both (Fig. 4a) muscle belly and (Fig. 4b) total muscle-tendon unit length (i.e. muscle belly length + external tendon length) because either can be used (depending on the preference of the researcher) to estimate fibre lengths in reconstructions of extinct animals. Considering the calculation of physiological cross-sectional area purely in theoretical terms, it makes more sense to consider the relationship between fibre length and muscle belly length, and to use this ratio predictively in extinct animals. However, most reconstructions of fossil animals (e.g. Bates & Falkingham, 2012; Gignac & Erickson, 2017) use the three-dimensional distance between origin and insertion as the muscle length value against which to estimate fibre length, which is strictly the total muscle-tendon unit length. Thus, utilising the fibre length to muscle belly length ratio would require *a priori* assumptions about the presence/absence of external tendon and values for tendon slack length. We therefore present both ratios (Fig 4a,b, Appendix S2). We attempted to be maximally inclusive of muscles and collated data from all regions of the body (limb, axial and cranial muscles) from as many groups of terrestrial vertebrates as possible.

Figure 4 demonstrates that fibre length to muscle length ratios vary enormously within both parallel fibre and pennate architectural types. Within parallel-fibred muscles (i.e. pennation = 0°) the fibre length to muscle belly ratio rangedfrom0.07to1(Fig. 4a); such variation likely reflects the strong functional signal observed in fibre and muscle lengths (e.g. Burkholder et al. 1994) but also the mixing of data from highly functionally and phylogenetically disparate taxa and body regions

(limbs,skulls,axial segments, etc.). Interestingly, we also observed variation across studies in terms of how fibre lengths were used to calculate physiological cross-sectional area in strictly parallel-fibred muscles. Some studies mirrored the approach of Gignac & Erickson (2017) and substituted muscle belly length for fibre length in Eq. 1 to calculate physiological cross-sectional area (e.g. Michilsens et al. 2009). Others studies did not take this approach, and although observing a parallel fibre architecture they followed Eq. 1 strictly and used their mean measured fibre lengths to derive a value for physiological crosssectional area, despite these values often being considerably shorter than muscle belly length (e.g. Smith et al. 2006; Williams et al. 2008a,b; Allen et al. 2010). In pennate muscles (i.e. pennation > 0) there is a broadly negative relationship between pennation angle and the ratio of fibre length to muscle length, as would be expected. In other words, as pennation angle increases the ratio of fibre length to (Fig. 4a) muscle belly and (Fig. 3b), total muscle tendon unit length tend to decrease. The same relationship, with a near identical slope, is observed when pennation angle vs. the ratio of fibre length to muscle-tendon unit length in masticatory muscles is considered (Fig. 4b). The fact that a positive relationship is observed between pennation angle vs. the ratio of fibre length to muscle belly length in masticatory muscles (Fig. 4a) almost certainly reflects the very low sample size (n = 5 muscles) and narrow range of pennation angles (8.5–16.9°) currently available in the literature. In all cases there is considerable scatter about RMA regressions lines (e.g. all r2 values < 0.4; see Supporting Information Table S1), which as noted above may at least partially reflect the mixing of data from different taxa and body regions.

This new dataset allows us to revisitthe issueofbiteperformance in T. rex to assess both the issue of quantitative estimates of bite force, and also the question of which soft tissue parameter currently represents the greatest source of uncertainty. This latter consideration is of consequence more widely in biomechanical reconstructions of extinct and extant animals (e.g. Hutchinson & Allen, 2009; Bates et al. 2010; Hutchinson, 2011; Bates, 2013; Maidment et al. 2014). To address both issues simultaneously we repeated the simulations of Bates & Falkingham (2012) and recalculated static bite force using the input data from Gignac & Erickson (2017) under two scenarios. First, we used the assumption of Gignac & Erickson (2017) that all jaw closing muscles are strictly parallel-fibred with a ratio of fibre length to muscle-tendon unit length equal to one. Second, we re-ran both analyses assuming a ‘relatively’ extreme pennate architecture, following Bates & Falkingham (2012). Specifically, for this latter scenario we assumed a pennation angle of 20°, which is approximately 3° higher than measured in Alligator (Porro et al. 2011), but considerably less than the maximum recorded for masticatory muscles from mammals in our dataset (Fig. 4, Appendix S2). To derive an average fibre length to muscle length ratio for a muscle with a 20° pennation angle we subsequently used the three negative regression slopes in Fig. 3, with the positive slope between pennation angle vs. the ratio of fibre length to muscle belly length in masticatory muscles (Fig. 4a) discounted due to small sample size (see above). The similarity of these three slopes leads to very similar fibre length to muscle length ratios for a muscle with 20° pennation angle (Table S1), and we subsequently used the mean slope value of 0.35 in our re-analysis of T. rex bite performance.

