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Developmental Modularity and the Marsupial–Placental Dichotomy

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ABSTRACT The contrasting evolutionary histories of marsupial and placental mammals have often been attributed to their different reproductive strategies. The speciose placentals develop mainly in utero and have radiated into diverse niches, whereas marsupials are born in a highly altricial state with immediate functional requirements and are limited in taxonomic, ecological, and morphological diversity. These differences have been tied to heterochrony, and it has been hypothesized that coordinated shifts in developmental timing occur among functionally- or developmentally related structures, such as forelimbs in marsupials. We use new ossification sequence data for 11 marsupial and 14 placental species to assess the integration of first ossification timing among skeletal elements. Although cranial elements fail to demonstrate significant coordination, marsupials and placentals differ markedly in postcranial integration. Marsupials display independent anterior and posterior developmental modules, whereas placentals show significant integration of the entire appendicular skeleton. This developmental integration of the placental postcranium is consistent with a recent study of phenotypic modularity in limbs of placental mammals, showing a potential correspondence between integration of developmental timing and of shape. The observed differences in postcranial integration between marsupials and placentals may reflect the disparate evolutionary histories of these two mammalian clades. *J. Exp. Zool. (Mol. Dev. Evol.) 312B:186–195, 2009.*

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The contrast in the evolutionary histories of the two extant therian mammal clades, marsupials and placentals, has been a rich source of study for centuries (Smith, 2006). Marsupials display only 5% of the taxonomic diversity of placental mammals (Nowak, '99), and although placental mammals have radiated into a wide variety of niches, from bats to whales, few marsupials stray from a purely terrestrial or arboreal ecology. This disparity in evolutionary ''success'' has primarily been attributed to the different reproductive modes that characterize these two clades (Fig. 1). Marsupials are born after a very short period of gestation, ranging from 12.5 days (Strahan, '97) to 45 days (Flannery, '95), and experience most of their growth outside of the womb. The young are born with disproportionately well-developed forelimbs and oral apparatus, which allow them to climb and attach to their mother's teat, where they proceed with most of their development (Smith, '97; Sánchez-Villagra, 2002; Sears, 2004). Placentals, however, undergo most of their

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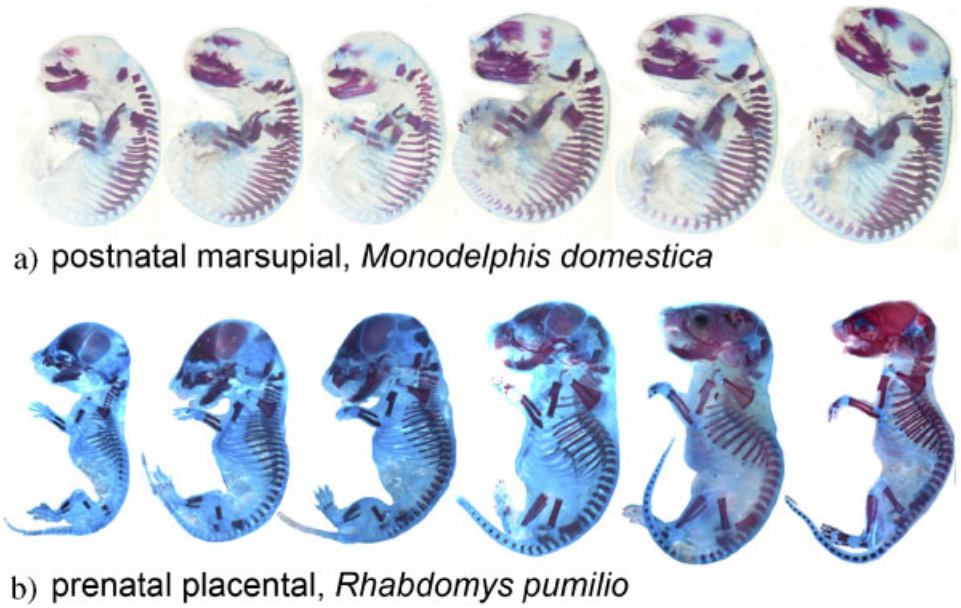

Fig. 1. Cleared-and-stained specimens, showing varying levels of skeletal ossification (shown by Alizarin red staining), of (**a**) a postnatal marsupial (*Monodelphis domestica*) and (**b**) a prenatal placental (*Rhabdomys pumilio*; M. Sánchez-Villagra, C. Mitgutsch, C. Schradin, unpublished data). Note the relatively well-ossified oral apparatus and forelimbs compared with the neurocranium and hindlimbs in the postnatal marsupial. The prenatal placental mammal shows much greater ossification of the entire skeleton, including the neurocranium and hindlimbs, than the postnatal marsupial.

development in utero, without the need for powerful forelimbs or functional mouths until birth, at which time the rest of their bodies are also sufficiently developed for life outside the womb.

