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Brief Communication: Methods of Sequence Heterochrony for Describing Modular Developmental Changes in Human Evolution

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ABSTRACT Interest in the developmental changes leading to apomorphic features of human anatomy is longstanding. Although most research has focused on quantitative measures of size and shape, additional information may be available in the sequence of events in development, including aspects of phenotypic integration. I apply two recently proposed techniques for analyzing developmental sequences to literature data on human and chimpanzee age of limb element ossification center appearance in radiographs. The event-pair cracking method of Jeffery et al. (Syst Biol 51 [2002] 478–491) offers little additional insight on sequence differences in this data set than a simpler difference of ranks. Both reveal shifts in timing that are likely related to locomotor differences between the two species. Poe's (Evolution 58 [2004] 1852–1855) test for modularity in a sequence identifies the ankle, wrist, and hind limb as developmental modules, which may correspond to localized combinations of developmental genes. Ossification patterns of the rays of the hand and foot show little modularity. Integrating these and other methods of sequence analysis with traditional metrics of size and shape remains an underdeveloped area of inquiry. Am J Phys Anthropol 138:231–238, 2009. © 2008 Wiley-Liss, Inc.

Evolutionary studies of processes of growth and development continue to reveal detailed features of species' adaptation and regulatory attributes in the structuring of development (Raff, 1996; West-Eberhard, 2003). The evolution of growth has implications for understanding primate and human locomotion, diet, cognition, social organization, and life history (e.g., Leigh and Shea, 1995; Smith and Tompkins, 1995; Harvati, 2000; Dean et al., 2001; Leigh, 2001, 2004) and may contribute to resolving phylogenetic relationships and hominin taxonomic diversity (Kitching et al., 1998; McCollum, 1999; Ackerman and Smith, 2007).

Recently proposed techniques in developmental biology offer new ways to explore primate growth. In this article, I apply two new methods to compare human and chimpanzee developmental sequences. The first of these methods is event-pair cracking (Jeffery et al., 2002), which is intended to identify events that have moved within a sequence between a presumed ancestor and descendant pair of species. Although Jeffery et al. (2002) and others have referred to changes in developmental sequence as sequence heterochrony, it is important to note that there is no obvious connection between these kinds of sequence change and the traditional categories of size-shape change used in heterochrony research (i.e., allometric heterochrony). Indeed, this remains an underdeveloped area of theoretical and empirical inquiry (Fiser et al., 2008). At the very least, knowledge of developmental sequence aids in identifying comparable developmental stages between ancestor and descendant (Bininda-Edmonds et al., 2002)—a requirement for traditional explorations of allometric heterochrony (Gould, 1977; Alberch et al., 1979). Furthermore, analysis of sequence data can address developmental features that cannot be assessed by metrics of size or shape, such as the initial expression of a particular gene product fusion of the neural folds (Smith, 2001).

A second technique of sequence heterochrony is a statistical test for modularity within a developmental sequence. Modularity—envisioning phenotypes as composed of many hierarchical and semi-independent subunits—is a ubiquitous concept in discussions of morphological evolution (Olson and Miller, 1958; Raff, 1996; West-Eberhard, 2003). The integration within modules and complementary dissociation of structures between them are widely appreciated to create "paths of least resistance" along which species tend to evolve—typically size and size-related shape variation (Schluter, 2000). An empirical difficulty remains as to how one identifies and tests for the existence of proposed modules. Current methods for morphometric data rely on manipulations of correlations or partial correlations, typically among linear distance measurements of skeletal elements (Magwene, 2001; Schlosser and Wagner, 2004). In contrast, Poe (2004) proposed a simple test based on developmental sequence data. His test relies on the assumption that a module is a set of events that must proceed in a certain order, though they need not be contiguous in the sequence (Alberch, 1985; Smith, 2001). The sequence of events in nonmodules can be rearranged at random with little effect on the resulting phenotype. Similar to the case with event-pair cracking, modules identified through linear measurements of the skeleton do not have obvious linkages with those that may be found in the sequence of developmental events (Goswami, 2007). Adult size will be determined by rates of

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growth as well as the timing of initiation and termination, which may be easier to index as clear developmental events (Alberch et al., 1979; Klingenberg, 1998). Both event-pair cracking and Poe's (2004) modularity test are methods for analyzing developmental sequence changes or sequence heterochronies. Event-pair cracking is related to sequence modularity by the simple fact that developmental delay or advancement of modules, which consist of multiple developmental events, will cause large sequence differences between taxa. Modular sequence changes can be seen as a special kind of sequence heterochrony.

I apply these new techniques to literature data on mean age of radiographic appearance of 61 postcranial ossification centers in humans and captive chimpanzees (Figs. 1 and 2; Pyle and Sontag, 1943; Nissen and Riesen, 1949; Gavan, 1953). The data were initially compared by Nissen and Riesen (1949) by difference in rank order with little attention to potential modularity in the developmental sequences. Applying new techniques to these data will allow the assessment of how well pair cracking identifies events moving in developmental sequence over the simpler methods. Additionally, it may offer previously unappreciated insights on the ontogenetic differences between humans and chimpanzees. Finally, it will aid in identifying the presence of developmental modules in the postcranium, which may be interpretable in light of recent discoveries in developmental genetics (Chiu and Hamrick, 2002) and offer comparisons with patterns of covariation among linear measurements traditionally used to address modularity in the primate skeleton (Marriog and Cheverud, 2001; Hallgrímsson et al., 2002).

