Specialize or risk disappearance – empirical evidence of anisomerism based on comparative and developmental studies of gnathostome head and limb musculature

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ABSTRACT

William K. Gregory was one of the most influential authors defending the existence of an evolutionary trend in vertebrates from a higher degree of polyisomerism (more polyisomeric or 'serial' anatomical structures arranged along any body axis) to cases of anisomerism (specialization or loss of at least some original polyisomeric structures). Anisomerism was the subject of much interest during the 19th and the beginning of the 20th centuries, particularly due to the influence of the Romantic German School and the notion of 'primitive archetype' and because it was conceptually linked to other crucial biological issues (e.g. complexity, *scala naturae*, progress, modularity or phenotypic integration). However, discussions on anisomerism and related issues (e.g. Williston's law) have been almost exclusively based on hard tissues. Here we provide the first detailed empirical test, and discussion, of anisomerism based on quantitative data obtained from phylogenetic and comparative analyses of the head and forelimb muscles of gnathostomes. Our results strongly support the existence of such a trend in both forelimb and head musculature. For instance, the last common ancestor (LCA) of extant tetrapods likely had 38 polyisomeric muscles (PMs) out of a total of 70 forelimb muscles (i.e. 54%), whereas in the LCAs of extant amniotes and of mammals these numbers were 38/73 (52%) and 21/67 (31%), and in humans are 11/59 (19%). Interestingly, the number of PMs that became specialized during the forelimb evolutionary transition from the LCA of extant tetrapods to humans (13) is very similar to the number of PMs that became lost (14), indicating that both specialization and loss contributed equally to the trend towards anisomerism. By contrast, during the evolution of the head musculature from the LCA of gnathostomes to humans a total of 27 PMs were lost whereas only one muscle became specialized. Importantly, the evolutionary trend towards anisomerism is not related to a general trend leading to the presence of fewer muscles in derived taxa, because for instance humans have more head muscles in total, but many less head polyisomeric muscles than early gnathostomes and extant fish such as sharks, and than early tetrapods and amphibians such as salamanders. This is because new muscles have also been acquired during gnathostome evolution (e.g. facial muscles of mammals). Interestingly, many new PMs have also been acquired during head evolution (but subsequently lost during the transitions towards humans), whereas only a few new PMs were acquired during forelimb evolution. Our comparisons and review of the literature indicate that there is also a trend towards anisomerism during development, thus providing a further example of a parallel between ontogeny and phylogeny, e.g. some forelimb PMs (e.g. contrahentes, intermetacarpales) become specialized or lost (re-absorbed) during human ontogeny and some head PMs (e.g. constrictores branchiales) become lost during salamander ontogeny. This review will inform future discussions on modularity, complexity, body plans, phenotypic integration and macroevolution, which should ideally include soft tissues and the use of new tools (e.g. anatomical networks) in order to provide a broader and more integrative understanding of these relevant subjects.

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I. INTRODUCTION

The American zoologist William K. Gregory was one of the most influential authors defending the existence of an evolutionary trend in vertebrates from a higher degree of polyisomerism (i.e. more polyisomeric or 'serial' anatomical parts arranged along any of the body axes) to cases of anisomerism (i.e. specialization or loss of at least some of the original polyisomeric structures). One of the examples provided by Gregory (e.g. 1929, 1934) in support of this idea concerns the presence of numerous similar teeth serially arranged in the jaws of early fossil gnathostomes, in contrast to the presence of few and highly specialized teeth in phylogenetically derived taxa such as mammals (e.g. incisors, molars, premolars, canines).

Evolutionary trends towards anisomerism were the subject of much interest at the end of the 19th century and the beginning of the 20th century, because they were conceptually linked to some of the most central subjects in the fields of evolutionary, comparative and developmental biology, as for example, the ideas of complexity, *scala naturae*, progress, modularity, body plans or phenotypic integration (e.g. Cope, 1871; Williston, 1914; Goodrich, 1930; Edgeworth, 1935). For instance, under the *Naturphilosophie* paradigm and the notion of 'primitive archetype' followed by some authors, such as Goethe (1790), Oken (1843), and later by Gegenbaur (1878) and Goodrich (1930), the head of vertebrates was seen as a continuation of the segmented trunk, the branchial arches being serially homologous to ribs, and the cranial nerves with spinal nerves (for a review, see Russell, 1916; Richards, 1992, 2002). At present, it is almost consensual that the vertebrate neurocranium is not a continuation of the segmented trunk and that the only cases of serial homology within the skeletal structures of the head (i.e. polyisomerism *sensu* Gregory) would be represented by the branchial arches, particularly the hyoid and more posterior arches (for a recent review see Kuratani *et al.*, 2013). For a recent review of the ideas of complexity, *scala naturae* and progress and their historical context, as well as of the terms 'phylogenetically basal *versus* derived taxa' and 'anatomically basal *versus* derived animals', see Diogo, Ziermann & Linde-Medina (in press).

Discussions on anisomerism faded out following the decline of comparative morphology in the second half of the 20th century, although some authors have continued to investigate the existence of evolutionary trends towards anisomerism and related issues, such as Williston's law (i.e. a general trend towards the reduction of the number of skull bones within vertebrate evolution) (e.g. Assis, 2009; Diogo & Wood, 2012*a*; Esteve-Altava *et al.*, 2013). For instance, recently the idea of anisomerism and, particularly, Williston's law, have been reassessed in the context of network theory. Conceptualizing the skull as a structural network where the bones and the suture joints of the skull represent nodes and links, respectively, some well-established network statistics can be calculated to investigate the existence of evolutionary trends in skull organization (Esteve-Altava *et al.*, 2013). Under this framework, Esteve-Altava *et al.* (2013) corroborated the existence of an evolutionary trend towards the reduction of skull bone number in mammals but suggested that this reduction was actually accompanied by a trend towards a more complex organization of the skull. However, some authors have contradicted both Williston's law and the existence of a general trend towards anisomerism. For example, according to Minelli (2009) the plesiomorphic condition for centipedes is to have a reduced rather than a high number of body segments, as illustrated by the house centipede (family Scutigeridae) with 15 pairs of legs, in contrast to derived geophilomorph centipedes, which can have up to 191 pairs of legs.

Some authors have suggested that the occurrence of evolutionary trends leading to anisomerism would have an adaptive basis. For example, according to Galis (1996) a structural decoupling among serial homologous parts (i.e. modularity) would release them from developmental or functional constraints, increasing their capability to evolve morphological adaptations. This structural uncoupling would facilitate the diversification and specialization of polyisomeric structures, leading to anisomerism. Thus, mutations that increased the degree of modularity of originally polyisomeric structures could be selectively advantageous for the species bearing them. An example of striking morphological diversity and speciosity with a structural

decoupling is provided by cichlid fishes (Galis, 1996), in which one of the branchial arches became modified into a highly specialized pharyngeal jaw apparatus. Developmental studies have shown that alterations in the expression of key regulatory genes might for instance underlie the evolutionary transformation from polyisomerism to anisomerism in the vertebrate limb (e.g. Drossopoulou *et al.*, 2000; Litingtung *et al.*, 2002; Lettice *et al.*, 2008; Towers *et al.*, 2008; Zhu *et al.*, 2008; Sheth *et al.*, 2012). Recently, it has been reported that alterations in the dose of distal *Hox* genes in the mouse limb bud produce a series of polydactylous limbs, i.e. limbs with supernumerary digits, and thus, with a higher degree of polyisomerism, some of them resembling the plesiomorphic (polyisomeric) condition of fish fins (Sheth *et al.*, 2012). Importantly, some studies have pointed out the relevance of taking into account the developmental processes of digit formation, and not only specific genetic mutations, for understanding the evolutionary alterations in digit number or the frequency distribution of polydactylous variants in populations (e.g. Sheth *et al.*, 2012; Lange, Nemeschkal & Muller, 2014).

