Ontogenetic Sequence Analysis: Using Parsimony to Characterize Developmental Sequences and Sequence Polymorphism

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ABSTRACT Ontogenetic sequences are a pervasive aspect of development and are used extensively by biologists for intra- and interspecific comparisons. A tacit assumption behind most such analyses is that sequence is largely invariant within a species. However, recent embryological and experimental work emphasizes that ontogenetic sequences can be variable and that sequence polymorphism may be far more prevalent than is generally realized. We present a method that uses parsimony algorithms to map hierarchic developmental patterns that capture variability within a sample. This technique for discovering and formalizing sequences is called the ''Ontogenetic Sequence Analysis'' (OSA). Results of OSA include formalized diagrams of reticulating networks, describe all most parsimonious sequences, and can be used to develop statistics and metrics for comparison of both intraspecific and interspecific sequence variation. The method is tested with examples of human postnatal skeletal ossification, comprising a time-calibrated data set of human hand and wrist epiphyseal unions, and a longitudinal data set of human wrist ossification. Results illustrate the validity of the method for discovering sequence patterns and for predicting morphologies not represented in analytic samples. OSA demonstrates the potential and challenges of incorporating ontogenetic sequences of morphological information into evolutionary analyses. *J. Exp. Zool. 310B, 2008.*

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Biologists have labored for centuries to characterize ontogeny in multicellular organisms in ways that promote new insights into the development of individual organisms and into the comparative development of different individuals and different species. Characterizing ontogeny, whether for intra- or interspecific comparison, generally requires a standard for calibrating the maturity of the various individuals, which are often preserved museum specimens, forming the basis for such work. One set of standards uses age or size as a proxy of maturity, whereas another uses polarized sequences of events that pass unidirectionally from immature into mature states. Although the two standards are reconcilable in many respects, each is problematic in its own ways, owing to combinations of heritable (e.g., Garn et al., '66) and environmentally or experimentally induced variation (e.g., Mabee et al., 2000; Grünbaum et al., 2007).

As discussed by Mabee ('93; p 191), sequences of discrete character transformations have traditionally been described using ''a size-ordered assemblage of preserved specimens [to] reconstruct the ontogeny of a ''typical'' individual of a species.'' This traditional method of characterizing ontogeny for a particular species is often formalized in a series of standardized developmental stages. In

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human development and for many model laboratory species, an important research goal has been the derivation of a standardized series of developmental stages (e.g., Witschi, '62 for *Rattus*; O'Rahilly and Müller, '87 for *Homo*; Theiler, '89; Kaufman, '92 for *Mus*; many staging summaries can be found online).

The stages serve to typify ontogenetic differentiation through time and during growth, and such schemes are employed by large research audiences to calibrate the maturity of individual specimens in their laboratories. No single rationale has proven to be a touchstone for the practice of staging, and considerable effort goes into reconciling the different criteria of age, size, and structural expression into single coherent staging schemes for any given species (e.g., Kaufman, '92; Table 1, for *Mus*). Although standardized stages are often acknowledged as artificial constructs (e.g., Alberch, '85), a recent historical survey of vertebrate embryology observed that ''Research on embryos is today inconceivable without normal stages, the pictures plus texts that define standard divisions of development'' (Hopwood, 2007; p 1).

A common assumption implicit in standardized staging, as well as in describing individual developmental sequences, has been that the temporal sequence of discrete developmental events is largely invariant within species (Alberch and Blanco, '95; Mabee et al., 2000). This assumption is also implicit in many comparative studies that use sequences of discrete developmental events to study heterochrony in the evolution of development (e.g., McKinney and McNamara, '91; Hall, '92). However, recent studies that examined large samples of developing individuals are discovering unexpected levels of intraspecific variation in sequences of developmental events. For instance, a recent survey of skeletal development in 47 individuals of the snapping turtle *Chelydra serpentina* revealed conspicuous differences in patterns of ossification in dermal cranial elements when compared with endochondral elements of the braincase as well as variability in patterns of ossification among metapodial and phalangeal bones (Sheil and Greenbaum, 2005). Similar patterns of sequence variability were observed in the zebrafish *Danio rerio* (Cubbage and Mabee, '96) and the Siamese fighting fish *Betta splendens* (Mabee and Trendler, '96), in membranous versus endochondral ossification patterns and in bones associated with the lateral line versus those that are not.

Experimental biologists have also begun to compare intraspecific variability in developmental timing in natural and experimentally manipulated populations, as they seek to tease apart sources of heritable variation from environmentally induced developmental variability. For example, when samples of *Danio* eggs were raised at three different incubation temperatures, the different cohorts were each found to have different ossification sequences for several different bones (Mabee et al., 2000).

The medical literature on human development has long recognized that certain groups of

TABLE 1. Matrix of ossification center appearances versus individuals based on data presented in Garn et al. ('66)

	Ossification center appearances					
Semaphoronts	Capitate	Hamate	Triquetral	Lunate	Trapezium	Trapezoid
1: 100000	1	0	0	0	0	0
2: 010000	0	1	0	0	0	0
3: 110000	1	1	0	0	0	0
4: 111000	1	1	1	0	0	0
5: 110100	1	1	0	1	0	0
6: 110010	1	1	0	1	0	0
7: 111100	1	1	1	1	0	0
8: 111010	1	1	1	0	1	0
9: 111001	1	1	1	0	0	1
10: 110110	1	1	0	1	1	0
11: 111110	1	1	1	1	1	0
12: 111101	1	1	1	1	0	1
13: 111011	1	1	1	0	1	1
14: 110111	1	1	0	1	1	1
mat:111111	1	1	1	1	1	1
juv:000000	0	0	0	0	0	0

structures, for example, carpal bones or epiphyseal ossifications, are variable in both strict timing and relative sequence of development. The term ''sequence polymorphism'' was introduced to describe this phenomenon (Garn et al., '66), but it has only very recently found its way into the language of comparative biology (Colbert, '99; Mabee et al., 2000).

Sequence polymorphisms violate the general assumption of temporal and sequential invariance in embryological staging. At the same time, this phenomenon invites the challenge of characterizing intraspecific ontogenetic variability. Sequence polymorphism also poses a new suite of questions about the nature of variability within and between species. For example, there are preliminary indications that ontogenetic sequences are themselves subject to heritable variation, and that sequence variability may be a novel source of data for phylogenetic inference (Colbert, '99). Emerging from this research is the realization that intraspecific variation in the relative timing and sequence of ontogenetic events must be considered when studying the evolution of development between species (Sheil and Greenbaum, 2005; p 266).

These studies highlight that staging, although a staple of biology for two centuries, remains problematic in practice. Staging necessarily ignores sequence variation and sequence polymorphisms that occur in natural populations (Garn et al., '66). In these limitations, staging misrepresents the consistency of a ''typical sequence.'' Moreover, given the widespread occurrence of ''static'' size variation, size ordering is itself problematic—as is the use of any single criterion that varies in its rate of expression. In essence, although ''size ordering'' can be used to approximate sequences, it may also distort or mischaracterize patterns of sequence variation. This same criticism applies to interspecific comparisons of sequential events used in studies of heterochrony. This realization has motivated a number of researchers to seek other measures of maturity that account for variability (e.g., Garn and Rohmann, '60; Garn et al., '66; Cubbage and Mabee, '96).