MATERIALS AND METHODS

Event-pair cracking

Standard methods of constructing event-pair matrices and performing pair cracking were followed from Jeffery et al. (2002). Initially, an event-pair matrix is formed by comparing developmental events in a table with each event listed down the leftmost column and across the top row. Pairings of events are coded within each cell of the table with a 2 for row event occurring after column event, 1 for occurring at the same time, and 0 for row event occurring before column event. Event-pair cracking directly compares these ancestor and descendant matrices. Male chimpanzees were used as a model ancestor for male humans and female chimpanzees for female humans. The comparison of matrices is done in several steps. First, each cell of the ancestral matrix is subtracted from its corresponding cell in the descendant matrix (descendant-ancestor). These differences are then scored as a 0, 1, or -1 based on their sign (i.e., the transition from 0 to 2 is thought of as equivalent to the transition from 1 to 2). Each developmental event then has its score of changes as a row event and as a column event individually summed. The difference of the row-event sum and column-event sum is defined as the total relative change (TRC = rowsum $-$ columnsum). The total absolute change is calculated as the sum of the absolute values of the row and column sums (TAC = |rowsum| + |columnsum|). The absolute value of the TRC is always equal to or less than the TAC. Events that show consistent movement in the sequence will have equal absolute values of their TRC and TAC. Cases where it is less than the TAC are those in which the event has advanced relative to some events and delayed relative to others in the developmental sequence—an inconsistent pattern of movement.

The second major step in pair cracking is to filter the list of events based on an arbitrarily selected cutoff value in the hope of identifying moving and nonmoving events. Often, the median of the absolute value of the TRCs is used as this cutoff but a form of sensitivity analysis can be carried out by progressively changing the cutoff. Four cutoffs were used in this analysis—quantiles 0.3, 0.5, 0.7, and 0.9. Further steps in pair cracking include only events with |TRC| greater the cutoff. Next, row and event score sums for these selected events are calculated using only event pairs between a selected and an unselected event. From these restricted sets of row and event score sums, new adjusted TRC and TAC values are calculated. Misidentified moving events will have adjusted TAC values of 0, whereas moving events will have TAC values greater or less than their unadjusted values. A measurement summarizing the "coherence of movement," J, is calculated as the ratio of adjusted TRC and TAC values. Nonmoving events will have undefined J values, whereas those events moving earlier in development relative to nonmoving events will have -1 and those moving later will have a J equal to 1. Although it is possible to arrive at J values not equal to 1 or -1 for moving events, it is very rare. All calculations were performed with scripts written by the author for R (R Development Core Team, 2007).

Modularity test

Poe's (2004) modularity test uses the bootstrap (Manly, 1997). It compares the observed nonparametric Kendall (1948) correlation (τ) of events between two species or individuals in a proposed module to an equivalently sized subset drawn at random from all of the events in the developmental sequence. A null distribution to test the observed correlation is built up by repeating the random draw and calculating its correlation. One thousand replicates were performed to generate the null distribution for each module tested. True modules will have very few randomly drawn sets that exceed their observed correlation. Only female data were used for the modularity tests.

A hierarchical list of proposed modules was chosen based on anatomical proximity of ossification centers or location within traditionally recognized modules (Table 4). The modules varied considerably in size with the majority of centers in the hands and feet. Hand and wrist modules were assessed including and excluding the distal ulna and radius. Similarly, the foot and ankle modules were analyzed including or excluding the distal tibia and fibula. Modularity of the digits of the hand and foot was tested both including and excluding the first ray. These modules contained metacarpals or metatarsals and phalangeal ossification centers. An additional set of seven modules formed by grouping across limbs (e.g., stylopod or ankle + wrist) was also tested. These are composed of homologous parts in the fore- and hind limb, which may be developmentally and genetically integrated (e.g., Shubin et al., 1997; Hallgrímsson et al., 2002; Young and Hallgrímsson, 2005).

RESULTS

The interspecific pair cracking for chimpanzee and human developmental sequences identifies relative advancement and delay of events similar to the assess-