In an influential but controversial paper, Schwartz (1999) proposed a specific hypothesis for the occurrence of a higher degree of polyisomerism in the plesiomorphic members of a clade. According to him, the recessive alleles of homeobox or other regulatory genes can spread silently in a population by heterozygosis. If there is a sufficient number of heterozygotes for the recessive mutation, their random mating can produce offspring homozygous for this specific allele. The spread of the new recessive allele could therefore be particularly quick and effective if the population is small. In accordance with this hypothesis, Schwartz (1999) cites an empirical study where a spontaneous mutation of the *Hox-13* gene resulted in the expansion of the polyalanine stretch and synpolydactyly in a laboratory colony of mice (Johnson *et al.*, 1998). Also according to Schwartz (1999), additional examples from the fossil record demonstrate not only that novel features can appear abruptly, but also that they are usually more fully expressed than in derived forms: e.g. the first tetrapods had more digits than any subsequent extant tetrapod (see also the recent review of Pierce, Hutchinson & Clack, 2013). More recently, it has been reported that some cases of polydactyly are the result of few point mutations in a conserved non-coding regulatory element of sonic hedgehog (*Shh*) gene (Lettice *et al.*, 2008), supporting the idea that evolutionary transformations leading to an increase of the number of polyisomeric structures can in fact occur suddenly in a geological time scale, rather than gradually.

As can be seen from the above, the study of issues related to polyisomerism *versus* anisomerism has been almost exclusively based on hard tissues. Some previous comparative myological studies have discussed these issues, but those studies were based on qualitative comparisons of muscles that did not include explicit phylogenetic or quantitative analyses (see review of Diogo & Abdala, 2010). Here, we explore the existence of an evolutionary trend towards anisomerism based on the results of cladistic and quantitative analyses of the musculature of the head and the forelimb of all the major vertebrate clades (e.g. Diogo, 2007; Diogo & Wood, 2011, 2012*a,b*, 2013). These myological phylogenetic works are part of a long-term project to investigate the comparative anatomy, homologies, evolution, development and phylogeny of the striated muscles of all of the major groups of vertebrates (e.g. Diogo, 2007; Diogo & Abdala, 2007, 2010; Diogo *et al.*, 2008, 2009*a,b*; Diogo & Tanaka, 2012, 2014; Diogo & Ziermann, 2014). Here we test the anisomerism hypothesis by: (*i*) comparing the estimated number of polyisomeric muscles (PMs) in the last common ancestor (LCA) of vertebrate clades and their closely related extant taxa; (*ii*) calculating the number of lost and specialized muscles between the LCAs of the different vertebrate clades and their extant taxa; (*iii*) calculating the total number of muscles of the head and of the forelimb (i.e. including both polyisomeric and non-polyisomeric muscles) for each extant taxon in order to explore whether the evolution of anisomerism is correlated to a general reduction in the number of muscles in different parts of the body.

For the identification of PMs in each anatomical region, we followed the methodology detailed in Diogo & Abdala (2010) and Diogo *et al.* (2013). For instance, regarding the human hand, three groups of PMs have been identified (lumbricales, interossei palmares and interossei dorsales) because the muscles included in each of these groups have a similar topology, attachments and function. For instance, the lumbricales 1, 2, 3 and 4 run respectively from the tendons of the flexor digitorum longus to the proximal phalanx and extensor expansions of digits 2, 3, 4 and 5, and all of them flex the proximal phalanges and extend the middle and distal phalanges of these digits (Fig. 1). It should be noted that we have recently provided an extensive discussion on serial homology (Diogo *et al.*, 2013), in particular between the forelimb and hindlimb (and specifically its refutation), and therefore, in the present work we will not repeat our discussions on whether the forelimb PMs (e.g. the lumbricales of the human hand) are, or are not, truly serial homologues to the hindlimb PMs (e.g. the lumbricales of the human foot). It should also be noted that the muscles discussed in the sections below (head and forelimb) were those included in our previous cladistic analyses, and therefore are those for which we have detailed information about the condition of the LCA for all the major groups listed in Tables 1 and 2.
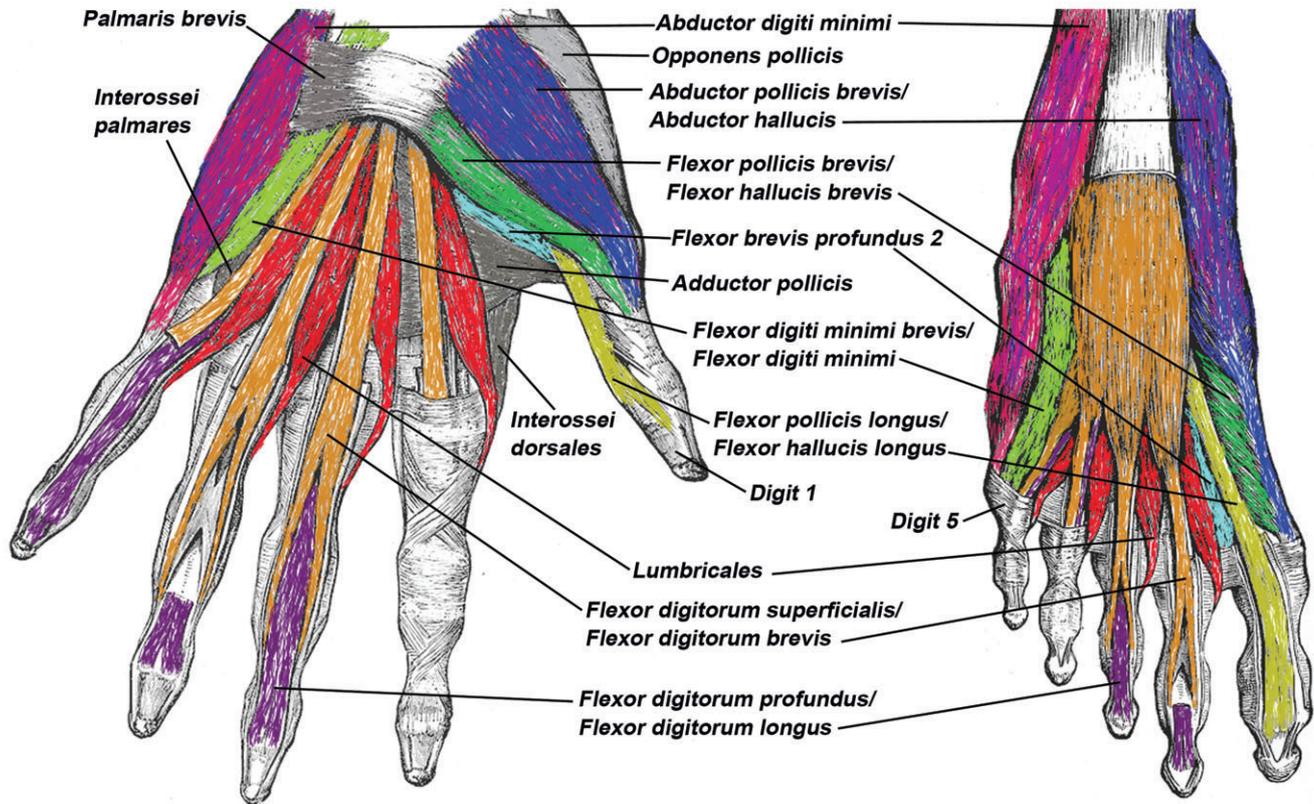
Fig. 1. Superficial musculature of the hand seen in palmar view (on the left) and of the foot seen in plantar view (on the right), showing the lumbricales (in red; both the hand and the foot have four lumbricales), which are one of the few groups of polyisomeric muscles found in the forelimb and hindlimb of modern humans (modified from Gray & Carter, 1858).