In this report, we describe and illustrate a new method for characterizing variability of sequential events called Ontogenetic Sequence Analysis (OSA), which can be used to determine and formalize intraspecific ontogenetic sequences and that can generate comparative sequence data for interspecific analyses. The sequence data employed by OSA can represent any of the discrete events that comprise ontogenetic sequences across the range of ontogeny (e.g., closure of the neural tube, appearance of limb buds, appearance of ossification centers, eruption of teeth, etc.). The OSA method uses a phylogenetic parsimony algorithm (PAUP; Swofford, '89) under restrictive conditions to infer relative timing of ontogenetic events. Results can be elaborated into graphic representations, or maps, of ontogenetic sequences, facilitating comparing sequences in different sample populations. This method expands upon Brochu's ('96) use of PAUP to derive maturity stages for crocodilian postcrania and complements efforts to use discrete sequences of events to make maturity estimates within species (e.g., Tappen and Severson, '71; Roth, '84; Anemone et al., '96; Kuykendall and Conroy, '96; Tompkins, '96). It is also complementary to ''ontogenetic trajectory'' methods that are widely used to identify heterochrony in interspecific comparisons (e.g., Alberch et al., '79; Kluge, '85, '88; O'Grady, '85; Mabee, '93; Cubbage and Mabee, '96; Shubin and Wake, '96).

Briefly, OSA uses PAUP to analyze a matrix scored for ontogenetic characters taken from individual organisms. The individuals are the terminal units employed in the PAUP analysis and represent a matrix generalization of organism phenotypes at a particular state of maturity (i.e., semaphoronts, sensu Hennig, '66). A restrictive condition of character irreversibility is imposed. The matrix is searched twice for the most parsimonious trees, first using the least mature condition as a polarizing outgroup, then the most mature condition(s) as the outgroup. Results are elaborated into a reticulating diagram depicting the entire diversity of ontogenetic sequences in the sampled population. These sequence diagrams place all individuals onto developmental paths that lead from the least to the most mature morphologies. These diagrammatic ''maps'' of reticulating networks allow description of all most parsimonious sequences and form predictive hypotheses for both intra- and interspecific sequence variation. Our results support earlier conclusions that sequence polymorphism is common (Garn et al., '66) and also show that the reticulation of sequences into networks is constrained around modal patterns.

Evaluation of determined sequence variation between samples can reveal limitations related to sampling artifacts as well as potential phylogenetic signals (Colbert, '99). OSA offers maturity

scores that can function in a similar way as conventional stages, but avoids the typology of staging methods and instead reflects the full range of variation present in a sample. OSA also affords a statistically robust means of calculating modal and average sequences within a sample, as a more rigorous baseline for comparisons between species using methods such as ''event-pair cracking'' (Jeffery et al., 2002), parsimov (Jeffery et al., 2005), or statistical comparisons of sequence similarity (e.g., Nunn and Smith, '98, 2001). Although the results of OSA are amenable to interspecific comparisons, we focus on establishing intraspecific characterizations of ontogeny in this initial report, which would form the basis for subsequent comparisons between species. OSA methodology and assumptions as well as the revised logical foundations required by this unorthodox use of a phylogenetic parsimony algorithm are discussed more fully below.

The method is demonstrated here using two examples: time-calibrated data on human hand and wrist epiphyseal unions (Pryor, '25), and longitudinal data (i.e., individuals tracked over extended periods of development) on human wrist ossification (Garn et al., '66). Although both are examples of human postnatal skeletal ontogeny, OSA is applicable to any of the multitude of developmental sequences occurring during the entire span of ontogeny in any taxon.

MATERIAL AND METHODS

Carpal ossification sequences: Garn et al. ('66) and Garn and Rohmann ('60) present data on sequence polymorphism in the relative timing of carpal ossification centers for 154 Caucasian males and females from Ohio (participants in the Fels Longitudinal Studies). This longitudinal sample includes six carpal elements (hamate (= unciform), capitate (= magnum), lunate (= lunar), triquetral (= cuneiform), trapezoid, and trapezium) that are scored as either ossification center absent (0) or present (1). To facilitate comparison with their work, carpal terminology follows Garn et al. ('66). Table 1 provides the OSA data matrix derived from their longitudinal data.

Hand and wrist epiphyseal fusion sequences: Data from Pryor ('25) document epiphyseal union patterns in the distal radius, distal ulna, metacarpals, and phalanges based on X-ray imagery of 144 Caucasians. The sample includes 63 males (ages 12.5–22.8 years), 33 of whom are neither least nor most mature semaphoronts, and 81 females (ages 12.1–22.5 years), 40 of whom are neither least nor most mature phenotypes. Pryor ('25) observed the following states in all six characters: distinct gap (0), indistinct gap (1), line (2), and completely fused with no demarcation (3). The OSA-coded data matrix is given in Table 2.

OSA methodology: Steps I–IV presented below are summarized in Figure 1.

Step I: *Scoring a matrix*: Discrete ontogenetic transformations are scored for individual organisms onto a ''character-by-individual'' matrix. The discrete ontogenetic characters can have two or more states (see Discussion below). Only irreversible ontogenetic transformations are included.

Step II: *PAUP analysis*: The scored data matrix is executed in PAUP, with character-type ''irreversible'' applied to all characters. Individuals that share identical states for all characters (i.e., a particular semaphoront condition) are culled before the analysis (Brochu, '96), after noting their relative frequencies. Adequate samples may be too large for ''Exhaustive'' searches and are analyzed using either ''Heuristic'' or ''Branch-and-Bound'' search options, keeping only minimal length trees.

Ontogenetic sequences are assumed to form a closed reticulum, with known starting and ending conditions (i.e., the least and most mature phenotypes). The branching trees generated by PAUP do not represent reticula, however, and terminals may be ''stranded'' on isolated branches (e.g., individual ''V'' in Fig. 1), with no indication of the most parsimonious sequence connecting them to the most mature phenotype. These branches, supported by characters, indicate sequence polymorphism. Conversely, lack of sequence polymorphism in a fully resolved sample is indicated by a completely pectinate tree that lacks character support for its side branches.

Deriving sets of alternately polarized trees allows generation of a most-parsimonious reticulum. One set, called the ''normal'' treatment, assumes the least mature condition (or hypothetical least mature condition) as the outgroup. The second set of trees, the ''reversed'' treatment, polarizes characters using the most mature condition (or hypothetical most mature condition) as the outgroup. These two treatments are logically justified because the sequences are composed of irreversible characters that are assumed to converge on either the most or least mature condition, passing through all observed conditions. Re-rooting the tree does not typically result in the same network topology as the reverse tree (e.g., see Fig. 1).