The results of this new analysis are shown in Fig. 5, which reveals a number of notable findings. First, standardisation of muscle fibre length and architecture based on our new dataset from living vertebrates (Fig. 4) has reversed the qualitative nature of the discrepancy in predicted bite forces between the two modelling studies (Fig. 5). If strictly long, parallel-fibred or pennate architectures are applied to both models, then the estimates of Gignac & Erickson (2017) are considerably higher than those of Bates & Falkingham (2012), which is consistent with the differences in other input parameters showninFig. 3.Ifpennate muscle architecture is reconstructed in T. rex, then fibre lengths are considerably reduced in the model of Gignac & Erickson (2017), thus muscle physiological cross-sectional area and subsequently muscle force are greatly increased in this model. As a result the

predicted force at posterior bite positions using the initial model inputs of Gignac & Erickson (2017) has more than doubled, rising from 24 272 N (Fig. 2) to 65 163 N (Fig. 5). Deriving fibre lengths for pennate muscles using our new data (Fig. 3) results in an increase in fibre lengths in the model of Bates & Falkingham (2012), and therefore a decrease in muscle physiological cross-sectional areas and subsequently muscle force. As a result, the predicted force at posterior bite positions using the initial model inputs of Bates & Falkingham (2012) has reduced by approximately 45%, from 44 940 N to 25 921 N (Fig. 5). If strictly long, parallel-fibred architectures are assumed in T. rex (as per Gignac & Erickson, 2017) then an even greater increase in fibre lengths occurs in the model of Bates & Falkingham (2012) and as a result, the average predicted bite force drops to just 9598 N (Fig. 5). Secondly, when standardised, the choice of muscle architectural type reconstructed for T. rex also impacts on the absolute, but not relative, magnitudes of the two studies (Fig. 5). When strictly long, parallel-fibred architectures are assumed in T. rex, the absolute difference between mean bite force predictions drops to 16 322 N (Fig. 5). If pennate muscle architecture is reconstructed in T. rex, the absolute difference between mean bite force predictions increases to 40 891 N (Fig. 5). In both instances the mean estimate derived from the model of Bates & Falkingham (2012) equates to 37% of that predicted by the model of Gignac & Erickson (2017). This highlights the third notable result shown in Figure 5: standardisation of muscle fibre lengths and architecture between these studies has actually increased the relative difference between their mean predictions; formerly, the estimate of Gignac & Erickson (2017) was 54% that of Bates & Falkingham (2012) (Fig. 1).

Fourthly, this analysis provides a new and wider context for the relative importance of individual soft tissue parameters on quantitative predictions of biomechanical performance in extinct vertebrates (Fig. 5). Indeed, this analysis is also highly informative for studies of extant taxa where it is not always logistically or ethically possible to measure all model input parameters directly. While previous conceptual discussions (e.g. Curtis et al. 2010) and modelling studies of extant taxa (e.g. Groning et al. 2013) have indicated that bite force predictions are sensitive to fibre length values, here we show that the absence of an objective and precise means of predicting fibre lengths in the absence of direct measurements (as is always the case in extinct animals) represents the single greatest source of uncertainty in model outputs. The few previous studies to have examined the effect of muscle fibre length on functional predictions for extinct animals have concluded that its impact is modest relative to other input parameters, particularly muscle mass and contractile properties (e.g. Hutchinson & Garcia, 2002; Hutchinson, 2004; Sellers & Manning, 2007; Gatesy et al. 2009; Bates et al. 2010; Bates & Falkingham, 2012). This almost certainly results from consideration of fibre lengths in isolation, without compensatory changes to physiological cross-sectional area, under which circumstances it has a modest effect on muscle force predictions relative to other parameters (Hutchinson & Garcia, 2002; Hutchinson, 2004; Bates et al. 2010; Bates & Falkingham, 2012). However, when its interaction with muscle physiological cross-sectional area is considered, muscle fibre length and architecture become the greatest source of uncertainty in quantitative bite force predictions (Fig. 5).