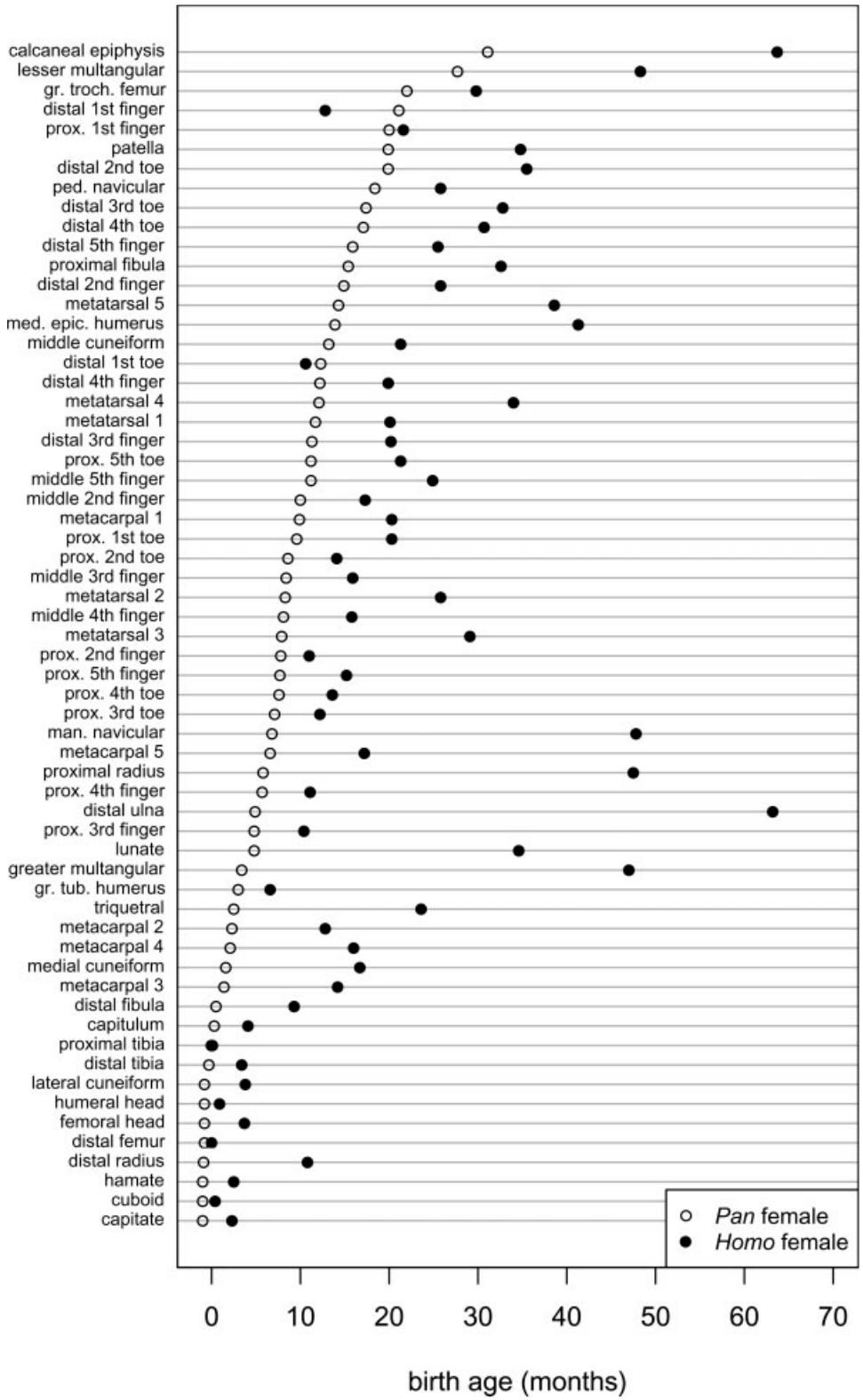

Fig. 1. Mean age of radiographic appearance of ossification centers in female chimpanzees (mean = 8.8) and humans (mean = 21.2). Developmental events are ordered by the presumed ancestral sequence for female chimpanzees.

ment of Nissen and Riesen (1949) based on difference in ranks (Tables 1–3). Furthermore, the centers that are identified as substantially changing position in the sequence are identical for both rank differences and pair cracking (e.g., distal 1st toe and finger, distal ulna, and greater multangular; Table 1). Most sites of movement are located in distal extremities: the forearms, hands, and feet (25/29 for females, 21/25 for males)—regions which only comprise about half (30/61) of the data set. The disproportionate representation of distal extremities