TABLE 2. Matrix of epiphyseal fusions for individual male and female humans based on data from Pryor ('25)

Male semaphoronts	(A) Distal radius	(B) Distal ulna	(C) Metacarpals	(D) First row phalanges	(E) Second row phalanges	(F) Third row phalanges
Male epiphyseal fusions						
Male 000000	0	0	0	0	0	0
Male 001111	0	0	1	1	1	1
Male 000022	0	0	0	0	2	2
Male 011111	0	1	1	1	1	1
Male 112222	1	1	2	2	2	2
Male 333332	3	3	3	3	3	2
Male 003333	0	0	3	3	3	3
Male 000002	0	0	0	0	0	2
Male 113333	1	1	3	3	3	3
Male 012222	0	1	2	2	2	2
Male 002222	0	0	2	2	2	2
Male 123333	1	2	3	3	3	3
Male 013333	0	1	3	3	3	3
Male 011333	0	1	1	3	3	3
Male 010111	0	1	0	1	1	1
Female semaphoronts						
Female epiphyseal fusions						
Female 000000	0	0	0	0	0	0
Female 003222	0	0	3	2	2	2
Female 003333	0	0	3	3	3	3
Female 002222	0	0	2	2	2	2
Female 001222	0	0	1	2	2	2
Female 011222	0	1	1	2	2	2
Female 013333	0	1	3	3	3	3
Female 133333	1	3	3	3	3	3
Female 000112		0	0	1	1	2
Female 012333	0	1	2	3	3	3
Female 223333	2	2	3	3	3	3
Female 122222	1	2	2	2	2	2
Female 011111	0	1	1	1	1	1
Female 233333	2	3	3	3	3	3
Female 333333	3	3	3	3	3	3

Step III: *Converting trees into partial sequences*: Tree topologies determined by PAUP (both ontogenetic ''normal'' and ''reversed'') are imported for analysis in MacClade (ver. 3.01; Maddison and Maddison, '92). Character support for particular branches is evaluated using the ''trace all changes'' command, utilizing the ''changes weighted by the cost of change'' option, and with the assumption of irreversibility applied to all characters. Zero-length branches are collapsed, placing their terminal semaphoronts directly onto the emerging sequence. MacClade ver. 3.01 requires that recovered polytomies are resolved before application of character irreversibility. Note that polytomies will always include a zero-length branch.

Typically, PAUP recovers multiple most-parsimonious trees, which are integrated into partially networked composite sequences (e.g., Fig. 1) representing partial sequences from ontogenetic ''normal'' and ''reversed'' analyses. The emerging sequence for either polarization is diagrammed here using Adobe Illustrator™, with terminal semaphoronts mapped onto a grid calibrated to maturity score (discussed below). Observed and optimized semaphoronts are graphically connected with sequence segment lines. Having the semaphoronts calibrated to ''maturity score'' greatly facilitates integrating partial sequences.

Step IV: *Integrating sequence maps*: All sampled individuals are assumed to lie on some sequence that leads from the least mature to the most mature outgroup condition. These sequences are completed by graphically combining the ontogenetic ''normal'' and ''reversed'' analyses using Adobe Illustrator™ (Fig. 1). Thus, the combined

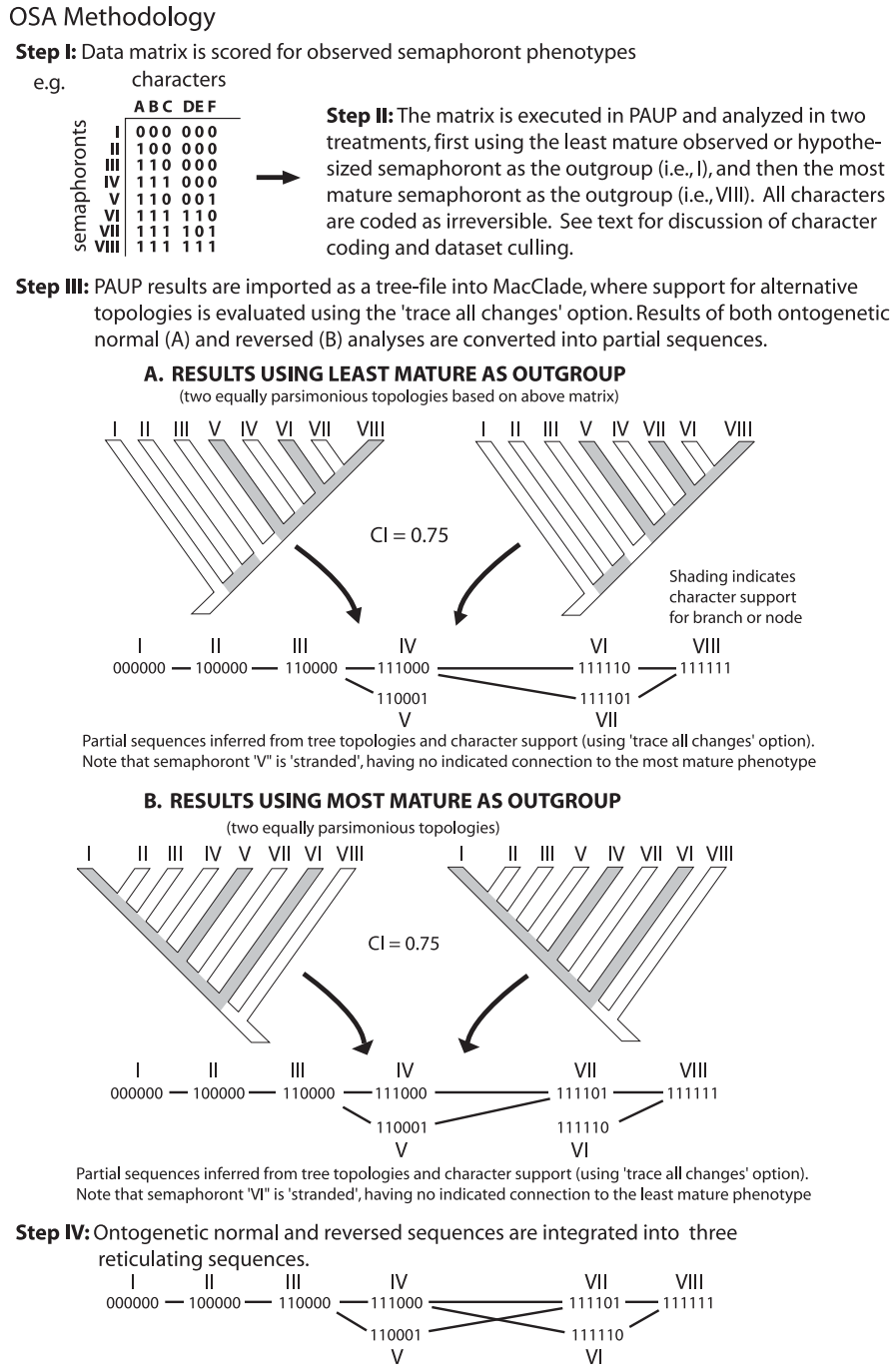

Fig. 1. Steps involved in generating sequence maps from cross-sectional data.

normal and reversed results will trace all parsimonious paths that lead from the least to the most mature condition and which include all observed semaphoront phenotypes.

Note that drafting OSA sequence maps can be labor intensive and time consuming, particularly with large data sets. However, it is clear that this aspect of OSA analysis, as well as the description of sequences discussed below, could be readily programmed and automated if there was sufficient demand.