II. TESTING THE TREND TOWARDS ANISOMERISM: DATA FROM FORELIMB MUSCULATURE

As can be seen in Table 1, regarding the forelimb muscles, the LCA of all extant tetrapods very likely had 70 muscles in total (based on the comparative data and phylogenetic reconstructions provided by Diogo, 2007; Diogo & Abdala, 2010; Diogo & Tanaka, 2012; Diogo *et al.*, 2013). Of those 70 muscles, 38 (54%) are PMs. This percentage of PMs is particularly remarkable if we take into account that all the 38 PMs are only localized in the hand (flexores breves superficiales and profundi, lumbricales, contrahentes, intermetacarpales and dorsometacarpales) and the distal portion of the extensor compartment of the forearm (extensores digitorum breves) (see Fig. 2). In fact, only one intrinsic hand muscle, the abductor digiti minimi, was not a PM. The first tetrapods with digits (e.g. *Acanthostega*) which are phylogenetically more plesiomorphic than the LCA of extant tetrapods (amniotes plus amphibians) had more than five digits (for a review see Pierce *et al.*, 2013). Therefore, it is very likely that these first tetrapods had an even higher percentage of PMs, assuming that they

already had the different groups of PM found in the LCA of extant tetrapods. Unfortunately, the musculature of these first tetrapods with digits is still poorly known and this hypothesis cannot be tested at present; we are currently undertaking a project to reconstruct in detail the forelimb muscles of taxa such as *Acanthostega* (J. Molnar, R. Diogo, S. E. Pierce, J. Hutchinson, P. Albergh & P. Johnston, in preparation).

In extant salamanders such as the axolotl (*Ambystoma mexicanum*), 24 out of 51 forelimb muscles are PMs (47%) (Table 1). These numbers support a trend towards anisomerism from the LCA of tetrapods to the axolotl, because this urodele not only lost the PMs associated with digit 5 (which is absent in this species) (see Fig. 2), but also some of the PMs associated with the remaining digits (i.e. the lumbricales and the dorsometacarpales). Interestingly, during the evolutionary transition from the LCA of extant tetrapods to the axolotl, two new PMs were acquired, the flexores digitorum minimi (this group of muscles is also present in anurans, so it was very likely present in the LCA of anurans and urodeles, see, e.g. Diogo & Ziermann, 2014). Remarkably, during the transitions from the LCA of extant tetrapods to the LCA of extant amniotes no PM

Table 1. Scheme illustrating the evolution and homologies of the forelimb (including pectoral) muscles of adults of representative tetrapod taxa (based on Diogo & Abdala, 2010; Diogo & Wood, 2011, 2012a; Diogo & Tanaka, 2012); Plesiomorphic tetrapod polyisomeric muscles (PM) are shown in bold; muscles specialized/modified from polyisomeric muscles are shown in blue; new, derived polyisomeric muscles are shown in red (for taxonomy and further details, see text). LCA, last common ancestor; LCAA, LCA of extant amniotes; LCAM, LCA of extant mammals; LCAP, LCA of extant primates; LCAT, LCA of extant tetrapods

	LCAT; 38/70 (54%) PMs	*Ambystoma mexicanum*; 24/51 (47%) PMs; from LCAT there are 14 fewer PMs because 16 were lost and 2 were acquired	LCAA: 38/73 (52%) (PMs); from LCAT there are 0 fewer PMs	*Timon lepidus*; 37/66 (56%) (PMs); from LCAA there is 1 fewer PMs because 1 was lost	LCAM: 21/67 (31%) (PMs); from LCAA there are 17 fewer PMs because 7 were lost and 10 became specialized	*Ornithorhynchus anatinus*; 13/56 (23%) (PMs); from LCAM there are 8 fewer PMs because 8 were lost	LCAP: 19/68 (28%) (PMs); from LCAM there are 2 fewer PMs because 2 became specialized	*Lemur catta*; 22/70 (31%) (PMs); from LCAP there are 3 more PMs because 1 was lost and 4 were acquired	*Homo sapiens*; 11/59 (19%) (PMs); from LCAP there are 8 fewer PMs because 7 were lost and 1 became specialized (in total, from LCAT to *H. sapiens* there are 27 fewer PMs because 14 were lost, 13 became specialized, and 0 were acquired)
Axial: Pectoral girdle	Serratus anterior + rhomboideus	Serratus anterior	Serratus anterior + rhomboideus	Serratus anterior	Serratus anterior + rhomboideus	Serratus anterior + rhomboideus	Serratus anterior + rhomboideus major + occipitalis	Serratus anterior + rhomboideus major + occipitalis	Serratus anterior + rhomboideus major + minor
	Levator scapulae + Levator claviculae	Levator scapulae	Levator scapulae + levator claviculae	Levator scapulae + levator claviculae	Levator scapulae + levator claviculae	Levator scapulae + levator claviculae	Levator scapulae + levator claviculae	Levator scapulae + levator claviculae	Levator scapulae
	—	—	Sternocoracoideus + costocoracoideus	Sternocoracoideus + costocoracoideus	Sternocoracoideus + costocoracoideus	Sternocoracoideus + costocoracoideus	Subclavius	Subclavius	Subclavius
Appendicular: Pectoral girdle and ARM	Pectoralis	Pectoralis	Pectoralis	Pectoralis	Pectoralis major + minor + panniculus carnosus (part)	Pectoralis major + minor + panniculus carnosus (part)	Pectoralis major + minor + panniculus carnosus (part)	Pectoralis major + minor + panniculus carnosus (part)	Pectoralis major + minor
	Supracoracoideus	Supracoracoideus	Supracoracoideus	Supracoracoideus	Infraspinatus + supraspinatus	Infraspinatus + supraspinatus	Infraspinatus + supraspinatus	Infraspinatus + supraspinatus	Infraspinatus + supraspinatus
	Deltoideus scapularis	Deltoideus scapularis	Deltoideus scapularis	Deltoideus scapularis	Deltoideus scapularis + teres minor	Deltoideus scapularis + teres minor	Deltoideus scapularis + teres minor	Deltoideus scapularis + teres minor	Deltoideus (part) + teres minor
	Procoracohumeralis	Procoracohumeralis	Deltoideus clavicularis + scapulo-humeralis anterior	Deltoideus clavicularis + scapulo-humeralis anterior	Deltoideus clavicularis + scapulo-humeralis anterior	Deltoideus clavicularis + scapulo-humeralis anterior	Deltoideus clavicularis	Deltoideus clavicularis	Deltoideus (part)
	Subcoracoscapularis	Subcoracoscapularis	Subscapularis + teres major	Subcoracoscapularis	Subscapularis + teres major	Subscapularis + teres major	Subscapularis + teres major	Subscapularis + teres major	Subscapularis + teres major
	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi	Latissimus dorsi
	Triceps brachii	Triceps brachii	Triceps brachii	Triceps brachii	Triceps brachii + dorsoepitrochlearis	Triceps brachii + dorsoepitrochlearis	Triceps brachii + dorsoepitrochlearis	Triceps brachii + dorsoepitrochlearis	Triceps brachii
	Humeroantebrachialis	Humeroantebrachialis	Brachialis	Brachialis	Brachialis	Brachialis	Brachialis	Brachialis	Brachialis
	Coracoradialis	Coracoradialis	Biceps brachii	Biceps brachii	Biceps brachii	Biceps brachii	Biceps brachii	Biceps brachii	Biceps brachii
	Coracobrachialis	Coracobrachialis	Coracobrachialis	Coracobrachialis	Coracobrachialis	Coracobrachialis	Coracobrachialis	Coracobrachialis	Coracobrachialis