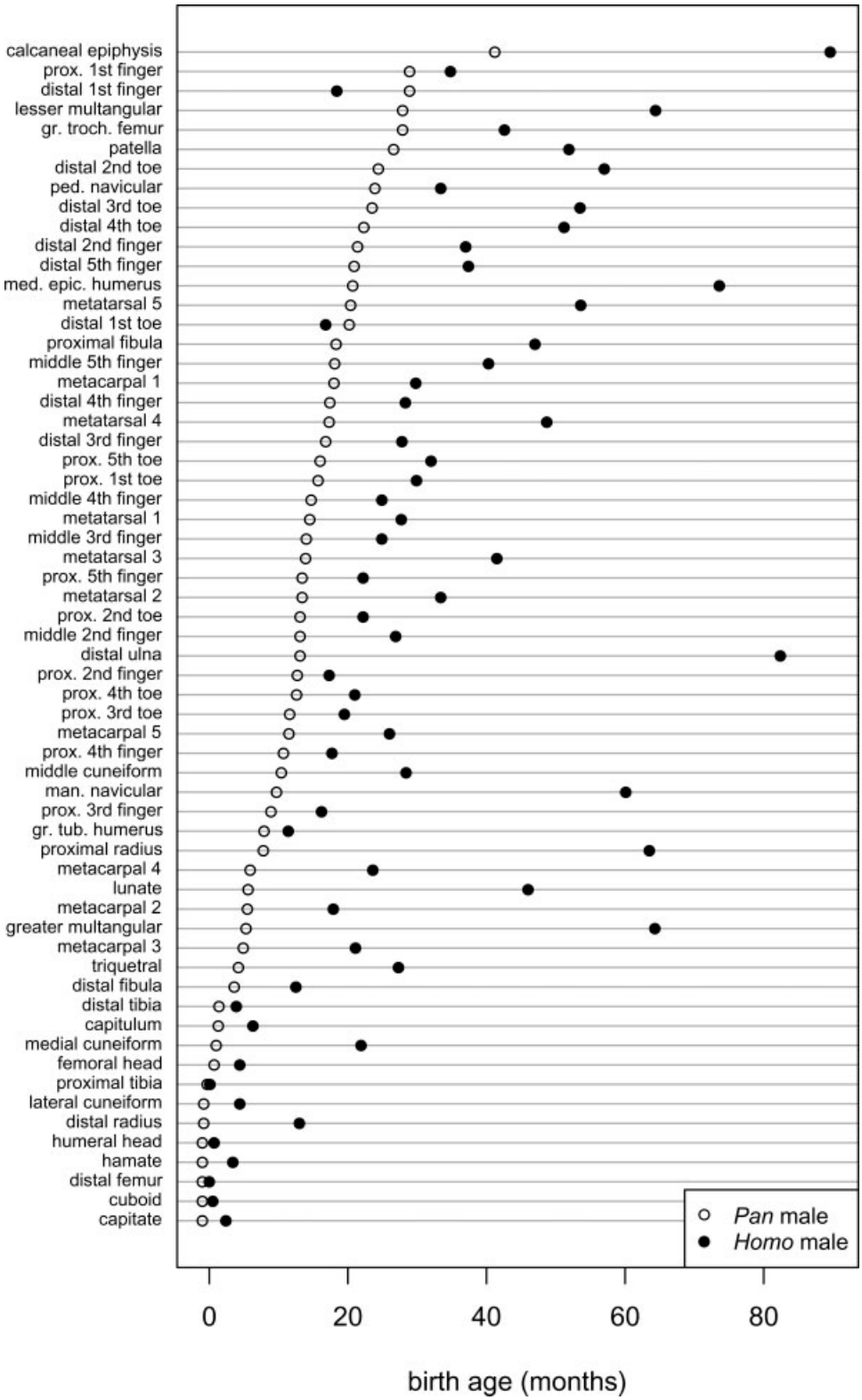

Fig. 2. Mean age of radiographic appearance of ossification centers in male chimpanzees (mean = 12.7) and humans (mean = 30.5). Developmental events are ordered by the presumed ancestral sequence for male chimpanzees.

in the set of moving events is statistically significant (χ^2 $P < 0.01$ for both males and females).

However, pair cracking and Nissen and Riesen's (1949) rank differences are not in complete agreement on the salient sequence differences. Pair cracking implicates an additional set of moving events in the developmental sequences. In females, humans are relatively advanced in the appearance of the proximal tibia and metatarsal 1, and they are relatively delayed in appearance of metatarsal 2, metacarpal 3, and the medial epicondyle of the humerus (Table 2). In males, humans are relatively advanced in the appearance of metacarpal 1 and the

TABLE 1. Ossification centers with large differences in sequence of radiographic appearance between humans and chimpanzees identified by Nissen and Riesen

Ossification center	Nissen and Riesen (rank difference)		Pair cracking (quantile)	
	Male	Female	Male	Female
Appearing relatively earlier in chimpanzees				
Distal ulna	+29	+44	0.7	0.9
Greater multangular	+41	+36	0.9	0.9
Prox. radius	+36	+34	0.9	0.9
Man. navicular	+32	+33	0.9	0.9
Lunate	+28	+30	0.9	0.7
Triquetral	+16	+22	0.7	0.7
Medial cuneiform	+13	+15	0.7	0.7
Metatarsal 3	+12	+13	0.7	0.7
Distal radius	+6.5	+9.5	0.3	0.7
Metacarpal 4	+8	+9	0.3	0.7
Metatarsal 4	+8	+9	0.3	0.3
Appearing relatively earlier in humans				
Distal 1st finger	−41	−39	0.9	0.9
Distal 1st toe	−33	−28	0.9	0.9
Prox. 1st finger	−18	−17	0.7	0.7
Ped. navicular	−16	−12	0.7	0.7
Prox. 2nd finger	−13	−15	0.7	0.7
Distal 4th finger	−11	−14	0.5	0.7
Gr. troch. femur	−12	−13	0.7	0.7
Distal 3rd finger	−9	−11	0.5	0.5
Middle 3rd finger	−9	−10	0.5	0.5
Prox. 2nd toe	−5	−12	0.3	0.7
Gr. tub. humerus	−10	−3	0.7	0.3
Prox. 5th finger	−10	−6	0.5	0.3
Middle 2nd finger	−10	−8	*	0.5
Middle 4th finger	−9	−8	0.7	0.3
Distal 2nd finger	−9	−5	0.5	0.3
Prox. 3rd finger	−8	−9	0.5	0.3
Prox. 3rd toe	−7	−9	0.3	0.5
Distal 5th finger	−6	−9	0.3	0.5
Appearing relatively earlier in male chimpanzees and female humans				
Middle cuneiform	+9	−10	0.7	0.5

Rank differences are calculated as human rank–chimpanzee rank. The pair-cracking quantile at which the center is identified as a moving event is indicated. Centers are ordered by rank differences for females. The * for males on the middle phalanx of the 2nd finger indicates that it was not selected as moving at the lowest quantile explored (0.3).