Describing sequences: Once reticulating sequence maps are established, all sequences can be listed. Every character transformation along each sequence can be assigned a relative sequence position. When the relative sequence position of

two or more characters is unresolved, they are given the average sequence position between their bracketing nodes (Nunn and Smith, '98). Determination of character sequence positions allows comparison of their relative timing between both polymorphic sequences and samples analyzed separately (e.g., males versus females, different species, etc.).

Although unresolved characters are initially assigned an average score defined by their bracketing nodes, their positional hierarchy can be further refined by comparison with other pathways or sequence maps, in which their relative sequence position has been resolved. This maximizes the fit between equally parsimonious sequences. If character transformations are consistently unresolved, they are potentially correlated.

The frequency distribution of variant sequences generally follows predictable patterns (see Garn et al., '66). With adequate samples, both the modal sequence and the average sequence can be identified (e.g., Fig. 2). These metrics are discussed below as aspects of sequence comparison.

Maturity scores: Maturity scores for individuals are calculated by summing the values of all coded character states scored for that individual. Thus, a character-state distribution of ''01034'' for five multistate characters receives a score of ''8'' (i.e., $0+1+0+3+4=8$), and ''20220'' receives a score of ''6'' (i.e., $2+0+2+2+0=6$). Assuming parity of character transformation, this tally of an individual's irreversible ontogenetic character transformations roughly calibrates its relative maturity (e.g., Roth, '84), although different semaphoronts can receive identical scores. This calculation is identical to the ''age rank'' calculation of Tappen and Severson ('71), but the term ''age'' is avoided here to emphasize that these values may not be well correlated with absolute age.

Maturity scores are here used to calibrate sequence maps, aligning semaphoronts of similar maturity along rows or columns (see Fig. 2). This facilitates visual appraisal of the amount of missing data and highlights the sequence variation of particular characters.

Sequence comparison: Descriptive statistics can be applied to a character's sequence position (e.g., sequence range, mode, median, standard deviation, etc.), and are the basis for most comparisons within and between samples.

With dense samples, the relative frequency of observed semaphoront phenotypes can provide likelihood estimates for the occurrence of predicted sequences. This assumes that the number of observed samples that occupies a particular path

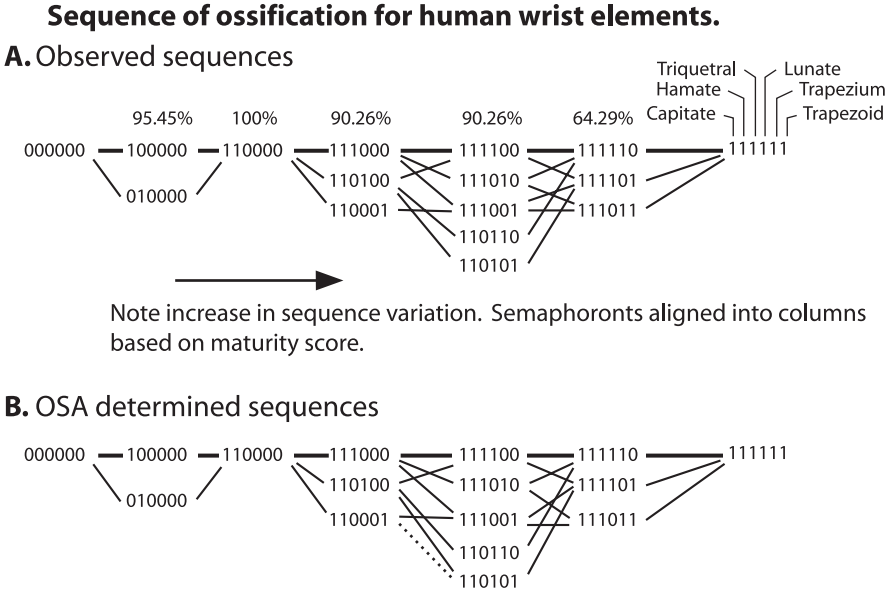

Fig. 2. Human carpal ossification sequences: (**A**) Observed sequences of wrist element ossification from a longitudinal series of 154 males and females. Percentages indicate the frequency of occurrence of the modal semaphoront (bold line represents modal sequence) at particular maturity scores. Modal sequence was followed by 55.2% of sampled individuals. (**B**) Sequences determined by OSA, using observed phenotypes. Data from Garn et al. ('66).

reflects the relative likelihood for the occurrence of that path as a whole. Here we use the following parameters to estimate a path's likelihood: the number of individuals at a particular maturity score on a particular path (**N**); the total number of individuals at a particular maturity score (**Nm**); and the number of paths predicted at a particular maturity score (**P**). Using these parameters, a likelihood estimate for an individual's occurrence on a particular path at a particular maturity score ($\mathbf{Lm_x}$) can be calculated as follows:

$$\mathbf{Lm_x} = (\mathbf{N} + \mathbf{1})/(\mathbf{Nm} + \mathbf{P}).$$

And the likelihood for each particular path or sequence (**L**) can be estimated as the product of all calculated $\mathbf{Lm_x}$ along that particular path:

$$\mathbf{L} = \mathbf{Lm_1Lm_2Lm_3Lm_4} \ldots . \mathbf{Lm_x}.$$

This estimate is a dimensionless value that can be used to compare relative sequence likelihood for different paths within a sequence map. This calculation applied below to the sequences of human carpal ossifications. Such estimates are best applied to large, well-sampled data sets.

When sample sizes are too small to accurately predict likelihood, simple tallies of the number of sampled individuals occupying each particular sequence can provide a rough estimate for relative sequence likelihood. Such a tally can indicate the modal sequence, which is here considered the one (or several) sequence(s) out of all sequences that incorporates the greatest number of sampled individuals. These tallies can be used to rank various sequences.

Finally, an average sequence can be calculated in which relative sequence is based on each character's average sequence position over all estimated sequences. This statistic is particularly useful when sample sizes are small and modal estimates are likely to be biased by sampling artifacts.

Semaphoront probabilities: In addition to calculating the modal sequence and ranking based on tallying individuals occupying each sequence, one can estimate the probability of seeing a particular distribution of semaphoronts relative to a random distribution of semaphoronts. The predicted random distribution is calculated as a function of the number of included characters and character states, assuming equal probability of change for all character transformations (see Fig. 3).