These differences in reproductive patterns have often been tied to differences in sequence heterochrony between marsupials and placentals. Heterochrony is the shift in timing of a developmental event relative to another event (Gilbert, 2003), and sequence heterochrony refers specifically to changes in the order of developmental events. An extensive suite of studies of cranial sequence heterochrony by Smith et al. (Smith, '96,'97; Nunn and Smith, '98; Smith, 2001, 2002) suggested that marsupials accelerate the ossification of the masticatory apparatus (dentary, maxilla, and premaxilla) and delay the development of the central nervous system (the brain), relative to the condition observed in placentals (Fig. 1). New methodologies (Jeffery et al., 2005), have improved the ability to identify active shifts and to locate the branches of a phylogenetic tree on which these changes have actually occurred. Using these improved methods, it was identified that marsupials actively accelerate the ossification of the maxilla, whereas placentals accelerate the ossification of the jugal and delay the exoccipital (Jeffery et al., 2002; Jeffery et al., 2005). However, a more recent analysis of a greatly expanded dataset found that no ossification sequence heterochronies in the cranium diagnose either marsupials or placentals (Sánchez-Villagra et al., 2008).

In the postcranial skeleton, forelimb development has been observed to far outpace hind limb development in marsupials (Sánchez-Villagra, 2002; Sánchez-Villagra and Maier, 2002, 2003; Sears, 2004; Bininda-Emonds et al., 2007). A recent study determined that marsupials actually delay the ossification of the hind limb, rather than accelerating the development of the forelimb (Weisbecker et al., 2008). A study using recently described method (Harrison and Larsson, 2008) reanalyzed an older dataset of mammalian postcranial ossification sequences (Sánchez-Villagra, 2002) and found a similar result. It has also been demonstrated that the marsupial mode of reproduction constrains the development of the forelimb and thus limits its morphological diversity (Sears, 2004), because of the requisite early development of these structures. Although a parallel argument can be constructed for the facial skeleton, there has been no similarly rigorous analysis of ontogenetic trajectories or morphological diversity of the cranium.

Although studies of sequence heterochrony usually treat developmental events as independent of each other, it is often noted that functionally- or developmentally integrated structures may display coordinated shifts in

developmental timing (Smith, '96; Schoch, 2006; Goswami, 2007; Harrison and Larsson, 2008). The concept of modularity (Bolker, 2000; Polly et al., 2001; Poe, 2004; Schlosser and Wagner, 2004; Goswami, 2007) in particular has been discussed in regard to developmental sequence data in a few recent studies (Schoch, 2006; Goswami, 2007; Harrison and Larsson, 2008). Modules, semi-autonomous sets of highly integrated traits or events within larger biological units, have been identified in diverse systems, and a major focus of research on modularity has been to examine the relationships among genetic, developmental, and functional modules. Modularity explains both integration within structures and autonomy among structures, and, for this reason, is a compelling idea in understanding morphological evolution. The modular organization of organisms allows unrelated components to vary separately, while integration within structures maintains necessary relationships (Wagner, '95,'96). Although most studies of modularity focus on the physical relationships among functionally- or developmentally related structures, changes in developmental timing are often considered one of the most important avenues of evolutionary change (McNamara and McKinney, 2005), and thus it is important to incorporate developmental timing into hypotheses of modularity and its evolutionary significance.

Sequence heterochrony requires that the relevant structures must be autonomous from each other in developmental timing (de Beer, '37; Gould, '77). Thus, heterochronic shifts in the sequence of developmental events may be expected to occur more often among different modules than within a single module. Schoch (2006) recently suggested that functionally- or developmentally related cranial bones display coordinated shifts in timing of ossification across tetrapod clades, although this was not explicitly tested. Few comparative studies have examined the relationship between modularity and heterochrony (Poe, 2004; Shubin and Davis, 2004; Goswami, 2007). Here, we use a new dataset on first ossification sequences of skeletal elements in marsupial and placental mammals (Weisbecker et al., 2008) to identify modules in developmental timing. Specifically, we test the hypothesis that traditional groupings of cranial and postcranial elements, as well as morphometrically derived cranial modules, display significantly greater coordination of timing of first ossification than observed between modules.