TABLE 2. Ossification centers identified as moving events in females with pair cracking cutoff set at the median (0.5 quantile, $|TRC| > 7$)

Center	TRC	Adjusted TRC	*J*
Distal 1st finger	−38	−16	−1
Distal 1st toe	−31	−11	−1
Prox. 1st finger	−19	−9	−1
Gr. troch. femur	−13	−7	−1
Proximal tibia	−8	−7	−1
Ped. navicular	−12	−6	−1
Distal 4th finger	−13	−5	−1
Prox. 2nd finger	−14	−5	−1
Distal 3rd finger	−8	−4	−1
Distal 5th finger	−10	−4	−1
Metatarsal 1	−10	−4	−1
Prox. 2nd toe	−13	−3	−1
Middle 2nd finger	−8	−2	−1
Middle cuneiform	−9	−2	−1
Prox. 3rd toe	−9	−2	−1
Middle 3rd finger	−8	−1	−1
Metatarsal 2	10	4	1
Metacarpal 3	10	5	1
Metatarsal 3	14	6	1
Med. epic. humerus	8	7	1
Medial cuneiform	14	7	1
Metacarpal 4	12	7	1
Distal radius	11	9	1
Triquetral	22	10	1
Lunate	30	14	1
Man. navicular	32	15	1
Proximal radius	33	16	1
Distal ulna	38	18	1
Greater multangular	37	18	1

Centers are ordered by their adjusted TRC. Negative values indicate relative advancement in the developmental sequence in humans; positive values indicate relative delay in humans. For these 29 events, the absolute value of the TRC was equal to the TAC in both the raw and adjusted TRCs.

proximal phalanx of the fourth finger, and they are relatively delayed in the ossification of the medial epicondyle of the humerus (Table 3). Pair cracking also suggests some of the events with modest rank order differences are not moving events. These are events only identified as moving at low cutoff quantiles (i.e., 0.3 or never selected). These include metatarsal 4 for males and females and the distal radius and metacarpal 4 for males—all of which appear to be relatively delayed in humans. Additional discordant centers between the two methods are indicated in Table 1.

Modularity tests highlight several integrated or coevolving regions of ossification centers (Table 4). The hind limb ($\tau = 0.693$, $P < 0.05$) and portions of it appear to be modules (foot: $\tau = 0.665$, $P = 0.11$; ankle: $\tau = 0.929$, $P < 0.05$). In contrast, the forelimb and most regions within it are not. The wrist is the only potential exception to this pattern ($\tau = 0.817$, $P = 0.10$). Forming potential modules by grouping homologous parts of the limbs only implicates the ankle + wrist as a module ($\tau = 0.751$, $P < 0.05$). None of the traditional modular groups across the limbs is identified as a significant module, although the ossification centers covary much more tightly in the stylopod ($\tau = 0.823$, $P = 0.10$) than the two distal regions of the limbs (zeugopod $\tau = 0.333$, autopod $\tau = 0.443$). The fingers and toes behave in a distinctly nonmodular fashion both in tests of their independent modularity (toes: $\tau = 0.451$, $P = 0.70$; fingers: $\tau = 0.481$, $P = 0.66$) or when grouping them as a single unit ($\tau = 0.437$, $P = 0.88$). Excluding the first ray raises correlations considerably, but even these limited sets of ossification centers are not significantly integrated (toes: $\tau = 0.636$, $P = 0.33$; fingers: $\tau = 0.600$, $P = 0.35$). Similarly, modules excluding the distal zeugopod centers from the ankle/hand or wrist/foot caused slight increases in correlations but reduced significance due to loss of degrees of freedom (e.g., ankle + wrist $\tau = 0.771$, $P = 0.06$).

DISCUSSION

The two methods explored in this article function adequately. However, the analysis does not point to any simple rules of thumb on the relationship between sequence changes and adult size and shape, such as later appearance results in smaller size (Gould, 1982). Lack of information on growth rates will limit the conclusions of strict study of developmental sequence (Smith, 2001). Furthermore, uncertainty of the functional meaning of ossification center appearance hinders

TABLE 3. Ossification centers identified as moving events in males with pair cracking cutoff set at the median (0.5 quantile, |TRC| >7)

Center	TRC	Adjusted TRC	*J*
Distal 1st finger	−41	−21	−1
Distal 1st toe	−32	−15	−1
Prox. 1st finger	−18	−10	−1
Gr. troch. femur	−12	−8	−1
Ped. navicular	−15	−8	−1
Prox. 2nd finger	−13	−6	−1
Distal 2nd finger	−9	−5	−1
Gr. tub. humerus	−10	−5	−1
Distal 4th finger	−9	−4	−1
Metacarpal 1	−8	−4	−1
Middle 4th finger	−11	−4	−1
Prox. 5th finger	−9	−4	−1
Distal 3rd finger	−8	−3	−1
Middle 3rd finger	−8	−3	−1
Prox. 3rd finger	−8	−3	−1
Prox. 4th finger	−8	−3	−1
Metatarsal 3	10	5	1
Med. epic. humerus	10	6	1
Middle cuneiform	11	6	1
Medial cuneiform	13	7	1
Triquetral	17	8	1
Lunate	29	12	1
Distal ulna	28	14	1
Man. navicular	32	18	1
Proximal radius	36	18	1
Greater multangular	41	20	1

Centers are ordered by their adjusted TRC. Negative values indicate relative advancement in the developmental sequence in humans; positive values indicate relative delay in humans. For these 26 events, the absolute value of the TRC was equal to the TAC in both the raw and adjusted sets of events.