Note that the theoretical distribution of semaphoront phenotypes ordered by maturity score forms a symmetrical distribution with minima at the least and most mature phenotypes. Given such a map of all possible sequences, the predicted frequencies for random semaphoront distributions can be calculated. For example, in Figure 3A, if all sequences were equally likely, half the samples of individuals at maturity score ''1'' should be semaphoronts ''01'', and the other half should be ''10''. Similarly, at maturity score ''2'', half the sample should randomly show semaphoront

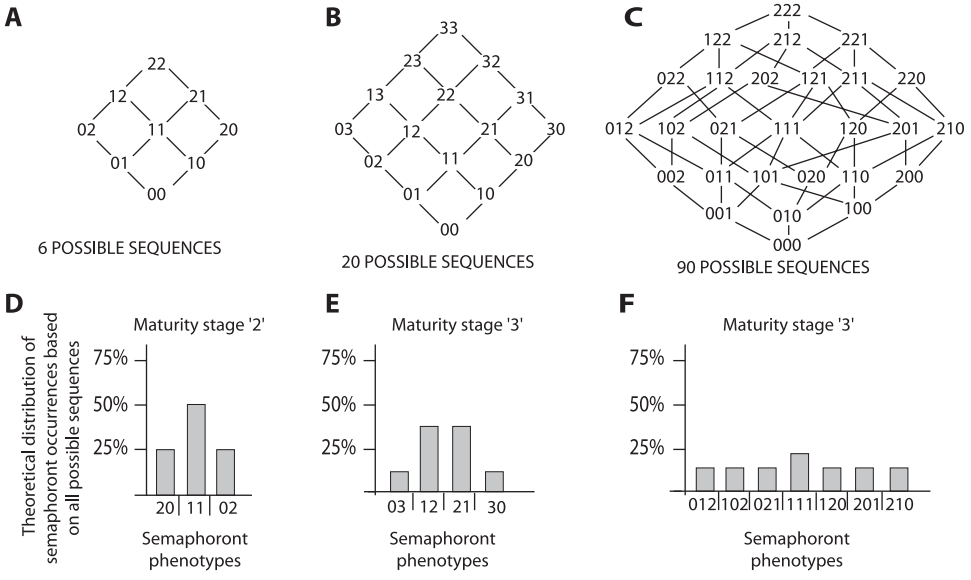

Fig. 3. Distribution of all possible developmental sequences in data sets having (**A**) two characters with three states, (**B**) two characters with four states, and (**C**) three characters with two states, all assuming equal transformation weights. **D–F** represent expected frequency distributions of sampled phenotypes for the above sequences at particular maturity stages if sequences are randomly generated. See explanation in text.

phenotype ''11'', whereas phenotypes ''20'' and ''02'', should each comprise one-quarter of the sample (Fig. 3D). Similarly, given two four-state characters, the random occurrences of maturity score ''3'' semaphoronts (03, 12, 21, and 30) would be distributed in a 1:3:3:1 ratio (Fig. 3E). Semaphoronts having a maturity score of ''3'' based on a three-character, three-state data set (012, 021, 102, 120, 201, 210, 111) should exhibit a random distribution in a 2:2:2:2:2:2:3 ratio, respectively (Fig. 3F). Such sampling distributions serve as null hypotheses against which the actual distribution of semaphoronts within particular maturity classes could be compared.

It is also possible to calculate a similar distribution for all semaphoronts regardless of maturity score, but this calculation is complicated by the potential that samples from different maturity classes are biased because of differential survivorship or collection. Corrections for these biases could be applied if actuarial survivorship statistics were available.

Sequence probabilities: If sequence order was random, and all sequences were equally likely, the likelihood of any particular sequence can be calculated based on the total number of theoretically possible sequences—which itself is calculated from the number of characters and characters states (see Fig. 3). If sequence order is not random, however, then the probability for a particular distribution of sequences compared with a random distribution can determined if the distribution of sequences was known or estimated based on adequate samples.

The probability for observing a particular distribution of sequences compared with a random distribution can be calculated using the following multinomial distribution:

$$\frac{n!}{k_1! + k_2! + \cdots + k_S!}\ p_1^{k_1} p_2^{k_2} \cdots\cdots p_S^{k_S}$$

where n equals the number of observed sequences,

$$p_1^{k_1}, p_2^{k_2}, \ldots\ldots, p_S^{k_S}$$

are the probabilities for each sequence's occurrence, and k_S is the number of times that a particular sequence is observed, such that $k_1! + k_2! + \cdots + k_S! = n$.

Assumptions of the OSA method

Ontogenetic hierarchy and reticulation: The primary assumption of OSA is that the relative orderliness of ontogenetic transformations results in a hierarchical pattern of character emergence during development. This hierarchy is a consequence of both causal and non-causal relationships (e.g., Alberch, '85). In other words, although the appearance of certain features is causally predicated on the appearance of earlier features, other hierarchic patterns only reflect temporal patterns of ontogenetic unfolding. In either case, ontogeny exhibits hierarchical patterns that can be characterized and compared using parsimony algorithms.

In non-causal sequences, there is a probabilistic correspondence between the amount of time separating events and sequence polymorphism; closely spaced events are more likely to overlap and/or change relative sequence, and more distantly separated events are less likely to change relative sequence position. The correlation of temporal spacing and sequence consistency can be qualitatively evaluated by comparison with normative data documenting absolute character appearance age.

The hierarchical signal of an ontogenetic data set can be estimated by the positional consistency of characters, combinations of characters, or by character transformation patterns seen in components of the sample. As in conventional phylogenetic analyses, characters may be included or removed from further analyses in PAUP depending on the level of generality and consistency desired, or by some otherwise determined importance of the feature.

The portrayal of multiple sequences in a sample as a reticulating sequence network reflects the tendency of biological populations or species to converge around a limited range of variation during growth (Creighton and Strauss, '86). Sequences inhabit a trajectory leading from the least to the most mature phenotype. The occurrence of adult phenotypic polymorphism (e.g., Shubin and Wake, '96), however, means that many sequences will not converge on a common mature condition. This may require special analytical treatment, such as the use of multiple mature outgroups. Clearly, sample populations having different ''most mature'' conditions, or those that cannot reasonably be scored using a common coding scheme should be analyzed separately. Although combining samples in which adult polymorphism reflects a more innocuous terminal addition or deletion along a similar trajectory may not affect determined sequence topologies, separate analyses are nevertheless recommended when significant variation exists between samples (i.e.,

variation reflecting sexual dimorphism, caste morphotype polymorphism, taxonomy, etc.). Subsequent combined analysis or comparison can illuminate the degree to which sequence consistency is affected by polymorphism.

Polarity assessment and the irreversibility of ontogenetic characters: OSA requires all included characters to be invariantly irreversible. Although many ontogenetic transformations are definitely irreversible (e.g., sutures between bones close, and do not open, during development: Moss, '58; Persson et al., '78; Oudhof, '82; Manzanares et al., '88; Beresford, '93), exceptions are not uncommon (e.g., the ''cell modulations'' of Hall, '92; the condylar cartilage of the mammalian dentary; Hall, '84,'92; Herring, '93; etc.). The assumption of ontogenetic irreversibility must be based on observation of the developmental process (Kluge, '88).

Even with irreversible sequences, it may be difficult to distinguish less from more mature character states (Kluge and Strauss, '85). For example, the lack of a prenasal (or internasal) process of the premaxilla in adult therian mammals is the result of non-differentiation of this process. In adult monotremes, however, the mature absence is the result of resorption by osteoclasts of an embryonic prenasal process that supports the os caruncle and assists in hatching (Hill and de Beer, '49; Rowe, '88). Thus, the irreversible monotreme sequence progresses from embryonic lack (0) to embryonic presence (1) to adult lack (2) of the prenasal process. Therians, in contrast, lack the process (0) throughout ontogeny. Proper use of these characters in OSA is contingent on the ability to discriminate between the embryonic lack (0) and the adult lack (2) of this process. Maturity scores are also predicated on the assumption of irreversibility and proper estimate of embryological polarity.