Table 1. Continued

Appendicular: Ventral forearm

LCAT: 38/70 (54%) PMs	Ambystoma mexicanum; 24/51 (47%) PMs; from LCAT there are 14 fewer PMs because 16 were lost and 2 were acquired	LCAA: 38/73 (52%) (PMs); from LCAT there are 0 fewer PMs	Timon lepidus; 37/66 (56%) (PMs); from LCAA there is 1 fewer PMs because 1 was lost	LCAM: 21/67 (31%) (PMs); from LCAA there are 17 fewer PMs because 7 were lost and 10 became specialized	Ornithorhynchus anatinus; 13/56 (23%) (PMs); from LCAM there are 8 fewer PMs because 8 were lost	LCAP: 19/68 (28%) (PMs); from LCAM there are 2 fewer PMs because 2 became specialized	Lemur catta; 22/70 (31%) (PMs); from LCAP there are 3 more PMs because 1 was lost and 4 were acquired	Homo sapiens; 11/59 (19%) (PMs); from LCAP there are 8 fewer PMs because 7 were lost and 1 became specialized (in total, from LCAT to H. sapiens there are 27 fewer PMs because 14 were lost, 13 became specialized, and 0 were acquired)
Palmaris profundus 1	Palmaris profundus 1	Palmaris profundus 1	Palmaris profundus 1	—	—	—	—	—
Pronator quadratus	Pronator quadratus	Pronator quadratus	Pronator quadratus + acessorius	Pronator quadratus	—	Pronator quadratus	Pronator quadratus	Pronator quadratus
Contrahentium caput longum	Contrahentium caput longum	—	—	—	—	—	—	—
Flexor accessorius lateralis	Flexor accessorius lateralis	—	—	—	—	—	—	—
Flexor accessorius medialis	Flexor accessorius medialis	—	—	—	—	—	—	—
Flexor digitorum communis	Flexor digitorum communis	Flexor digitorum longus	Flexor digitorum longus	Flexor digitorum longus (part)	Flexor digitorum longus (part)	Flexor digitorum profundus + superficialis (part) + palmaris longus	Flexor digitorum profundus + superficialis (part) + palmaris longus	Flexor digitorum profundus + superficialis (part) + flexor pollicis longus + palmaris longus
Flexor carpi ulnaris + epitrochleoanconeus	Flexor anconeus et carpi ulnaris (but epitrochleoanconeus found in other urodeles)	Flexor carpi ulnaris + epitrochleoanconeus	Flexor carpi ulnaris + epitrochleoanconeus	Flexor carpi ulnaris + epitrochleoanconeus	Flexor carpi ulnaris + epitrochleoanconeus	Flexor carpi ulnaris + epitrochleoanconeus	Flexor carpi ulnaris + epitrochleoanconeus	Flexor carpi ulnaris
Flexor carpi radialis + pronator teres	Flexor anconeus et carpi radialis (but pronator teres found in other urodeles)	Flexor carpi radialis + pronator teres	Flexor carpi radialis + pronator teres	Flexor carpi radialis + pronator teres	Flexor carpi radialis + pronator teres	Flexor carpi radialis + pronator teres	Flexor carpi radialis + pronator teres	Flexor carpi radialis + pronator teres

Table 1. Continued

Appendicular: Ventral and dorsal hand	LCAT; 38/70 (54%) PMs	*Ambystoma mexicanum;* 24/51 (47%) (PMs); from LCAT there are 14 fewer PMs because 16 were lost and 2 were acquired	LCAA; 38/73 (52%) (PMs); from LCAT there are 0 fewer PMs	*Timon lepidus;* 37/66 (56%) (PMs); from LCAA there is 1 fewer PMs because 1 was lost	LCAM: 21/67 (31%) (PMs); from LCAA there are 17 fewer PMs because 7 were lost and 10 became specialized	*Ornithorhynchus anatinus;* 13/56 (23%) (PMs); from LCAM there are 8 fewer PMs because 8 were lost	LCAP: 19/68 (28%) (PMs); from LCAM there are 2 fewer PMs because 2 became specialized	*Lemur catta;* 22/70 (31%) (PMs); from LCAP there are 3 more PMs because 1 was lost and 4 were acquired	*Homo sapiens;* 11/59 (19%) (PMs); from LCAP there are 8 fewer PMs because 7 were lost and 1 became specialized (in total, from LCAT to H. sapiens there are 27 fewer PMs because 14 were lost, 13 became specialized, and 0 were acquired)
Flexores breves superficiales (5)	Flexores breves superficiales (5)	Flexores breves superficiales (4)	**Flexores breves superficiales (5)**	**Flexores breves superficiales (5)**	Flexor digitorum longus (4 tendons)	Flexor digitorum longus (4 tendons)	Flexor digitorum superficialis (part of the muscle: 4 tendons) + palmaris brevis	Flexor digitorum superficialis (part: 4 tendons) + palmaris brevis	Flexor digitorum superficialis (part: 4 tendons) + palmaris brevis
Lumbricales (4)	Lumbricales (4)	— (but lumbricals found in some urodeles and in anurans)	Lumbricales (4)	Lumbricales (3) (but 4 or 5 lumbricals found in some reptiles)	Lumbricales (4)	Lumbricales (3) (but 4 or 5 lumbricals found in echidnas)	Lumbricales (4)	Lumbricales (4)	Lumbricales (4)
Contrahentes digitorum (5)	Contrahentes digitorum (5)	Contrahentes digitorum (4)	Contrahentes digitorum (5)	Contrahentes digitorum (5)	**Contrahentes digitorum (3) + adductor pollicis**	Adductor pollicis	**Contrahentes digitorum (3) + adductor pollicis**	**Contrahentes digitorum (2) + adductor pollicis**	Adductor pollicis + 'volaris primus of Henle'
Flexores breves profundi (10)	Flexores breves profundi (10)	Flexores breves profundi (8)	Flexores breves profundi (10)	Flexores breves profundi (10)	Flexores breves profundi (10)	Flexores breves profundi (10)	Flexor pollicis brevis + opponens pollicis + flexor digiti minimi brevis + opponens digiti minimi + flexores breves profundi (8)	Flexor pollicis brevis + opponens pollicis + flexor digiti minimi brevis + opponens digiti minimi + flexores breves profundi (8) + interossei accessorii (4)	Flexor brevis profundus 2 ('deep head flexor pollicis brevis') + flexor pollicis brevis + opponens pollicis + flexor digiti minimi brevis + opponens digiti minimi + interossei palmares (=some flexores breves profundi) (3)
—	—	Flexores digitorum minimi (2)	—	—	—	—	—	—	—
—	—	Interphalangeus III	—	—	—	—	—	—	—
Abductor digiti minimi	—	—	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis	Abductor pollicis brevis
Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi	Abductor digiti minimi
Intermetacarpales (4)	Intermetacarpales (4)	Intermetacarpales (3)	Intermetacarpales (4)	Intermetacarpales (4)	Intermetacarpales (4)	—	Intermetacarpales (4)	Intermetacarpales (4)	Interossei dorsales (=some flexores breves profundi + intermetacarpales) (4)
Dorsometacarpales (5)	Dorsometacarpales (5)	— (but dorsometacarpales found in some urodeles and in anurans)	Dorsometacarpales (5)	Dorsometacarpales (5)	—	—	—	—	—