TABLE 4. Modularity test based on sequence of radiographic appearance of ossificaion centers following Poe (2004)

Module	No. centers	τ	Bootstrap *P*
Arm	33	0.420	0.922
Leg	28	0.693	0.025
Hand	28	0.359	0.966
Hand*	26	0.365	0.953
Foot	22	0.665	0.112
Foot*	20	0.603	0.286
Ankle	8	0.929	0.034
Ankle*	6	1.000	0.051
Wrist	9	0.817	0.102
Wrist*	7	0.878	0.099
Man. rays 1–5	19	0.481	0.662
Man. rays 2–5	16	0.600	0.347
Ped. rays 1–5	14	0.451	0.695
Ped. rays 2–5	11	0.636	0.327
Ankle + wrist	17	0.751	0.037
Ankle + wrist*	13	0.771	0.062
Toes + fingers	33	0.437	0.876
Knee + elbow	7	0.524	0.609
Hip + shoulder	4	0.913	0.303
Stylpod	7	0.823	0.096
Zeugopod	7	0.333	0.813
Autopod	46	0.443	0.971

The value τ is the Kendall correlation between female chimpanzees and humans for ossification centers within the proposed module. The bootstrap *P*-value indicates the proportion of randomly drawn groups of centers from the full sequence of events that had $|\tau|$ exceeding the observed $|\tau|$. One thousand bootstraps samples were taken for each test. Module names with * indicate the exclusion of centers from the distal zeugopod.

further linking of these results with known modular patterns in size and shape data.

Event-pair cracking identifies differences in developmental sequence between humans and chimpanzees. However, these differences are essentially the same as those recognized with simpler methods by Nissen and Riesen (1949). An important deficiency of this method is that is blind to changes in the timing of development that do not result in the reordering of events. King (2004) has advocated the application of discriminant functions to developmental sequence data. His analysis of the timing of primate dental eruption, craniofacial suture closure, and epiphyseal fusion identified large-scale conservation of sequence but important shifts in the overall pace of development among major groups. Ossification center appearance data for humans and chimpanzees do not entirely fit this pattern. The overall correlation between the chimpanzee and human sequences is much lower than in King's study (female τ = 0.53; male τ = 0.55; both $P < 0.001$), but the entire human sequence is generally delayed relative to chimpanzees (Figs. 1 and 2). Ultimately, the utility of pair cracking, or rank order comparisons, is in identifying particular moving events (Bininda-Edmonds et al., 2002; Jeffery et al., 2002), which may be difficult to extract from the multivariate workings of discriminant functions; these different methods complement one another for analyzing developmental sequences (King, 2004).

The sequence differences identified by pair cracking, or rank order comparisons, suggest tentative interpretations (Nissen and Riesen, 1949), although one should be aware of the limitations of such a simple two-species comparison (Garland and Adolph, 1994). The ossification centers appearing much earlier in chimpanzees are concentrated in the wrist and forearm and are likely involved in resisting the stresses on the forelimbs during arboreal climbing and quadrupedal knuckle-walking (Hunt, 1991, 1992; Dainton and Macho, 1999). Human centers appearing earlier tend to be concentrated in the fingers and toes, which may reflect developmental patterns that increase manual dexterity (Marzke, 1997) and accommodate bipedal locomotion (Harcourt-Smith and Aiello, 2004). The human advance in appearance of the greater trochanter of the femur could be interpreted as a similar adaptation for bipedalism (Harmon, 2007), though no comparative data exist to determine whether humans or chimpanzees have a derived or ancestral sequence.

However, limited ossification sequence data on captive macaques (*Macaca nemestrina* and *M. mulatta*) provide an indication of character polarity in some human and chimpanzee sequence differences (Newell-Morris et al., 1980). First, they imply the general appearance of tarsal and carpal centers prior those of the digits is an ancestral catarrhine feature that chimpanzees have maintained. Second, they suggest that humans are apomorphic in having a very early appearance of hallical and pollical ossification centers. Additional comparative sequence information and detailed biomechanical models will be necessary to substantiate any of the interpretations of what, given available data, appear to be derived features of human development (Lauder, 1995).

Poe's (2004) modularity test also highlights regions of the limbs that are understood to develop and function as modules primarily from studies of mammalian developmental genetics. Tetrapod fore- and hind limb identity are established, in part, by localized expression of differ-

ent *Tbx* and *Pitx* genes in the developing limb bud (Weatherbee and Carroll, 1999). This, and likely other differences in gene expression, allows for independence in limb development and evolution. The difference in modularity between the arm and leg in chimpanzee and human ossification center appearance is likely related to the large difference in function of the forelimb, which is released from locomotor activities in humans. Alternatively, it may simply be a result of hind limb development being more tightly integrated in mammals. Comparison of chimpanzee ossification center appearance with that of gorillas or other hominoids could resolve this question directly. Nevertheless, in a comparison of covariation patterns in linear measurements of limb elements, Young and Hallgrímsson (2005) found that quadrupedal mammals had much more tightly integrated fore- and hind limb development than the bats or gibbons in their sample that use their forelimbs in different capacities, which argues against this alternative.