MATERIALS AND METHODS

Ossification sequences

Two recent studies compiled the largest existing dataset on cranial and postcranial ossification sequences (Sánchez-Villagra et al., 2008; Weisbecker et al., 2008) for therian mammals and selected sauropsid outgroups. Raw data on absolute timing of onset of ossification for cranial and postcranial elements was converted to sequences of ranked or relative timing to allow for comparisons across disparate taxa with vast differences in the duration of skeletal development. The cranial dataset includes relative timing of ossification for 17 elements in 7 marsupial and 13 placental species, as well as 7 sauropsids as outgroups (Sánchez-Villagra et al., 2008). The postcranial dataset includes relative timing of ossification for 25 elements for 11 marsupial and 13 placental species, with 3 sauropsids as outgroups (Weisbecker et al., 2008). These data were acquired through a variety of sources, including previous studies (Nunn and Smith, '98; Sánchez-Villagra, 2002; Goswami, 2007) and original data collection from cleared-and-stained or CT-scanned whole specimens (Fig. 1) and sectioned specimens in historical collections (detailed in Sánchez-Villagra et al., 2008 and Weisbecker et al., 2008). Included species are shown in Figure 2.

Although there are many aspects of developmental timing that could be tested for modularity, the use of sequence data for first ossification of skeletal elements offers many benefits. Published data on first ossification are readily available in the literature, particularly from historical studies of comparative embryology. New data on ossification are obtainable with nondestructive sampling from rare museum specimens, allowing for sampling of many nonmodel organisms. Because first ossification is a discrete event that can be identified reasonably objectively, it is easily comparable across a broad range of species, and recent studies have shown that the onset of ossification is well correlated with other developmental events. Fröbisch (2008) recently reviewed ossification patterns in the tetrapod limb and concluded that the sequence of onset of ossification is at least partially correlated to that of chondrification. Another study has demonstrated that differences in ossification timing correlate with differences in the timing of FGF8 expression in marsupials and placentals (Sears, 2003), also suggesting that ossification timing reflects earlier development