Discrete versus continuous data: Although characters used here are considered discrete, it is recognized that character continuity is correlated with transformation rate and sampling artifacts. Depending on the scale of observation, character states emerging over narrow time periods may profitably be viewed as discrete. Conversely, intermediate states are more likely to be sampled in more slowly transforming elements, potentially demanding additional coded states. An obstacle to recognizing completely continuous characters in OSA relates to the differential weight afforded particular characters having more coded states. Theoretically, it would be possible to incorporate more continuous ontogenetic transformations into OSA with the appropriate statistical techniques (see Swiderski et al., '98).

Discrimination of multiple character states should be based on observed intermediate morphologies. It is presumed that, with adequate samples, the probability of sampling character states is correlated with character transformation rate (see discussion of sampling by Thorington and Vorek, '76). For example, in guinea pigs, the proximal radius begins epiphyseal union at ca. week 16–17 and is completely united by ca. week 23–24, taking at most 8 weeks to fuse, whereas the distal radius begins at ca. week 49–52 and is united by ca. week 86–96, taking from 34 to 47 weeks to fuse (Zuck, '38; Fig. 4). Assuming equal probabilities for sampling age groups and a relatively constant rate of fusion within each element, it is four to six times more likely to sample a partially fused distal than proximal radius (Fig. 4). Accordingly, because more intermediate steps would be sampled in the distal radius than the proximal radius, the distal radius could be assigned more intermediate character states based on degrees of partial fusion. This potentially provides a more realistic estimate of its temporal hierarchical signal by proportionally weighting transformations as a function of transformation time. Application of OSA methodology to more continuous data and the associated issue of character weighting are avenues for future investigations.

Parsimony criteria: The parsimony criterion required by ontogenetic data differs from PAUP's assumption of parsimony for phylogeny reconstruction. PAUP considers the preferred phylogeny to be that which requires the least amount of character homoplasy between taxa. In OSA, the preferred sequence topologies minimize the number of discordant ontogenetic events between sampled individuals.

Cladistic consistency statistics: Although character consistency indices (Ci) and overall consistency indices (CI) are generally inversely proportional to the number of alternate sequences

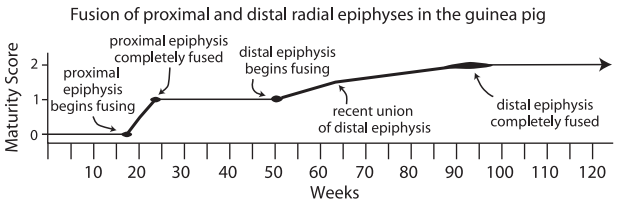

Fig. 4. Timing of union of epiphyseal elements in the guinea pig, based on data of Zuck ('38).

predicated by OSA, they do not accurately reflect the degree of sequence order violation. Thus, although the aberrant precocious maturation of a character may only marginally affect its Ci, it might drastically impact the sequence range for that feature. Note that, unlike a cladogram, Ci and CI in OSA do not indicate homoplasy (convergence or reversal), but rather the degree to which the data conform to a particular sequence in sampled individuals.

RESULTS

Carpal ossification sequences

OSA, beginning with a PAUP analysis of Garn et al.'s ('66) data, yields 768 trees in the ontogenetic normal treatment with a CI of 0.40, and 1,152 trees in the ontogenetic reversed treatment, with a CI of 0.40. The sequence maps generated from these trees (Fig. 2B) stipulate 26 equally parsimonious ontogenetic sequences (Table 3).

Based on these analyses, the average sequence (i.e., the order based on the average sequence position over all sequences) is [hamate, capitate] ⇒ triquetral ⇒ [lunate, trapezium] ⇒ trapezoid (the relative position of elements listed in brackets is not resolved).

These human longitudinal samples demonstrate that OSA can both determine sequence polymorphism and recover observed sequences. Sequence maps based on observed sequences, and sequences determined by OSA, are shown in Figures 2A and B, respectively. OSA reproduces all transformation sequences observed in the longitudinal sample and further predicts an additional transformation that was not observed in the original sample (dashed line in Fig. 2B).

OSA predicts 11 sequences in addition to the 15 actually observed by Garn et al. ('66). Although a seemingly great increase in predicted sequence polymorphism, note that 87% of their sampled individuals fall on just two sequences, with all other sequences observed in only 13% of the population (see below). Indeed, the four observed

TABLE 3. Sequences of ossification center appearance in humans predicted by the OSA method using longitudinal data presented in Garn et al. ('66)

Sequences	Capitate	Hamate	Triquetral	Lunate	Trapezium	Trapezoid	Observed frequency (%)	Predicted frequency (%)
G I	1	2	3	4	5	6	55.2	50.0
G II	1	2	3	4	6	5	24.7	26.3
G III	1	2	3	5	4	6	2.6	2.2
G IV	1	2	3	5	6	4	3.2	1.3
G V	1	2	3	6	4	5	1.3	0.1
G VI	1	2	3	6	5	4	0.6	0.1
G VII	1	2	4	3	5	6	3.2	5.0
G VIII	1	2	4	3	6	5	2.6	2.6
OSA I	1	2	4	5	6	3	0.0	0.0
G XI	1	2	4	5	3	6	0.6	0.0
G IX	1	2	4	6	3	5	0.6	0.0
G X	1	2	6	3	4	5	0.6	0.0
OSA II	1	2	5	4	6	3	0.0	0.0
OSA III	2	1	3	5	4	6	0.0	0.1
G XII	2	1	3	4	5	6	2.4	1.9
G XIII	2	1	3	4	6	5	0.6	1.1
OSA IV	2	1	3	6	4	5	0.0	0.0
OSAV	2	1	3	5	6	4	0.0	0.0
OSA VI	2	1	3	6	5	4	0.0	0.0
OSA VII	2	1	3	5	4	6	0.0	0.0
G XIV	2	1	4	3	5	6	0.6	0.2
G XV	2	1	4	3	6	5	1.2	0.1
OSA VIII	2	1	5	3	6	4	0.0	0.0
OSA IX	2	1	4	5	6	3	0.0	0.0
OSA X	2	1	4	6	5	3	0.0	0.0
OSA XI	2	1	5	4	6	3	0.0	0.0

Observed frequency as recorded by Garn et al. ('66), predicted frequency using likelihood estimate presented in text; OSA, Ontogenic Sequence Analysis.

sequences in which the hamate appears first (i.e., those passing through semaphoront ''010000'') comprise only 5% of sampled sequences but account for half of the sequences predicted by OSA. Thus, a small minority of the data account for a substantial increase in and predicted sequence polymorphism. The OSA predicts that the sequences that were not observed by Garn et al. ('66) occur with very low frequencies (see below), which implies that they might eventually be recovered with repeated sampling.

The occurrence of sequence polymorphism demonstrates that development is not completely canalized. At a fundamental level, it also implies a general lack of causality in the order of ossification center appearances. The only pattern for which a strictly causal relationship may still be postulated is the appearance of the capitate and hamate before all other ossifications. Because the capitate and hamate ossification centers appear much earlier than do those of the other carpal elements considered (e.g., Lewis, '36), it is possible that sequence invariance simply reflects their timing of appearance. It is interesting to note that the sequences of carpal ossification center appearance have little direct correlation and may even be inversely correlated with the order of chondrogenic condensations postulated by Oster et al. ('88).