The identity of segments within the limb is organized primarily by the localized expression of combinations of *Hox* genes (Chiu and Hamrick, 2002; Wellik and Capecchi, 2003). The regions receiving the most support as developmental modules using Poe's (2004) test—the ankle and wrist—are an area of overlapping expression of *Hoxa11-13* and *Hoxd11-13*. However, no clearly delimited region of *Hox* expression corresponds to these proximal regions of the autopod, as these genes are expressed throughout the autopod and distal zeugopod, suggesting other developmental genes may play more important roles in affecting ankle and wrist development (Chiu and Hamrick, 2002). Elsewhere, on the basis of the location of *Hox* gene expression in mice and a covariation study of primate forelimbs, Reno et al. (2008) have suggested the existence of a two modules in the hand and distal forearm. The first includes the distal radius and the metacarpals and phalanges of all but the first ray. The second module consists of only the first ray metacarpal and first proximal phalanx. Presence of these two modules and increasing or decreasing action of certain *Hox* genes in the developing hand was implicated in explaining the evolution of the characteristically short fingers and long thumb of living and fossil humans. The difference in τ between modules of ossification centers tested that included or excluded the first ray is consistent with their model. However, the generally low correlations in the hand found here, and the well known high amount of intraspecific and interspecific variation in distal limb elements (i.e., the autopod and zeugopod), suggest that these are not strong limitations on evolutionary alterations of development and may be common targets for selection (Shubin et al., 1997; Hallgrímsson et al., 2002).

However, finding modules in ossification center appearance data is somewhat unexpected. For example, Goswami's (2007) study of ossification centers in the mammalian cranium failed to find any significant modules although cranial modularity is well documented by covariation in morphometric data (Cheverud, 1982; Goswami, 2006). It is possible that this discordance arises because ossification is a single and relatively late event in bone development, which may not be integrated by the same mechanisms as growth rates.

Poor understanding of what radiographic appearance of ossification centers indicates functionally or developmentally hinders relating sequence differences or modularity in ossification center appearance to patterns of covariation in skeletal distances or limb proportions. Rollian (2008) evaluated a mathematical model of bone growth based on chondrocyte size, frequency of cell division, and number of cells in the proliferating zone in an ontogenetic series for two rodent species. He associated variation in the number of proliferating chondrocytes with limb proportions. In the femur, tibia, and humerus, larger elements had a larger initial set of proliferating chondrocytes. In more distal regions (the hand and foot), length differences were related to rates of loss of proliferating chondrocytes. However, the largest differences noted between the chimpanzee and human sequences here do not show any consistent pattern with bone size. For example, human digits 2–5 are smaller than those of chimpanzees, and the appearance of their ossification centers is accelerated, but the pollical and hallical centers, which are larger in humans, are also accelerated (Smith, 1995). This discordance of timing and terminal size is a general developmental feature, not restricted to bone. Bininda-Edmonds et al. (2003) found that the embryonic development of human cerebral hemispheres is delayed in sequence relative to other primates, despite humans having much larger brains. Similarly, tarsiers were found to have relatively large eyes despite being delayed in their developmental appearance.

Nevertheless, the identification of modules from human and chimpanzee ossification sequences suggests that sequence changes can have important effects on patterns of modularity in later portions of development and resulting adult phenotypes. Furthermore, ossification may reflect underlying modularity in the expression of genes active much earlier in development. However, clarifying these effects will require attention to the full developmental pattern and include detailed information on the timing of growth prior to and after the beginning of ossification and growth rate data to relate it to quantitative changes in size and shape (Alberch et al., 1979; Klingenberg, 1998).

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LITERATURE CITED

Ackerman RR, Smith RJ. 2007. The macroevolution of our ancient lineage: what we know (or think we know) about early hominin diversity. Evol Biol 34:72–85.

Alberch P. 1985. Problems with the interpretation of developmental sequences. Syst Zool 34:46–58.

Alberch P, Gould SJ, Oster GF, Wake DB. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317.

Bininda-Edmonds ORP, Jeffery JE, Coates MI, Richardson MK. 2002. From Haeckel to event-pairing: the evolution of developmental sequences. Theory Biosci 121:297–320.

Bininda-Edmonds ORP, Jeffery JE, Richardson MK. 2003. Is sequence heterochrony an important evolutionary mechanism in mammals? J Mammal Evol 10:335–361.

Cheverud JM. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516.

Chiu CH, Hamrick MW. 2002. Evolution and development of the primate limb skeleton. Evol Anthropol 11:94–107.

Dainton M, Macho GA. 1999. Did knuckle-walking evolve twice? J Hum Evol 36:171–194.

Dean C, Leakey MG, Reid D, Schrenk F, Schwartz GT, Stringer C, Walker A. 2001. Growth processes in teeth distinguish modern humans from Homo erectus and earlier hominins. Nature 414:628–631.