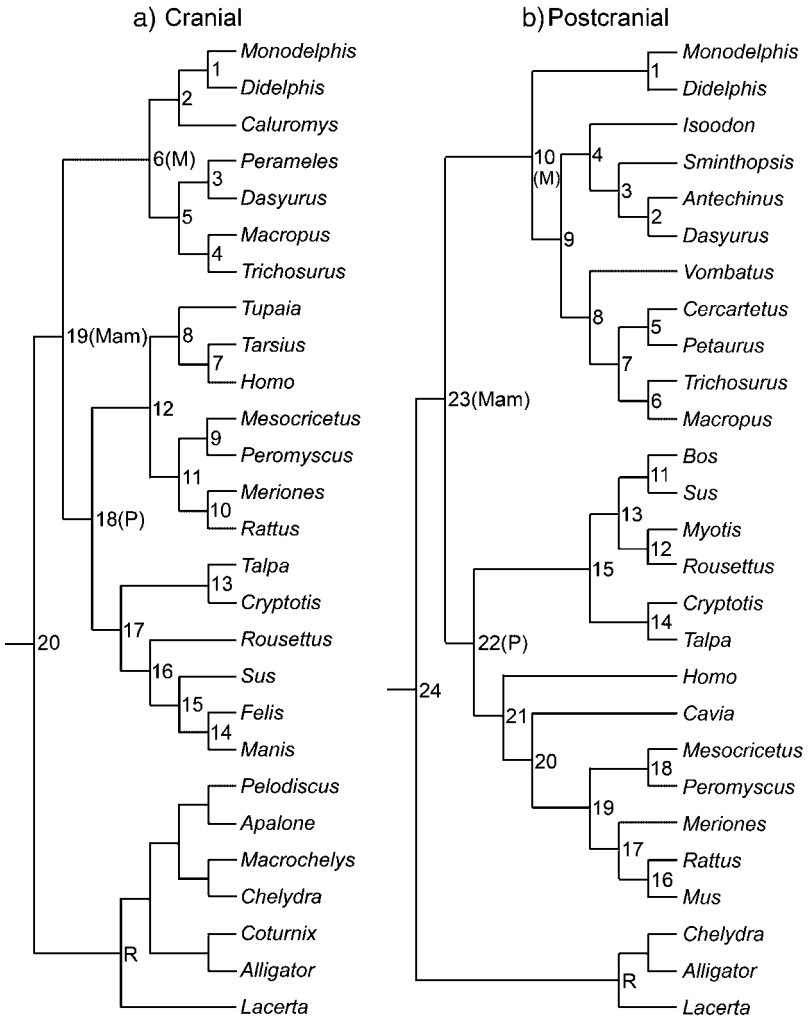

Fig. 2. Composite phylogenetic trees of species included in the (**a**) cranial and (**b**) postcranial analyses, based on recent molecular studies (detailed in Methods). M, Marsupialia; P, Placentalia; Mam, Mammlia; R, Reptilia (outgroups). Numbers refer to specific sister group comparisons and correspond to the nodes used in Table 2.

events. Ossification onset is linked to many different aspects of skeletal growth, and it is a practical and meaningful metric that is ideal for large-scale comparative analyses of developmental timing. Thus, the established differences in ossification sequence between marsupials and placentals provide an ideal framework for testing for modularity in developmental timing.

Modules

To test for coordinated shifts in ossification timing, theoretical functional and developmental modules were constructed. Theoretical modules are composed of sets of elements that are hypothesized to display coordinated timing of first ossification based on a strong functional or developmental relationship. Theoretical cranial modules were based on morphometric analyses of adult cranial modularity, which have identified six modules in the therian skull: anterior oral–nasal, molar, orbit, zygomatic-pterygoid, vault, and basicranium (Goswami, 2006). In addition, three traditional cranial regions (oral, face, and neurocranium) were also analyzed as theoretical modules. Because morphometric analyses of cranial modularity are based primarily on biologically homologous landmarks, such as sutures, which involve multiple elements, some elements contribute to multiple theoretical cranial modules. For example, the jugal contributes to the orbit module along its anterodorsal margin and the zygomatic-pterygoid module along its posterior margin.

All previous studies of postcranial modularity focus entirely on limb elements only (Magwene, 2001; Hallgrimsson et al., 2002; Young and

Hallgrímsson, 2005; Reno et al., 2007; Lawler, 2008). For this reason, the 15 theoretical postcranial modules tested here are based on hypothesized functional and developmental relationships, primarily reflecting traditional divisions of the skeleton into anterior and posterior elements, appendicular and axial elements, and proximal and distal elements. All analyzed theoretical cranial and postcranial modules are listed in Table 1, and specific theoretical module associations of elements are listed in Supplementary Tables 1 and 2, respectively.

Data analysis

Analysis of the integration of ossification events followed Poe's (2004) extension of Nunn and Smith's ('98) rank analysis method. Poe's method tests for the conservation of sequences, or rank order, within sets of traits (i.e. theoretical modules) in a phylogenetic framework. Specifically, Poe's method compares sequences in pairs of sister taxa using Kendall's τ, a nonparametric rank correlation measure. This statistic measures the similarity between two sequences of, in this study, first ossification. Kendall's τ is calculated as:

$$\tau = \frac{n_c - n_d}{(n_c + n_d + n_x)(n_c + n_d + n_y)^{1/2}}$$

where n_c is the number of concordant pairs of ranks, n_d is the number of discordant pairs, n_x is the number of tied events in the first taxon, and n_y is the number of tied events in the second taxon.

TABLE 1. Hypothetical or empirically-derived modules tested

Cranial modules	Postcranial modules
Anterior Oral–Nasal	Forelimb+pectoral girdle
Molar	Forelimb all
Orbit	Forelimb—long bones only
Zygomatic-Pterygoid	Hindlimb+pelvic girdle
Vault	Hindlimb all
Basicranium	Hindlimb—long bones only
Face	Pelvis
Neurocranium	Appendicular (limbs+girdles)
Oral	Forelimb+hindlimb
	Both girdles
	Axial+girdles
	Axial (vertebrae, ribs, and sternum)
	Anterior axial+girdle
	Posterior axial+girdle
	Vertebrae only

The element associations with each module are listed in Supplementary Tables 1 (cranial elements) and 2 (postcranial elements).