Table 1. Continued

Appendicular: Dorsal forearm

	LCAT: 38/70 (54%) PMs	Ambystoma mexicanum; 24/51 (47%) PMs; from LCAT there are 16 fewer PMs because 16 were lost and 2 were acquired	LCAA: 38/73 (52%) (PMs); from LCAT there are 0 fewer PMs	Timon lepidus; 37/66 (56%) (PMs); from LCAA there is 1 fewer PMs because 1 was lost	LCAM: 21/67 (31%) (PMs); from LCAA there are 17 fewer PMs because 7 were lost and 10 became specialized	Ornithorhynchus anatinus; 13/56 (23%) (PMs); from LCAM there are 8 fewer PMs because 8 were lost	LCAP: 19/68 (28%) (PMs); from LCAM there are 2 fewer PMs because 2 became specialized	Lemur catta; 22/70 (31%) (PMs); from LCAP there are 3 more PMs because 1 was lost and 4 were acquired	Homo sapiens; 11/59 (19%) (PMs); from LCAP there are 8 fewer PMs because 7 were lost and 1 became specialized (in total, from LCAT to H. sapiens there are 27 fewer PMs because 14 were lost, 13 became specialized, and 0 were acquired)
	Extensor carpi radialis + brachioradialis	Extensor carpi radialis (but brachioradioalis found in some urodeles)	Extensor carpi radialis longus + brevis + brachioradialis	Extensor carpi radialis	Extensor carpi radialis longus + brevis + brachioradialis	Extensor carpi radialis + brachioradialis	Extensor carpi radialis longus + brevis + brachioradialis	Extensor carpi radialis longus + brevis + brachioradialis	Extensor carpi radialis longus + brevis + brachioradialis
	Supinator; Extensor carpi ulnaris + anconeus	Supinator; Extensor anconeus et carpi ulnaris (but anconeus found in some urodeles)	Supinator; Extensor carpi ulnaris + anconeus	Extensor antebrachii et carpi ulnaris (but anconeus found in some lepidosaurs)	Supinator; Extensor carpi ulnaris + anconeus	Supinator; Extensor carpi ulnaris + anconeus	Supinator; Extensor carpi ulnaris + anconeus	Supinator; Extensor carpi ulnaris + anconeus	Supinator; Extensor carpi ulnaris + anconeus
	Extensor digitorum; Extensores digitorum breves (5)	Extensor digitorum; Extensores digitorum breves (3)	Extensor digitorum; Extensores digitorum breves (5)	Extensor digitorum; Extensores digitorum breves (5)	Extensor digitorum; Extensor digiti minimi + extensor digiti quarti + extensor digiti III proprius + extensor indicis + extensor pollicis longus	Extensor digitorum; Extensor digiti minimi + extensor digiti III proprius + extensor indicis + extensor pollicis longus	Extensor digitorum; Extensor digiti minimi + extensor indicis + extensor pollicis longus	Extensor digitorum; Extensor digiti minimi + extensor indicis + extensor pollicis longus	Extensor digitorum; Extensor digiti minimi + extensor indicis + extensor pollicis longus
	Abductor pollicis longus	Abductor et extensor digiti 1	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus	Abductor pollicis longus + extensor pollicis brevis

Table 2. Scheme illustrating the evolution and homologies of the head and neck (not including the occular) muscles of adults of representative gnathostomes (based on Diogo & Abdala, 2010; Diogo & Wood, 2011, 2012a; Ziermann & Diogo, 2013, 2014)

	LCAG; 15/24 (63%) PMs	*Squalus acanthias*; 17/30 (57%) PMs; from LCAG there are 3 more PMs because 5 were lost and 7 were acquired	LCAR; 20/35 (57%) PMs; from LCAG there are 5 more PMs because 6 were lost and 11 were acquired	*Neoceratodus forsteri*; 20/33 (61%) PMs; from LCAR there are 0 fewer PMs because 0 were lost	LCA of extant tetrapods (LCAT); 14/31 (45%) PMs; from LCAR there are 6 fewer PMs because 8 were lost and 2 were acquired	*Ambystoma mexicanum*; 12/31 (39%) PMs; from LCAT there are 2 fewer PMs because 5 were lost, 1 became specialized, and 4 were acquired	LCAA; 0/20 (0%) PMs; from LCAT there are 14 fewer PMs because 13 were lost and 1 became specialized	*Homo sapiens*; 0/60 (0%) PMs; from LCAA there are 0 fewer PMs (in total, from LCAG to *H. sapiens* there are 15 fewer PMs because 27 were lost, 1 became specialized, and 13 were acquired)
Mandibular	Intermandibularis	Intermandibularis	Intermandibularis posterior	Intermandibularis	Intermandibularis posterior	Intermandibularis posterior	Intermandibularis posterior	Mylohyoideus + digastricus anterior
	—	—	Intermandibularis anterior	—	Intermandibularis anterior	Intermandibularis anterior	Intermandibularis anterior	—
	—	—	Adductor mandibulae A3′	Adductor mandibulae A3′	Pseudotemporalis + pterygomandibularis (present in e.g. some caecilians)	Pseudotemporalis	Pseudotemporalis + pterygomandibularis	Pterygoideus medialis + tensor veli palatini + tensor tympani
	Adductor mandibulae A2	Adductor mandibulae A2	Adductor mandibulae A2 + adductor mandibulae A2-PVM	Adductor mandibulae A2 + adductor mandibulae A2-PVM	Adductor mandibulae A2 + adductor mandibulae A2-PVM	Adductor mandibulae A2 + adductor mandibulae A2-PVM	Adductor mandibulae A2 + adductor mandibulae A2-PVM	Masseter + temporalis + pterygoideus lateralis
	Levator arcus palatini	Levator arcus palatini + spiracularis	Levator arcus palatini	—	Levator bulbi	Levator bulbi	Levator bulbi	—
	—	Preorbitalis	—	—	—	—	—	—
Hyoid	Interhyoideus	Interhyoideus	Interhyoideus	Interhyoideus	Interhyoideus	Interhyoideus	Interhyoideus	23 facial muscles (part)
	—	—	Depressor mandibulae + levator hyoideus	Depressor mandibulae + levator hyoideus	Depressor mandibulae (incl. levator hyoideus)	Depressor mandibulae + branchiohyoideus	Depressor mandibulae	Stylohyoideus + digastricus posterior + stapedius + 23 facial muscles (part)
	Constrictor hyoideus dorsalis	Constrictor hyoideus dorsalis	Constrictor hyoideus dorsalis	Constrictor hyoideus	—	—	—	—
	Adductor arcus palatini	Adductor arcus palatini	—	—	—	—	—	—