Fiser C, Bininda-Edmonds OP, Blejec A, Sket B. 2008. Can heterochrony help explain the high morphological diversity within the genus *Niphargus* (Crustacea: Amphipoda)? Org Divers Evol 8:146–162.
Garland TG, Adolph SC. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. Physiol Zool 67:797–828.
Gavan JA. 1953. Growth and development of the chimpanzee: a longitudinal and comparative study. Hum Biol 25:93–143.
Goswami A. 2006. Cranial modularity shifts during mammalian evolution. Am Nat 168:270–280.
Goswami A. 2007. Cranial modularity and sequence heterochrony in mammals. Evol Dev 9:290–298.
Gould SJ. 1977. Ontogeny and phylogeny. Cambridge: Belknap Press of the University of Harvard Press.
Gould SJ. 1982. Change in developmental timing as a mechanism of macroevolution. In: Bonner JT, editor. Evolution and development. New York: Springer-Verlag. p 333–346.
Hallgrímsson B, Willmore K, Hall BK. 2002. Canalization, developmental stability, and morphological integration in primate limbs. Yearb Phys Anthropol 45:131–158.
Harcourt-Smith WEH, Aiello LC. 2004. Fossils, feet and the evolution of human bipedal locomotion. J Anat 204:403–416.
Harmon EH. 2007. The shape of the hominoid proximal femur: a geometric morphometric analysis. J Anat 210:170–185.
Harvati K. 2000. Dental eruption sequence among colobine primates. Am J Phys Anthropol 112:69–85.
Hunt KD. 1991. Mechanical implications of chimpanzee positional behavior. Am J Phys Anthropol 86:521–536.
Hunt KD. 1992. Positional behavior of *Pan troglodytes* in the Mahale mountains and Gombe stream national parks, Tanzania. Am J Phys Anthropol 87:83–107.
Jeffery JE, Richardson MK, Coates MI, Bininda-Emonds OR. 2002. Analyzing developmental sequences within a phylogenetic framework. Syst Biol 51:478–491.
Kendall MG. 1948. Rank correlation methods. London: Charles Griffin and Co.
King SJ. 2004. Relative timing of ontogenetic events in primates. J Zool 264:267–280.
Kitching, IJ, Forey PL, Humphries CJ. 1998. Cladistics: the theory and practice of parsimony analysis, 2nd ed. New York: Oxford University Press.
Klingenberg CP. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. Biol Rev 73:79–123.
Lauder GV. 1995. On the inference of function from structure. In: Thomason J, editor. Functional morphology in vertebrate paleontology. Cambridge: Cambridge University Press. p 1–18.
Leigh SR. 2001. Evolution of human growth. Evol Anthropol 10:223–236.
Leigh SR. 2004. Brain growth, life history, and cognition in primate and human evolution. Am J Primatol 62:139–164.
Leigh SR, Shea BT. 1995. Ontogeny and the evolution of adult body size dimorphism in apes. Am J Primatol 36:37–60.
Magwene PM. 2001. New tools for studying integration and modularity. Evolution 55:1734–1755.
Manly BFJ. 1997. Randomization, bootstrap, and monte carlo methods in biology. London: Chapman and Hall.
Marriog G, Cheverud JM. 2001. A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology, and ontogeny during cranial evolution of New World monkeys. Evolution 55:2576–2600.
Marzke MW. 1997. Precision grips, hand morphology, and tools. Am J Phys Anthropol 102:91–110.
McCollum MA. 1999. The robust australopithecine face: a morphogenetic perspective. Science 284:301–305.
Newel-Morris L, Tarrant LH, Fahrenbruch CE, Burbacher TM, Sackett GP. 1980. Ossification in the hand and foot of the pigtail macaque (*Macaca nemestrina*). II. Order of appearance of centers and variability in sequence. Am J Phys Anthropol 53:423–439.
Nissen HW, Riesen AH. 1949. Onset of ossification in the epiphyses and short bones of the extremities in chimpanzee. Growth 13:45–70.
Olson EC, Miller RL. 1958. Morphological integration. Chicago: University of Chicago Press.
Poe S. 2004. A test for patterns of modularity in sequences of developmental events. Evolution 58:1852–1855.
Pyle I, Sontag LW. 1943. Variability in the onset of ossification in the epiphyses and short bones of the extremities. Am J Roentgenol Radium Ther 49:795–798.
R Development Core Team. 2007. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN3-900051-07-0.
Raff RA. 1996. The shape of life. Chicago: University of Chicago Press.
Reno PL, McCollum MA, Melanie A, Cohn MJ, Meindl RS, Hamrick M, Lovejoy CO. 2008. Patterns of correlation and covariation of anthropoid distal forelimb segments correspond to Hoxd expression territories. J Exp Zool B 310:240–258.
Rollian C. 2008. Developmental basis of limb length in rodents: evidence for multiple divisions of labor in mechanisms of endochondral bone growth. Evol Dev 10:15–28.
Schlosser G, Wagner GP. 2004. Modularity in development and evolution. Chicago: University of Chicago Press.
Schluter D. 2000. The ecology of adaptive radiation. New York: Oxford University Press.
Shubin N, Tabin C, Carroll S. 1997. Fossils, genes and the evolution of animal limbs. Nature 388:639–648.
Smith BH, Tompkins RL. 1995. Toward a life history of the Hominidea. Annu Rev Anthropol 24:257–279.
Smith KK. 2001. Heterochrony revisited: the evolution of developmental sequences. Biol J Linn Soc 73:169–186.
Smith SL. 1995. Pattern profile analysis of hominid and chimpanzee hand bones. Am J Phys Anthropol 96:283–300.
Weatherbee SD, Carroll SB. 1999. Selector genes and limb identity in arthropods and vertebrates. Cell 97:283–286.
Wellik DM, Capecchi MR. 2003. Hox10 and Hox11 genes are required to globally pattern the mammalian skeleton. Science 301:363–367.
West-Eberhard MJ. 2003. Developmental plasticity and evolution. New York: Oxford University Press.
Young NM, Hallgrímsson B. 2005. Serial homology and the evolution of mammalian limb covariation structure. Evolution 59:2691–2704.