Conclusions and future perspectives

Overcoming uncertainty in quantitative predictions of functional performance derived from poorly constrained input values for unfossilised soft tissue parameters (Figs 3 and 5) currently represents the greatest challenge facing the field of evolutionary biomechanics (e.g. Hutchinson & Allen, 2009; Bates et al. 2010; Hutchinson, 2011; Bates, 2013; Maidment et al. 2014). Hutchinson & Allen (2009) termed this paradigm an ‘interpretive asymptote’, and acknowledged that functional interpretations of extinct organisms may one day reach a threshold for quantitative precision beyond which no further progress can be made (Fig. 1b). Our case study on bite performance in T. rex is testament to

the challenges posed by quantitative soft tissue reconstruction; however, like Hutchinson & Allen (2009), we would argue that the means of achieving progress and pushing back the 'interpretive asymptote' are clear.

Reconstructions of soft tissues in fossils would be more informed, and potentially more tightly constrained, with more quantitative anatomical and functional data from living vertebrates. It is our hope that the muscle architecture dataset that we have collated here (Fig. 4, Appendix S2) will in this way contribute directly, but also indirectly by highlighting where important data are lacking in the literature on living animals. For example, the literature we collated on muscle architecture is dominated by limb musculature (hind limb 54% and forelimb 41% of the data), with jaw and axial musculatures extremely poorly represented (approximately 2% each). This large sample size for limb musculature should enable more detailed analyses of the relationship between muscle architecture and muscle function within limbs that could guide fibre length estimation on a joint-by-joint or even muscle-by-muscle basis in locomotion studies of extinct animals. Indeed, the spread of fibre length to muscle length ratios seen in this dataset (Fig. 4) almost certainly represents a functional signal in terms of the different or specialised roles that muscles perform within the musculoskeletal system (e.g. Burkholder et al. 1994); for example, whether they function as joint 'motors', 'brakes', 'stabilisers' or 'springs' within a limb (e.g. Ahn & Full, 2002; Rankin et al. 2016). That such specialisation of muscle function should be manifested in FL : ML has been inferred from anatomical and functional experiments (e.g. Burkholder et al. 1994; Biewener, 1998; Biewener & Gillis, 1999; Gillis & Biewener, 2001; Ahn & Full, 2002; Wilson & Lichtwark, 2011; Bates & Schachner, 2012) and predicted in theoretical and simulation studies of muscle contraction (e.g. Falk et al. 2016). The volume of data available that we have collated for limb muscles (Fig. 4) may allow subclassification of muscles, leading to assignment of function-specific fibre lengths to limb muscles in models of extinct vertebrates. However, clearly such a detailed analysis of jaw and axial musculature is not currently possible based on published data (Fig. 4). Without more data on jaw muscle architecture in living animals, future biomechanical models of extinct vertebrates will suffer from similar magnitudes of uncertainty to those identified here for T. rex (Fig. 5) if researchers are maximally inclusive of error estimates in their reconstructions.

Hutchinson & Allen (2009) also cite the extension, wider validation and development of new methodological approaches as being key to refining functional predictions of extinct animals. New methodological approaches might act at both the 'front-end' and 'back-end' of biomechanical reconstructions of fossils. At the front-end, new approaches would serve to refine and minimise the range of plausible input values for soft tissues. For example, although our dataset (Fig. 4) may enable better future estimations of muscle fibre length in extinct animals, there is no evidence at present that using the ratio of fibre length to muscle length is the best or most appropriate reconstructive method to use, particularly given the considerable scatter about trendlines (Fig. 4). In an alternative approach, Sellers et al. (2013) analysed the relationship between fibre length and the length change endured by muscles during motion in a small number of living taxa. This subsequently allowed the statistically supported mean ratio of fibre length to muscle length change to be used to estimate fibre lengths in computer models of extinct taxa (Sellers et al. 2013). One notable benefit of this approach is that it allows tuning of individual muscle fibre lengths and tendon lengths with the length changes endured by muscles in vivo, thereby ensuring that individual muscles have fibre lengths that allow them to function (i.e. generate force) over a wide range of joint angles. This is not explicitly guaranteed when fibre length is based solely on a 'generic' mean ratio of fibre length to muscle length values.