Table 2. Continued

	LCAG; 15/24 (63%) PMs	Squalus acanthias; 17/30 (57%) PMs; from LCAG there are 3 more PMs because 5 were lost and 7 were acquired	LCAR; 20/35 (57%) PMs; from LCAG there are 5 more PMs because 6 were lost and 11 were acquired	Neoceratodus forsteri; 20/33 (61%) PMs; from LCAR there are 0 fewer PMs because 0 were lost	LCA of extant tetrapods (LCAT); 14/31 (45%) PMs; from LCAR there are 6 fewer PMs because 8 were lost and 2 were acquired	Ambystoma mexicanum; 12/31 (39%) PMs; from LCAT there are 2 fewer PMs because 5 were lost, 1 became specialized, and 4 were acquired	LCAA; 0/20 (0%) PMs; from LCAT there are 14 fewer PMs because 13 were lost and 1 became specialized	Homo sapiens; 0/60 (0%) PMs; from LCAA there are 0 fewer PMs; from LCAG to H. sapiens there are 15 fewer PMs because 27 were lost, 1 became specialized, and 13 were acquired
Branchial + epibranchial	Cucullaris	Cucullaris	Levatores arcuum branchiarum (5) + protractor pectoralis	Levatores arcuum branchiarum (5) + protractor pectoralis	Levatores arcuum branchiarum (4) + protractor pectoralis	Levatores arcuum branchiarum (4) + protractor pectoralis	Trapezius + sternocleidomastoideus	Trapezius + sternocleidomastoideus
	Coracobranchiales (5)	Coracobranchiales (5)	Coracobranchiales (4)	Coracobranchiales (4)	—	—	—	—
	—	—	Subarcuales recti (2)	Subarcuales recti (2)	Subarcuales recti (4)	Subarcuales recti (4) (two of them often designated as subarcuales obliqui)	Ceratohyoideus + hyobranchialis (both these muscles derived from the subarcualis rectus 1)	Stylopharyngeus
	—	—	Transversi ventrales (4)	Transversi ventrales (4)	Transversi ventrales (2)	Transversus ventralis IV	—	—
	Adductores branchiales (5) + constrictores branchiales (5)	Adductores branchiales (5) + interarcuales laterales (4)	Constrictores branchiales (5)	Constrictores branchiales (5)	Constrictores branchiales (4)	— (only larval)	—	—
	—	—	—	—	—	Levators & depressors branchiarum (4)	—	—
	—	Interpharyngo branchiales (3) + subspinalis	—	—	—	—	—	—
Pharyngeal	—	—	—	—	—	—	—	Constrictor pharyngis medius, inferior & superior + cricothyroideus + palatopharyngeus + levator veli palatini + salpingopharyngeus + musculus uvulae

Table 2. Continued

	LCAG; 15/24 (63%) PMs	***Squalus acanthias;* 17/30 (57%) PMs; from LCAG there are 3 more PMs because 5 were lost and 7 were acquired**	**LCAR; 20/35 (57%) PMs; from LCAG there are 5 more PMs because 6 were lost and 11 were acquired**	***Neoceratodus forsteri;* 20/33 (61%) PMs; from LCAR there are 0 fewer PMs because 0 were lost**	**LCA of extant tetrapods (LCAT); 14/31 (45%) PMs; from LCAR there are 6 fewer PMs because 8 were lost and 2 were acquired**	***Ambystoma mexicanum;* 12/31 (39%) PMs; from LCAT there are 2 fewer PMs because 5 were lost, 1 became specialized, and 4 were acquired**	**LCAA: 0/20 (0%) PMs; from LCAT there are 14 fewer PMs because 13 were lost and 1 became specialized**	***Homo sapiens;* 0/60 (0%) PMs; from LCAA there are 0 fewer PMs (in total, from LCAG to *H. sapiens* there are 15 fewer PMs because 27 were lost, 1 became specialized, and 13 were acquired)**
Laryngeal	—	—	Constrictor laryngis	Constrictor laryngis	Constrictor laryngis	Constrictor laryngis / Laryngeus	Constrictor laryngis	—
	—	—	—	—	—	—	—	Thyroarytenoideus + cricoarytenoideus lat. + vocalis + arytenoideus transversus + arytenoideus obliquus
	—	—	Dilatator laryngis	Dilatator laryngis	Dilatator laryngis	Dilatator laryngis	Dilatator laryngis	Cricoarytenoideus posterior
Hypobranchial	Coracomandibularis	Coracomandibularis	Coracomandibularis	Coracomandibularis	Geniohyoideus	Geniohyoideus	Geniohyoideus	Geniohyoideus
	—	—	—	—	Genioglossus	Genioglossus	Genioglossus	Genioglossus
	—	—	—	—	Hyoglossus	Hyoglossus	Hyoglossus	Hyoglossus + styloglossus + palatoglossus
	—	—	—	—	—	Interradialis	—	—
	Sternohyoideus	Sternohyoideus + coracoarcualis	Sternohyoideus	Sternohyoideus	Sternohyoideus + omohyoideus	Sternohyoideus + omohyoideus	Sternohyoideus + omohyoideus	Sternohyoideus + omohyoideus + sternothyroideus + thyrohyoideus

Plesiomorphic gnathostome polyisomeric muscles are shown in bold; muscles specialized/modified from polyisomeric muscles are shown in red; new, derived polyisomeric muscles are shown in blue (for taxonomy and further details, see text).
LCA, last common ancestor; **LCAA**, LCA of extant amniotes; **LCAG**, LCA of extant gnathostomes; **LCAR**, LCA of extant rhipidistians (dipnoans + tetrapods); **LCAT**, LCA of extant tetrapods; **PMs**, polyisomeric muscles.
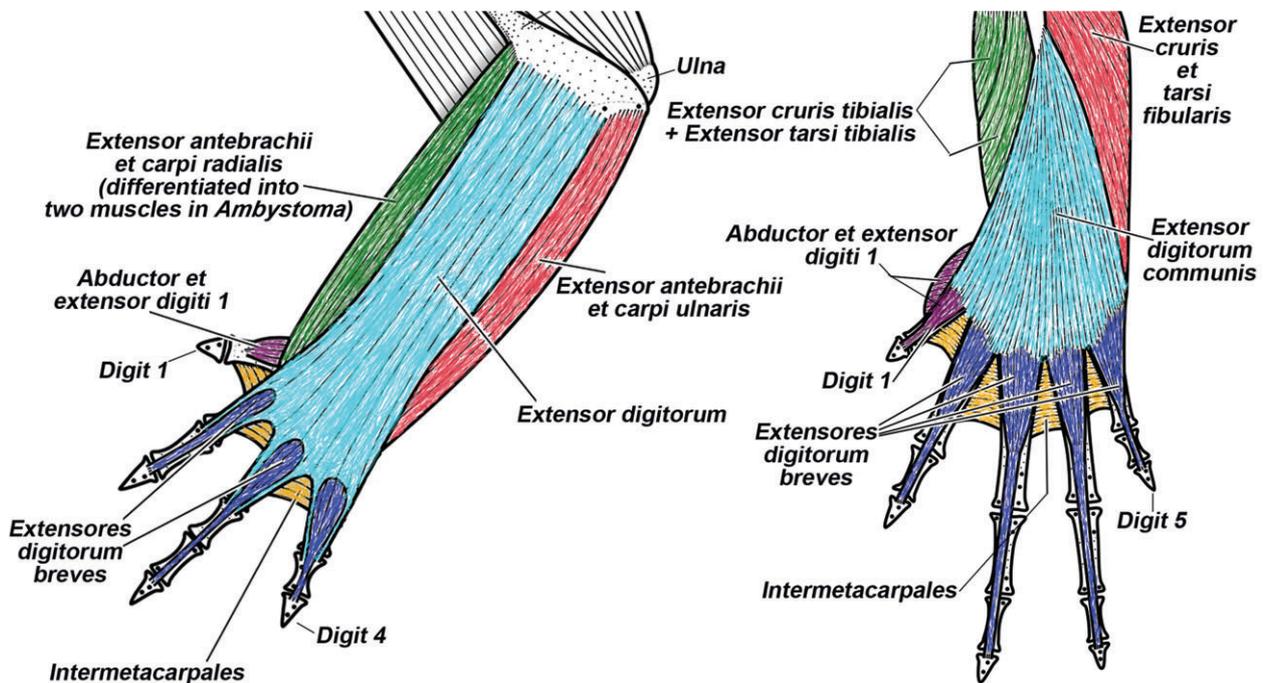
Fig. 2. Superficial musculature of the distal portion of the urodele *Taricha torosa* forelimb (on the left) and hindlimb (on the right) in dorsal view, showing the intermetacarpales (in orange; the hand and foot have, respectively, three and four intermetacarpales) and the extensores digitorum breves (in dark blue; the hand and foot have, respectively, three and four extensores digitorum breves), which are among the various groups of polyisomeric muscles found in the forelimb and hindlimb. The urodele *Taricha torosa* differs little from *Ambystoma mexicanum* in its limb muscles [modified from Walthall & Ashley-Ross (2006); N.B., as this is a dorsal view whereas Fig. 1 is a palmar/plantar (i.e. ventral) view, there is no correspondence between the colours used in the two figures, i.e. they do not represent homologous structures between urodeles and modern humans].