The significance of Kendall's τ was determined by comparison with a null distribution of comparably sized sets of first ossification events. Because the sets of events were relatively small (ranging from 3 to 18 events per theoretical module), it was possible to generate a distribution of all possible groupings of events, rather than using random sets (Goswami, 2007). If the theoretical module, a set of first ossification events, is in fact shifting in timing as a module, it should have a significantly higher correlation value than a random, similarly sized grouping of ossification events that include elements from different modules. This study used a 0.05 significance level, necessitating that the correlation among theoretical modules should be greater than 95% of all possible groupings. All analyses were conducted in Mathematica 6.0 (Wolfram Research, Inc., Champaign, IL).

Because this method only conducts pairwise comparisons of sister taxa, it is necessary to construct ossification sequences for nonterminal nodes, representing ancestral states. Ossification sequences for ancestral nodes were determined by averaging the sequences (the temporal ranks of each event) of sister taxa joined at a particular node (Fig. 2). The averaged sequence then became the hypothetical ancestral sequence of first ossification events for that node. This new sequence was then compared with the next most closely related taxon or node. A composite phylogenetic tree, based on several recent molecular analyses (Gaffney and Meylan, '88; Rest et al., 2003; Asher et al., 2004; Springer et al., 2005; Steppan et al., 2005), was used to provide the topology for reconstructing ancestral nodes. Kendall's τ was calculated separately for each pair of sister taxa for all nine subsets of cranial ossification events and 15 subsets of postcranial ossification events.

RESULTS

Of the 20 sister group comparisons (Fig. 2a) for nine cranial sets (Table 1, Supplementary Table 1), only one was correlated (the facial module in Eulipotyphla, Kendall's $\tau = 1$, $P = 0.02$). No other sets were significant at the $P = 0.05$ significance level, while six other sister group comparisons were marginally significant ($0.1 > P > 0.05$) for four modules: orbit (*Mus* vs. *Rattus*, *Peromyscus* vs. *Mesocricetus*), zygomatic-pterygoid (*Tarsius* vs. *Homo*), basicranium (*Mus* vs. *Rattus*), face (*Macropus* vs. *Trichosurus, Felis-Manis-Sus* node vs. *Rousettus*).

TABLE 2. Results of analyses for 15 postcranial modules

	Marsupials										Placentals													
M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
FP	**1.00**	1.00	0.97	**1.00**	0.85	0.97	1.00	**1.00**	**1.00**	0.90	0.83	0.82	0.96	0.91	0.65	0.92	0.92	0.90	0.90	0.94	0.91	0.76	0.81	0.91
FA	1.00	1.00	0.96	**1.00**	0.87	0.96	1.00	1.00	1.00	1.00	0.88	**1.00**	0.93	0.88	0.57	0.96	0.96	0.88	0.92	0.89	0.83	0.55	0.75	0.83
FL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–	–	–	–	–	0.82	0.82	–	0.82
AAG	0.87	1.00	0.67	0.67	**1.00**	1.00	1.00	1.00	0.67	0.78	0.93	0.39	0.93	0.96	0.75	0.87	0.87	1.00	0.90	0.85	0.93	1.00	0.83	0.87
HP	0.93	1.00	0.93	0.85	0.89	0.81	0.92	0.88	0.94	0.94	0.87	0.84	0.84	0.80	0.65	0.95	0.83	0.75	0.80	0.79	0.71	0.86	0.73	0.69
HA	1.00	1.00	**1.00**	**1.00**	**1.00**	1.00	0.96	0.87	1.00	0.96	0.92	0.93	0.93	0.82	0.64	0.96	0.78	0.83	0.81	0.96	0.85	0.97	0.75	0.97
HL	–	–	–	–	–	–	–	–	–	–	0.50	–	0.82	–	0.82	–	–	–	–	–	–	0.82	–	0.82
P	0.18	0.91	0.91	0.55	1.00	1.00	0.55	1.00	0.91	1.00	0.91	0.91	0.67	0.80	1.00	1.00	1.00	1.00	1.00	0.91	1.00	1.00	0.67	0.33
PAG	0.56	0.82	0.72	0.71	0.97	0.81	0.54	0.90	0.85	0.87	0.75	0.91	0.41	0.69	0.90	0.78	0.83	0.92	0.88	0.72	0.91	0.95	0.71	0.41
AP	0.77	0.95	**0.92**	0.85	0.84	0.90	0.87	0.94	0.92	**0.93**	**0.88**	0.83	**0.92**	0.88	0.72	0.89	0.87	0.84	0.85	0.87	0.85	**0.87**	0.75	0.74
FH	0.86	0.97	0.93	0.90	0.86	0.95	0.97	0.95	0.90	**0.97**	0.89	0.87	0.92	0.81	0.60	0.84	0.81	0.82	0.81	0.89	0.82	0.78	0.80	0.72
G	0.69	0.96	0.96	0.82	0.93	1.00	0.82	1.00	0.96	0.97	0.93	0.89	0.87	0.93	**1.00**	**1.00**	**1.00**	1.00	1.00	0.93	1.00	**1.00**	0.87	0.80
AG	0.82	0.92	0.88	0.82	0.86	0.95	0.84	0.96	0.91	0.93	0.85	0.70	0.65	0.89	**0.82**	0.86	0.85	0.91	0.87	0.84	**0.95**	**0.92**	**0.86**	0.80
A	0.92	0.94	0.97	0.97	0.80	1.00	0.94	0.97	1.00	0.95	0.86	0.44	0.59	0.95	0.82	0.70	0.65	0.84	0.79	0.95	0.98	0.98	0.88	**0.98**
V	0.89	0.88	0.94	0.94	0.71	1.00	0.88	0.94	1.00	0.95	**1.00**	0.87	0.53	0.94	0.89	0.67	0.53	0.88	0.78	0.89	0.95	1.00	1.00	0.95