New methodological developments acting at the 'backend' of functional analyses would act to desensitise or even detach the final estimates of biomechanical performance (bite force, running speed etc.) from soft tissue input values. For example, Sellers et al. (2017) recently extended their evolutionary robotics simulations to include consideration of the bone-loading magnitudes in gait reconstructions. This allowed them to demonstrate that although it is plausible to reconstruct large theropod dinosaurs like T. rex with sufficient limb musculature to achieve slow-to-moderate running speeds, such gaits would lead to unacceptably high loads on the limb bones (Sellers et al. 2017). There is obvious potential to extend this approach to other taxa and other regions of the musculoskeletal system, including bite force estimation. Indeed, we predict that higher-end muscle and bite forces currently predicted for T. rex (Fig. 5) might lead to unacceptably high loads on the skull and/or mandible and thus could potentially be excluded through use of the 'multiphysics' approach of Sellers et al. (2017).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Appendix S1. Additional discussion, data and model code. Table S1. Reduced major axis statistics for analysis of relationship between fibre length and muscle belly and muscle-tendon unit length (Fig. 4 in main text).

Appendix S2. Raw muscle architecture data

Figures

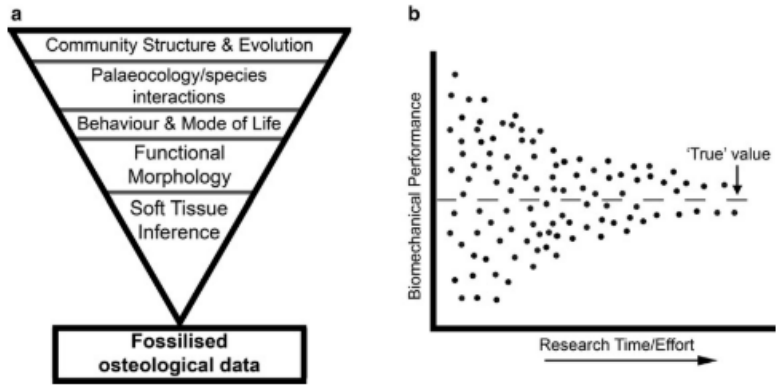

Fig. 1 (a) The palaeontological pyramid of inference (modified from Witmer, 1995). Witmer (1995) conceptualised this scientific process as a hierarchy of sequential steps in the form of an 'inverted pyramid of inference', in which progression from actual fossilised hard tissues to 'higher level' ecological and macroevoluationary conclusions requires extrapolation and inference in the form of soft tissue and functional reconstructions. (b) Conceptualisation of biomechanical predictions for an extensively studied 'exemplar' taxon over time. In this scenario, continued research effort and methodological refinements lead predictions of biomechanical performance to converge on an increasingly narrower range, which hopefully lies close to the actual functional capabilities of that organism, signified by the dashed horizontal line.