was lost or gained (Table 2). However the percentage of PMs/total number of muscles in reptiles such as the lizard *Timon lepidus* (56%) is actually higher than that estimated for the LCA of extant tetrapods (54%). This is because various non-PMs were lost during the transitions from the LCA of extant amniotes to *Timon lepidus*, whereas only one PM was lost during these transitions; the total number of PMs in *Timon lepidus* is however still lower than those found in the LCA of extant tetrapods and amniotes.

A particularly notable change towards anisomerism occurred during the transition from the LCA of extant amniotes to the LCA of extant mammals where 7 PMs were lost and 10 PMs became specialized, i.e. fused to other muscles (e.g. the flexores breves superficiales) or anatomically different from the remaining muscles of their group (e.g. the adductor pollicis of mammals) results from a specialization of the contrahens of digit 1 and it is anatomically very different, and broader, than the contrahentes of the other digits (Fig. 1; Table 1). The trend towards anisomerism is also seen in the transitions from the LCA of mammals to the monotreme platypus (*Ornithorhynchus anatinus*), in which eight forelimb PMs were lost (Table 1), and to the LCA of extant primates, in which two forelimb PMs became

specialized (i.e. the flexor brevis profundus became divided into a flexor pollicis brevis and an opponens pollicis, and the flexor brevis profundus became divided into a flexor digiti minimi brevis and an opponens digiti minimi: Table 1).

The forelimb transition from the LCA of extant primates to the strepsirrhine *Lemur catta* represents an exception to the general trend of the forelimb musculature towards anisomerism: four new PMs were acquired (interossei accessorii, which seem to represent a synapomorphy of strepsirrhines: e.g. Diogo & Wood, 2012a) whereas only one PM was lost (one contrahens) increasing the proportion of PMs. That is, both the number of PMs (22) and the percentage of PM/total number of muscles (31%) are greater in this strepsirrhine than in the LCA of primates (19/68 or 28%). But in the context of the present work this is clearly one of those 'exceptions that confirm the rule', because if we analyse the forelimb transitions from the LCA of extant primates to humans (seven PMs lost and one PM specialized), or from the LCA of extant tetrapods to humans (14 PMs lost and 13 PMs specialized, resulting in 27 fewer PMs in total), it is clear that there is an overall trend towards anisomerism (Table 1). The fact that the number of PMs that became specialized during the transition from the

LCA of extant tetrapods to humans (13) is very similar to the number of PMs that became lost during this transition (14) indicates that, in the case of the forelimb musculature, both muscle specialization and muscle loss contributed equally to the trend towards anisomerism.

One issue that is often not discussed in the literature about anisomerism concerns the parallel between phylogeny and ontogeny. However, our comparisons and review of the literature allow us to address this subject in the context of the evolution and development of the forelimb of tetrapods. In *Ontogeny and Phylogeny* Gould (1977) argues that although Haeckel's hypothesis that the ontogeny of one organism recapitulates the adult stages of its ancestors (i.e. recapitulation) has been refuted, researchers often use this idea as a 'straw-man' to deny that there is often a parallel between ontogeny and phylogeny. According to Gould (1977) such a parallel exists and is probably driven more by phylogenetic/ontogenetic constraints than by adaptive plasticity. According to data on the development of the human forelimb provided by authors such as Cihak (1972) such a parallel does exist, at least regarding some muscles. For instance, as happened during the evolutionary transitions leading to humans (Table 1), during human ontogeny the intermetacarpales also become fused with some flexores breves profundi to form the dorsal interossei, and the contrahentes become specialized (the one to digit 1) or lost (i.e. reabsorbed; the ones to the other digits) (Fig. 3; Cihak, 1972). This is, of course, not recapitulation in the Haeckelian sense: the contrahentes digitorum and the intermetacarpales of human embryos do not correspond to the muscles of adult mammals such as chimpanzees or lemurs, but instead to the muscles of the embryos of these latter taxa. That is, the developmental pathways that generate these muscles in the adults of the latter taxa have not been completely lost in modern humans, even after several millions of years, probably because these pathways are related to those involved in the development of other structures that are present and functional in modern human adults (ontogenetic constraints; for further details, see recent review by Diogo & Wood, 2012*b*).

III. TESTING THE TREND TOWARDS ANISOMERISM: DATA FROM HEAD MUSCULATURE

In general, the data obtained from the study of the evolution of the head musculature from the LCA of extant gnathostomes to the LCA of extant tetrapods, and particularly, to the LCA of extant amniotes, also provides support for a trend towards anisomerism. The percentage of PMs for these three LCAs is, respectively, 63% (15 of a total of 24 muscles), 45% (14 of a total of 31 muscles) and 0% (0 of a total of 20 muscles) (Table 2). However, it should be noted that during the transitions from
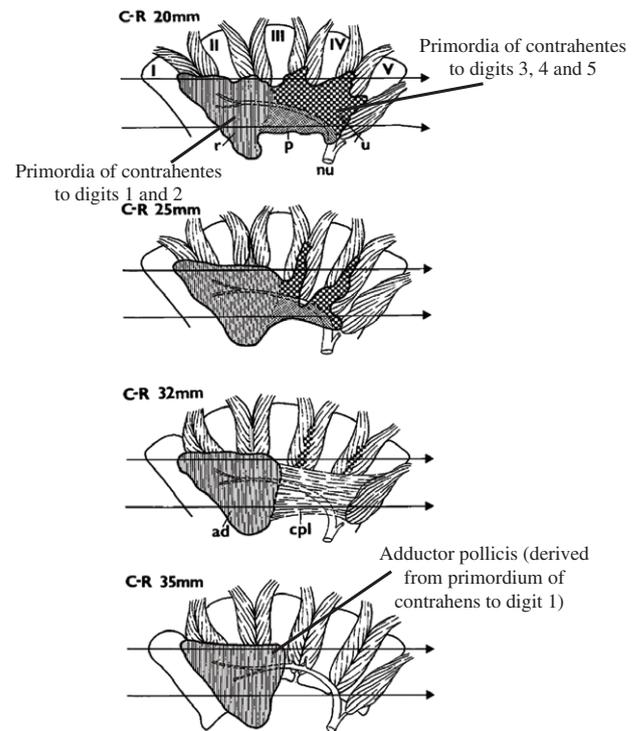
Fig. 3. Ontogeny of the contrahentes digitorum in the hand of a karyotypically normal human embryo showing how the contrahentes to digits other than digit 1 are usually lost (reabsorbed) early in development (modified from Cihak, 1972). Part of the interossei primordia (i.e. the flexores breves profundi layer) are shown between the metacarpals. r, p, u: radial, proximal and ulnar parts, respectively, of contrahentes layer; nu: ulnar nerve; ad: adductor pollicis; cpl: contrahens plate; I–V: metacarpals I–V; C-R: crown–rump length of the embryos.