FP, forelimb+pectoral girdle; FA, forelimb all; FL, forelimb—long bones only; AAG, anterior axial + pectoral girdle; HP, hind limb+pelvic girdle; HA, hind limb all; HL, hind limb—long bones only; P, pelvis; PAG, posterior axial+pelvic girdle; AP, appendicular (limbs+girdles); FH, forelimb+hind limb; G, both girdles; AG, axial+girdles; A, axial only; V, vertebrae only. Nodes as in Fig. 2. Shaded cells indicate $0.1 > P > 0.05$. Bold and filled cells indicate $P < 0.05$. The first eight modules (above the line) involve dissociation of the anterior and posterior postcranial skeleton, whereas the last seven modules involve integration of the anterior and posterior skeleton. – denote those sets (forelimb and hind limb long bone only) for which Kendall's τ was indeterminate, owing to low element numbers and high conservation of sequence.

Out of 24 sister group comparisons (Fig. 2b) for 15 postcranial sets, 26 were significantly correlated (Table 2, Fig. 3). 28 comparisons were marginally significantly correlated (Table 2). Six hypothesized sets (forelimb long bones, hind limb long bones, hind limb+pelvis, pelvis only, axial only, posterior axial+girdle) were not significant in any sister group comparison. The two sets with the fewest elements involved, forelimb long bones and hind limb long bones, three elements each, often returned indeterminate results because all of three elements ossified simultaneously, or close enough temporally to appear simultaneous with the available ontogenetic sampling (Table 2). Of the 26 significant correlations, one was between therian mammals and reptiles (axial), one was between marsupials and placentals (axial+girdles), 12 were within marsupials, and 12 within placentals (Table 2, Fig. 3). The significant postcranial results displayed surprising differences between marsupials and placentals. Nine of the 12 significant correlations within marsupials are in sets that are restricted to either the anterior or the posterior postcranial skeleton (anterior axial+girdle, forelimb all, hind limb all, forelimb+pectoral). Only one of the 12 significant results within placentals is restricted to only the anterior or posterior skeleton (bats show a significant forelimb module), whereas the other 11 group appendicular and/or axial traits across the anterior and posterior skeleton (Table 2, Fig. 3).