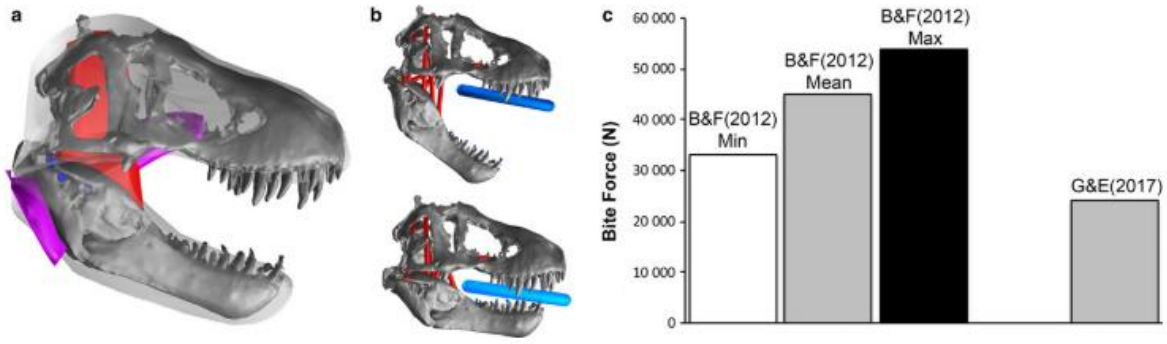

Fig. 2 Example of the generic workflow used to construct biomechanical models of *T. rex* to estimate bite force (modified from Bates & Falkingham, 2012). (a) Computer-aided design approaches are used to estimate muscle volumes, before (b) muscle lines of actions relative to the jaw joints and biting positions (i.e. teeth) are mathematically reconstructed. (c) This basic workflow was used to derive highly disparate estimates of static or sustained bite force for the same specimen of *T. rex* by Bates & Falkingham (2012) and Gignac & Erickson (2017).

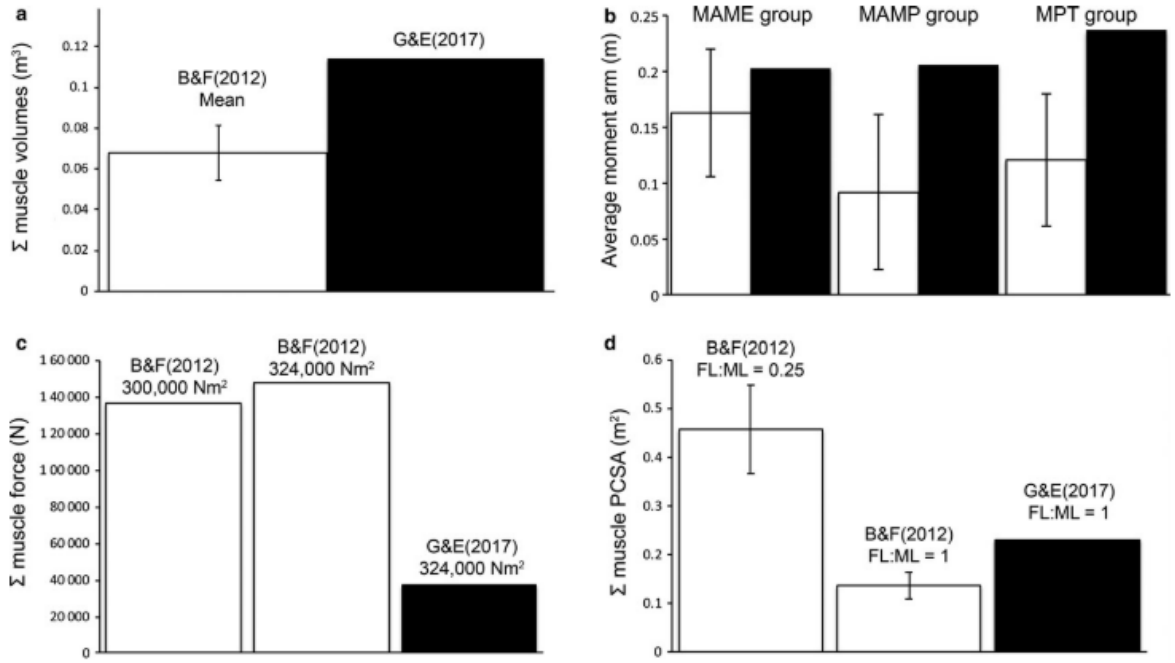

Fig. 3 Comparison of muscle properties reconstructed for the same specimen of *T. rex* by Bates & Falkingham (2012, 2018) and Gignac & Erickson (2017). In each graph, white bars are values from Bates & Falkingham (2012, 2018), where error bars correspond to ranges predicted from their anatomical reconstructions and tested in their sensitivity analysis. Black bars are values from Gignac & Erickson (2017). These graphs show that the range of (a) muscle volumes, (b) muscle moment arms and (c) the value for maximum isometric stress used by Bates & Falkingham (2012) are lower than those of Gignac & Erickson (2017), indicating that these parameters cannot be responsible for the higher bite forces predicted by the former study. For example, (c) standardising the value maximum isometric stress across these studies actually increases, rather than decreases, the disparity in muscle and bite force predictions. However, despite (a) reconstructing smaller muscle volumes, Bates & Falkingham (2012) derived (d) much larger muscle physiological cross-sectional areas for jaw-closing muscles due to their use of a fibre length to muscle length (FL : ML) ratio of 0.25, vs. the higher ratio of 1 used by Gignac & Erickson (2017). This explains the larger muscle and bite forces predicted by Bates & Falkingham (2012). Recalculating the physiological cross-sectional areas of muscle volumes reconstructed by Bates & Falkingham (2012) using FL : ML = 1 results in values lower than those of Gignac & Erickson (2017).