Although not completely canalized, these samples conform to restricted patterns of ossification center appearances. Observed sequences show a strong modality centered on two sequences, with 55% of the sampled individuals following the modal sequence (bold line in Fig. 2) and 25% of the sample following the second most common sequence, which differs from the modal only in the relative order of appearance of the trapezium and trapezoid ossification centers. Of the remaining 31 individuals, nine have sequences in which the ossification center of the hamate is the first to appear.

The occurrence of a modal sequence and other particular sequences can be estimated based on OSA results. This involves estimating the frequency of occurrence for a particular predicted sequence as a function of the number of individuals inhabiting that sequence. For predicted sequences of carpal ossification center appearances, this can be approximated by considering the observed frequencies of different semaphoronts presented by Garn et al. ('66) as cross-sectional data. As seen in Figure 6, estimates of sequence frequencies agree well with observed sequence frequencies. Note that all of the predicted sequences that were not observed were not predicted to be observed given a sample size of 154 individuals.

Hand and wrist epiphyseal fusion sequences

Branch-and-bound analysis of the female data matrix yields 16 trees with a CI of 0.67 in positive polarity treatment and eight trees with a CI of 0.64 in the reverse polarity treatment. Resulting female sequence maps predict 22 most parsimonious sequences. Branch-and-bound PAUP analysis of the male data results in 192 trees with a CI of 0.40 in the positive polarity treatment and two trees with a CI of 0.42 in the reverse polarity treatment. Resulting male sequence maps predict 17 most parsimonious sequences. Male and female sequence maps are presented in Figure 5 and sequences are listed in Table 4.

It has long been recognized that human females show accelerated skeletal maturation relative to males (e.g., Mall, '06). The questions posed here are whether males and females show differences in the degree of sequence polymorphism; sequence order; or the correlation between maturity scores and chronological age.

Both sequence maps (Fig. 5) and sequence position scores (Fig. 7) indicate greater male sequence polymorphism and variability compared with female samples. Increased male variability is evidenced by the greater number of observed and predicted male semaphoronts compared with female semaphoronts. There are 25 observed and a minimum of 65 predicted male semaphoront phenotypes, of which 11 are resolved as nodes (Fig. 5). In contrast, there are only 15 observed and a minimum of 36 predicted female phenotypes, of which seven are resolved as nodes (Fig. 5). Males also show a greater distance between inferred sequences (Fig. 5) as well as a greater range in sequence position for character transformations (Fig. 7). The greater male polymorphism is further reflected in males having a lower correlation between age and skeletal maturity than the female sample (Fig. 8). The poor correlation between age and skeletal maturity in both samples, however, emphasizes the peril of inferring age from the relative skeletal maturity of hand and wrist elements.

Although there is more variation predicted for male sequences than for females, Garn et al. ('66) suggest that a similar pattern of greater male

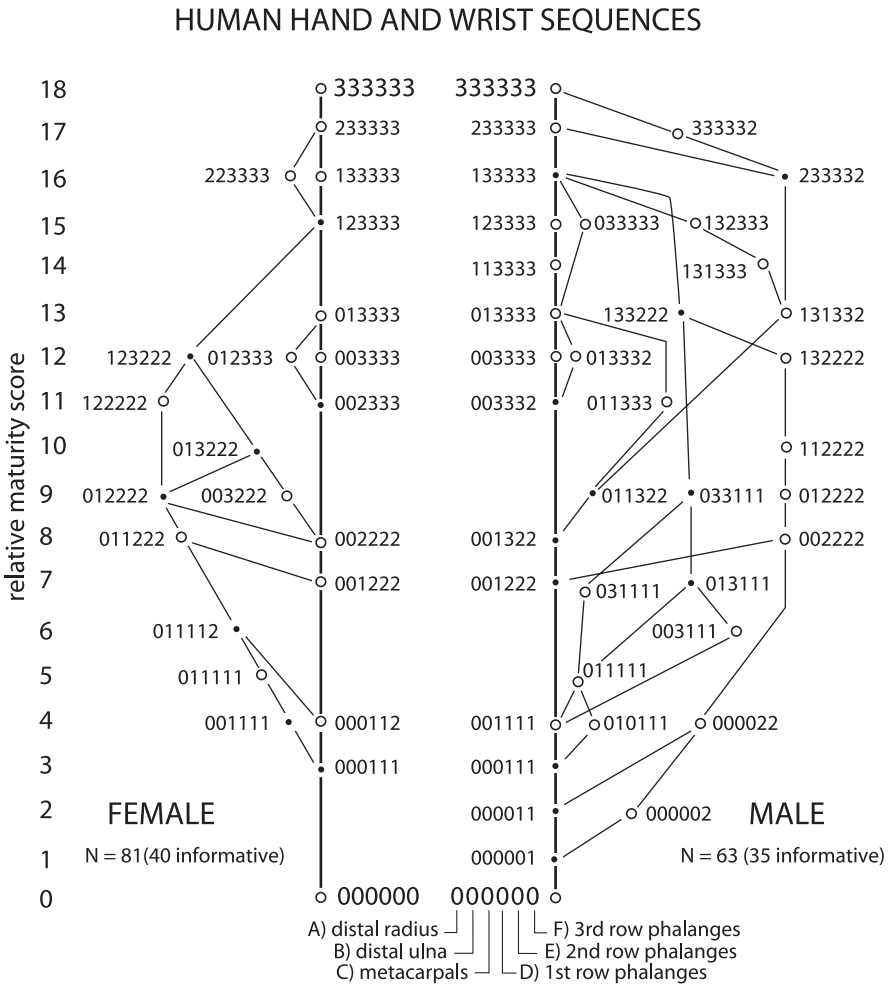

Fig. 5. Human hand and wrist epiphyseal union order. Predicted sequence map of epiphyseal union in females (left) and males (right), based on cross-sectional observations of Pryor ('25). Observed semaphoronts, and semaphoronts predicted by optimization, are represented as open and filled circles on the sequence map, respectively. Semaphoront phenotypes are listed for observed and predicted semaphoronts. All six included characters were multistate characters. See discussion in text.

variation in wrist ossification center sequences is a sampling artifact. They postulate that male phenotypes are better sampled as a consequence of retarded male maturation. OSA does not support this interpretation for Pryor's ('25) data. Because the less variable female sample sizes are larger (40 females to 33 males, excluding the most and least mature phenotypes) and also have a longer maturation period (5 years and 8 months versus 3 years and 11 months for the youngest and oldest informative female and male semaphoronts, respectively), it is unlikely that differences in variability are a result of sampling artifacts.

The ratio of observed to predicted phenotypes is low, indicating poor sampling of the actual population. Predicted male and female modal sequences can only be considered tentative. Notwithstanding, the overall similarity between inferred male and female modal sequences (out of the 18 possible positions on the male and female modal sequences, there are at least nine identical semaphoronts, and possibly 13 identical semaphoronts) supports a occurrence of a predictable sequence distribution for both samples.