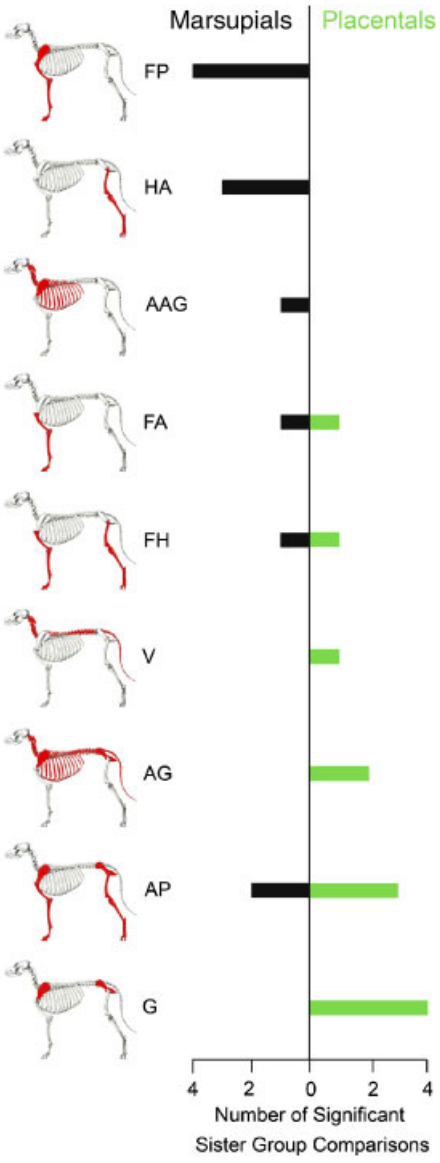

Fig. 3. Number of significant sister group comparisons for postcranial modules. Postcranial modules without any significant comparisons are not included. Elements involved in each postcranial module are shown in red on dog skeletons. Marsupials (in black) show more coordination of modules that involve either anterior or posterior elements, whereas placentals (in green) predominantly display significant coordination of modules that involve both anterior and posterior elements. Abbreviations as in Table 2.

DISCUSSION

In the majority of analyses, no significant coordination of ossification timing was detected. Only one of 180 analyses was significant for the cranium, and 26 of 330 for the postcranium. This may well reflect a real lack of modularity in ossification timing, but it also likely reflects the conservativeness of the methodology (Goswami, 2007). Because the significance of rank correlations is determined through comparison with other possible groupings of the same dataset, the lack of significance may be owing to a high level of similarity in ossification sequence across species. This conservation of sequence has been demonstrated in cranial ossification among therian mammals (Sánchez-Villagra et al., 2008) and has been previously noted by Smith ('97) in a study that found that 43% of cranial event pairs, including cartilage, bone, muscle, and central nervous system development, were uniform across therian mammals. This lack of variation may well explain the lack of significant coordination of ossification sequence within the cranium.

Another potential issue was that six of the nine phenotypic cranial modules tested were based on cranial morphometric data (Goswami, 2006). As mentioned above, these morphometric data are based primarily on sutures that involve more than one bone. Most module boundaries are thus

defined by the intersection of multiple bones, meaning that individual bones were often involved in multiple modules (Goswami, 2007). For this reason, each ossification event was included in all sets with which it is involved in the empirically derived modules of the adult skull, resulting in a range of one–three sets per bone (Supplementary Table 1). Therefore, this conservative method may obscure significant modularity in first ossification timing for the six morphometrically defined cranial modules.

Nonetheless, recent analyses of cranial sequence heterochrony have demonstrated that the bones mainly involved in sequence heterochrony are essentially random with regard to phenotypic modularity of the cranium. For example, the consensus Parsimov reconstruction of heterochronic shifts for therian mammals involves the maxilla, dentary, parietal, and pterygoid bones, representing some, but not all, of three modules. Thus, it is likely that the lack of statistical significance for modularity in timing of cranial ossification accurately reflects the lack of a relationship between phenotypic modularity and ossification timing in the therian mammal cranium.