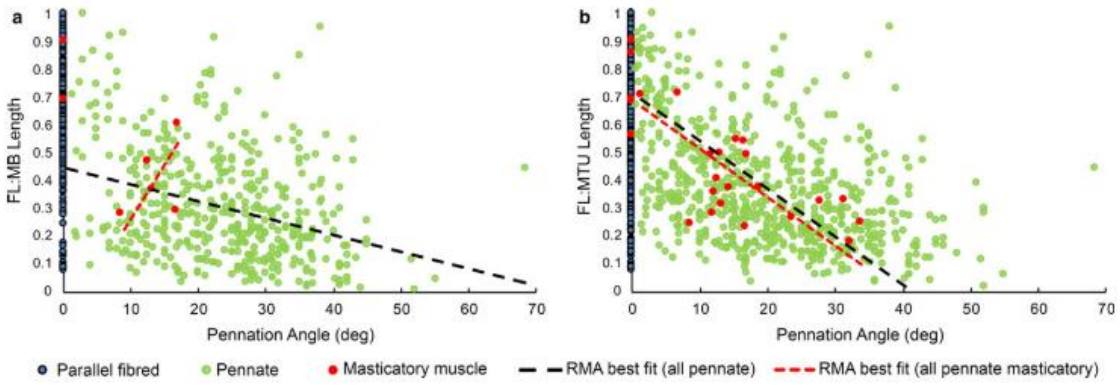

Fig. 4 Analysis of the ratio of fibre length to (a) muscle belly length and (b) muscle-tendon unit length in 1130 measurements of muscle architecture in extant vertebrates (see Appendix S2 for data). Best fit lines were calculated using reduced major axis (RMA) regression.

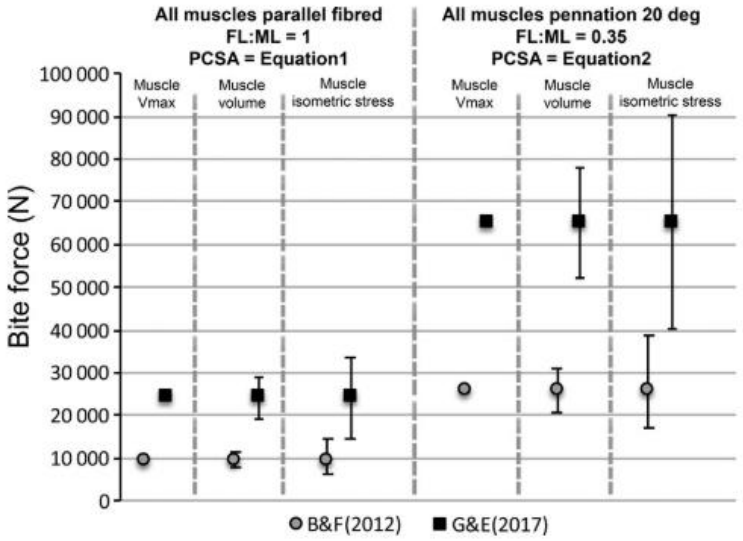

Fig. 5 Reanalysis of the biomechanical models of Bates & Falkingham (2012) and Gignac & Erickson (2017) based on our analysis of relative muscle fibre length and architecture in Fig. 4 and previous data on ranges for soft tissue input values used in studies of extinct animals (Bates et al. 2010; Bates & Falkingham, 2012). Error bars have been calculated for the model of Gignac & Erickson based on error estimates for individual parameters used in Bates & Falkingham (2012) and Bates et al. (2010).