the LCA of gnathostomes to extant chondrichthyans such as sharks (17 PMs), as well as to the LCA of rhipidistians (dipnoans + tetrapods) (20 PMs), there was actually an increase of the total number of PMs, because while some PMs were lost, numerous new PMs were acquired (interarcuales laterales and interpharyngobranchiales in chondrichthyans, and levatores arcuum branchiarum, transversi ventrales and subarcuales recti during the transitions to rhipidistians; Table 2). This clearly contrasts with the evolution of the forelimb muscles, in which only a few new PMs were acquired, and only in some specific (less inclusive) groups such as the strepsirrhines or the amphibians (see Section II and Table 1).

Another major difference between the evolution of the head and forelimb muscles is that, as explained above, the number of PMs that became specialized during the forelimb transitions from the LCA of extant tetrapods to humans (13) is similar to the number of PMs that became lost during those transitions (14), while during the head transitions from the LCA of gnathostomes to humans a total of 27 PMs were lost

while only one became specialized (Table 2). That is, there is much less specialization of specific PMs in the head, and therefore less diversity and functional decouplings than in the forelimb.

The study of the head muscles also indicates that the evolutionary trend towards anisomerism is not related to the existence of a general trend leading to a reduction of the number of muscles in derived taxa. For example, humans have more head muscles in total (60), but many less polyisomeric head muscles (0), than early gnathostomes and fish such as sharks (17 PM out of 30 muscles), and than early tetrapods and amphibians such as salamanders (12 out of 31 muscles). This is because new, non-PMs have also been differentiated during evolution (e.g. the facial muscles of mammals).

Interestingly, as in the case of the forelimb muscles (see Section II), the study of the head muscles provides examples of a trend towards anisomerism during development and therefore of a parallel between ontogeny and phylogeny. For instance, in salamanders, some head PMs (e.g. constrictores branchiales) originate early in ontogeny but are subsequently lost at later developmental stages (Edgeworth, 1935), as they were during the evolutionary history of rhipidistians (Table 2; e.g. Diogo & Abdala, 2010). As pointed out by Diogo & Wood (2012b) and explained above, such examples of muscles, particularly of PMs, which are lost during development are probably related to ontogenetic constraints (e.g. Gould, 1977, 2002), rather than to adaptive embryonic changes.

IV. CONCLUSIONS

(1) The inclusion of soft-tissue-based information in comparative and phylogenetic investigations strongly supports the existence of a trend where some of the original PMs associated with the branchial arches in the head, and with the digits in the forelimb, became specialized or lost during the evolution of gnathostomes and tetrapods, respectively. For instance, the LCA of extant tetrapods likely had 38 PMs out of the 70 forelimb muscles (i.e. 54%), whereas the LCA of extant amniotes and the LCA of mammals had likely 38/73 (52%) and 21/67 (31%), respectively. In humans the percentage of PMs is reduced to 19% (11/59).

(2) Remarkably, the number of PMs that became specialized in the forelimb during the transition from the LCA of extant tetrapods to humans (13) is very close to the number of PMs that became lost during this transition (14). This indicates that, in the case of the forelimb, both muscle specialization and muscle loss contributed equally to the trend towards anisomerism. This contrasts with the evolution of the head muscles from the LCA of gnathostomes to humans, in which a total of 27 PMs were lost whereas only one muscle became specialized.

These different patterns are at least in part likely related to the fact that the number of forelimb bones of the LCA of extant tetrapods was very similar to that of humans, whereas there was a significant reduction of the number of cranial bones from the LCA of gnathostomes to humans (e.g. Diogo, 2007). This reduction is mainly linked to the dramatic loss of bones of the branchial arches during the water–land transitions that led to the origin of tetrapods and then to amniotes; these transitions are the ones where there was a greater loss of PMs (Table 2).

(3) The evolutionary trend towards anisomerism is not related to the existence of an evolutionary trend leading to a reduction in the number of muscles in derived taxa. For example, humans have more head muscles in total, but many less PMs than early gnathostomes and fish such as sharks, and than early tetrapods and amphibians such as salamanders. This is because new muscles have also differentiated during the evolution of the head musculature (e.g. facial muscles of mammals). Interestingly, many new PMs were also acquired during the evolution of head muscles, but they were subsequently lost during the transitions towards humans. By contrast, a very reduced number of new PMs were acquired during forelimb evolution, the few exceptions being the interossei accessorii muscles of strepsirrhine primates and the flexores digitorum minimi of amphibians.

(4) In the case of the forelimb musculature, only during the transition from the LCA of extant primates to the strepsirrhine *Lemur catta* did the total number of PMs increase, representing the sole exception to a general trend towards anisomerism. Regarding the head muscles, there are two cases of a trend towards polyisomerism: from the LCA of extant gnathostomes to extant chondrichthyans, such as sharks, and to the LCA of rhipidistians (dipnoans + tetrapods).

(5) Interestingly, trends towards anisomerism are also observed during muscle development. For example, during human ontogeny some forelimb PMs (e.g. contrahentes, intermetacarpales) become specialized or lost (re-absorbed). During salamander ontogeny some head PMs (e.g. constrictores branchiales) are also lost, representing a further example of a parallel between ontogeny and phylogeny.

(6) We are currently undertaking muscle reconstructions of early tetrapods in order to investigate whether these animals have more forelimb PMs than any extant tetrapods as predicted by their polydactyly (see Section II). One other example of promising lines of future research to evaluate anisomerism and related issues is the use of anatomical networks. As explained above, by using such networks Esteve-Altava *et al.* (2013) corroborated the existence of an evolutionary trend towards the reduction of skull bone number in mammals, but suggested that this reduction was actually accompanied by a trend towards a more complex organization of the skull.

Together with B. Esteve-Altava and colleagues, we plan to investigate if the trends towards anisomerism reported here are associated with a greater, or a lower, network organization of the head/forelimb.

(7) These examples of some possible future research directions emphasize that there are numerous fascinating subjects within the fields of evolutionary, comparative and developmental biology that can be informed from myological data. These include the notions/ideas of complexity, *scalae naturae*, progress, modularity, integration and body plans. We hope that this review will stimulate such discussions and particularly pave the way for the integration of myological data on those discussions.

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