In contrast, there is more variation in ossification sequence among postcranial elements (Weisbecker et al., 2008), and, similarly, there is more significant coordination of timing. Out of 15 hypothesized sets (Table 2), only nine are significant in any sister group comparison (Fig. 3). Because the 15 sets overlap considerably (for example, the humerus is included in forelimb long bones, forelimb all, forelimb+pectoral, appendicular all, and forelimb+hind limb; Supplementary Table 2), it is unsurprising that most of the sets do not show significant coordination. Indeed, only five sister clade comparisons show significant coordination of overlapping sets (e.g. both girdles are involved in two significantly coordinated modules across Laurasiatheria; Table 2, Fig. 3). As discussed above, the significance of the rank correlations is determined by comparison with random sets of elements. Therefore, if there is a large amount of coordination among large sets of traits, it will be difficult for small sets to achieve statistical significance. Furthermore, although many of the hypothetical sets are hierarchical, inclusion of any poorly coordinated elements will return nonsignicant results. Therefore, the relatively high number of nonsignificant results seen here is expected and does not dilute the larger pattern, which is perhaps more striking given the conservativeness of the method. Of the 23 sister clade comparisons within therian mammals, only six failed to demonstrate some significant coordination of postcranial elements. As three of these six comparisons are among closely related real species and not reconstructed ancestral nodes, it is possible that this lack of significance again reflects similarity of sequence, as in the cranial dataset.

The most striking pattern is that the postcranium appears to be well integrated in first ossification timing and displays marked differences between the two major clades of extant mammals. Although 11 of 12 significant results within placentals involve both anterior and posterior elements, nine of the 12 significant results within marsupials involve only the anterior or posterior skeleton (Table 2, Fig. 3). This difference in the developmental modularity of the postcranial skeleton in marsupials and placentals complements previous studies of ossification sequence heterochrony in therian mammals and suggests that a fundamental shift in the developmental modularity of the marsupial postcranial skeleton occurred in the evolution of the unique marsupial reproductive strategy. Because the comparison of the hypothetical therian mammal ancestor and the sauropsid outgroups also revealed significant modularity of the full axial skeleton, with no separation of the anterior and posterior segments, it is suggested that the marsupial pattern of postcranial modularity is the derived condition. There are few skeletal correlates of reproductive strategy, making identification of the primitive condition for therian mammals from fossil evidence difficult. The results of these analyses provide an alternative methodology and identify a subtle imprint of reproductive strategy in ossified elements that can be used to link morphology to developmental attributes of extinct taxa.

Interestingly, quantitative study of covariation among the postcranial elements in adult placental mammals (Young and Hallgrímsson, 2005) has found that there is significant covariation between forelimb and hind limb elements in most taxa. Notably, bats were the one exception in showing weak and nonsignificant covariation between forelimb and hind limb elements. Microchiropterans and megachiropterans also showed one of the largest numbers of heterochronic differences in postcranial ossification sequences between therian mammal sister clades (Weisbecker et al., 2008). Bats are of course unusual among placental mammals in their modification of the forelimb

into highly specialized wings, and a recent study has demonstrated that elongation of bat digits can be attributed to upregulation of the Bmp pathway in bat forelimbs, but not in bat hind limbs, again reflecting developmental dissociation among these elements (Sears et al., 2006). Thus, there is striking correspondence between modularity in ossification timing of bat postcranial ossification (presented here), dissociation of molecular controls on bat limb development (Sears et al., 2006), and phenotypic modularity in the bat postcranial skeleton (Young and Hallgrímsson, 2005). These results also demonstrate that shifts in functional relationships among elements are reflected in shifts in their developmental relationships, discussed further below. We predict that quantitative analysis of limb covariation in marsupials would show a pattern similar to bats, with low covariation among forelimb and hind limb elements, consistent with studies of the pectoral and pelvic girdles (Sears, 2004), and consistent with the different functional requirements of the forelimb and hind limb in marsupial neonates.

On a more fundamental level, developmental constraints are often considered to be a major factor limiting variation, which provides the raw material for natural selection and, thus, morphological evolution. More specifically, developmental associations among traits are hypothesized to be a principal way that developmental constraints are manifested, by limiting the variation of individual traits. Although the root cause for associations may be functional, as in the limb covariance structure of bats, Young and Hallgrimson (2005) note that ''functional association may drive selection for integration, but the underlying target of this selection is development. To produce high levels of covariation, selection must act on the underlying developmental architecture of traits''. Shifts in developmental timing, or heterochrony, are probably the best-studied and among the most pervasive pathways for evolutionary modifications to adult morphology. Because comparative data on ossification sequences are more easily attainable than other forms of developmental data, they offer a rare opportunity to study large-scale shifts in the coordination of development and its impact on large-scale patterns of morphological evolution. This study identifies one mechanism, dissociation of ossification timing, that is likely related to differences in reproductive strategy, functional associations, and morphological disparity previously described among therian mammals. Future work along these lines will continue to unravel the complex relationship between development integration and morphological evolution.

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