DISCUSSION

Sequence likelihood and constraint: The degree to which the estimated number of sequences is restricted compared with the possible number of sequences reflects constraint in the developmental system. In the example of human wrist ossification, constraint is indicated by the capitate and hamate always ossifying before the other considered elements. Conversely, the remaining four elements show little sequence constraint after the

TABLE 4. OSA predicted sequences of human hand epiphyseal fusion based on data from Pryor ('25)

Male Sequences (Sequence I is modal)

I. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ (C2+C3+E3)[9-11] ⇒ F3[12] ⇒ B1[13] ⇒ A1[14] ⇒ B2[15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
II. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ (C2+C3+E3)[9-11] ⇒ F3[12] ⇒ B1[13] ⇒ (B2[14]+B3[15]) ⇒ A1[16] ⇒ A2[17] ⇒ A3[18]
III. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ (C2+C3+E3)[9-11] ⇒ B1[12] ⇒ F3[13] ⇒ A1[14] ⇒ B2[15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
IV. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ (C2+C3+E3)[9-11] ⇒ B1[12] ⇒ F3[13] ⇒ (B2[14]+B3[15]) ⇒ A1[16] ⇒ A2[17] ⇒ A3[18]
V. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ B1[9] ⇒ (E3+F3)[10-11] ⇒ (C2[12]+C313]) ⇒ A1[14] ⇒ B2[15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
VI. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ B1[9] ⇒ (E3+F3)[10-11] ⇒ (C2[12]+C313]) ⇒ (B2[14]+B3[15]) ⇒ A1[16] ⇒ A2[17] ⇒ A3[18]
VII. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ B1[9] ⇒ (A1+B2+B3+E3)[10-13] ⇒ F3[14] ⇒ C215]) ⇒ C3[16] ⇒ A2[17] ⇒ A3[18]
VIII. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ B1[9] ⇒ (A1+B2+B3+E3)[10-13] ⇒ (A2+C2+C3)[14-16] ⇒ F3[17] ⇒ A3[18]
IX. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ D3[8] ⇒ B1[9] ⇒ (A1+B2+B3+E3)[10-13] ⇒ (A2+C2+C3)[14-16] ⇒ A3[17] ⇒ F3[18]
X. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (D2+E2+F2)[5-7] ⇒ C2[8] ⇒ B1[9] ⇒ A1[10] ⇒ (B2[11]+B3[12]) ⇒ C3[13] ⇒ (D3+E3+F3)[14-16] ⇒ A2[17] ⇒ A3[18]
XI. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ B1[5] ⇒ (B2[6]+B3[7]) ⇒ (C2[8]+C3[9]) ⇒ (A1+D2+E2+F2)[10-13] ⇒ (D3+E3+F3)[14-16] ⇒ A2[17] ⇒ A3[18]
XII. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ B1[5] ⇒ (C2[6]+C3[7]) ⇒ (B2[8]+B3[9]) ⇒ (A1+D2+E2+F2)[10-13] ⇒ (D3+E3+F3)[14-16] ⇒ A2[17] ⇒ A3[18]
XIII. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ C1[4] ⇒ (C2[5]+C3[6]) ⇒ B1[7] ⇒ (B2[8]+B3[9]) ⇒ (B2[8]+B3[9]) ⇒ (A1+D2+E2+F2)[10-13] ⇒ (D3+E3+F3) [14-16] ⇒ A2[17] ⇒ A3[18]
XIV. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ B1[4] ⇒ C1[5] ⇒ (B2[6]+B3[7]) ⇒ (C2[8]+C3[9]) ⇒ (A1+D2+E2+F2)[10-13] ⇒ (D3+E3+F3)[14-16] ⇒ A2[17] ⇒ A3[18]
XV. F1[1] ⇒ E1[2] ⇒ D1[3] ⇒ B1[4] ⇒ C1[5] ⇒ (C2[6]+C3[7]) ⇒ (B2[8]+B3[9]) ⇒ (A1+D2+E2+F2)[10-13] ⇒ (D3+E3+F3)[14-16] ⇒ A2[17] ⇒ A3[18]
XVI. F1[1] ⇒ E1[2] ⇒ (E2+F2)[3-4] ⇒ (C1+C2+D1+D2)[5-8] ⇒ B1[9] ⇒ A1[10] ⇒ (B2[11]+B3[12]) ⇒ C3[13] ⇒ (D3+E3+F3)[14-16] ⇒ A2[17] ⇒ A3[18]
XVII. F1[1] ⇒ F2[2] ⇒ (E1[3]+E2[4]) ⇒ (C1+C2+D1+D2)[5-8] ⇒ B1[9] ⇒ A1[10] ⇒ (B2[11]+B3[12]) ⇒ C3[13] ⇒ (D3+E3+F3)[14-16] ⇒ A2[17] ⇒ A3[18]

Female Sequences (Sequence I is modal)

I. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ (D3+E3+F3)[9-11] ⇒ C3[12] ⇒ B1[13] ⇒ (A1+B2)[14-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
II. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ (D3+E3+F3)[9-11] ⇒ C3[12] ⇒ B1[13] ⇒ (A1+B2)[14-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
III. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ (D3+E3+F3)[9-11] ⇒ B1[12] ⇒ C3[13] ⇒ (A1+B2)[14-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
IV. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ (D3+E3+F3)[9-11] ⇒ B1[12] ⇒ C3[13] ⇒ (A1+B2)[14-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
V. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ C3[9] ⇒ B1[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
VI. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ C3[9] ⇒ B1[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
VII. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ B1[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
VIII. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ B1[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
IX. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ B1[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
X. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ C2[8] ⇒ B1[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
XI. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ B1[8] ⇒ C2[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
XII. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ B1[8] ⇒ C2[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
XIII. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ B1[8] ⇒ C2[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
XIV. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (C1+D2+E2)[5-7] ⇒ B1[8] ⇒ C2[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
XV. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (B1+C1)[5-6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
XVI. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (B1+C1)[5-6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
XVII. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (B1+C1)[5-6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
XVIII. (D1+E1+F1)[1-3] ⇒ F2[4] ⇒ (B1+C1)[5-6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
XIX. (D1+E1+F1)[1-3] ⇒ C1[4] ⇒ (B1)[5] ⇒ (F2)[6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
XX. (D1+E1+F1)[1-3] ⇒ C1[4] ⇒ (B1)[5] ⇒ (F2)[6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ C3[10] ⇒ (A1+B2)[11-12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]
XXI. (D1+E1+F1)[1-3] ⇒ C1[4] ⇒ (B1)[5] ⇒ (F2)[6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ B3[16] ⇒ A2[17] ⇒ A3[18]
XXII. (D1+E1+F1)[1-3] ⇒ C1[4] ⇒ (B1)[5] ⇒ (F2)[6] ⇒ (D2+E2)[7-8] ⇒ C2[9] ⇒ (A1+B2)[10-11] ⇒ C3[12] ⇒ (D3+E3+F3)[13-15] ⇒ A2[16] ⇒ B3[17] ⇒ A3[18]

Characters coded as in Figure 5 and Table 2. The relative sequence position of characters grouped in rounded brackets is not resolved by OSA. Numbers in square brackets document each character's inferred modal sequence position; OSA, Ontogenic Sequence Analysis.

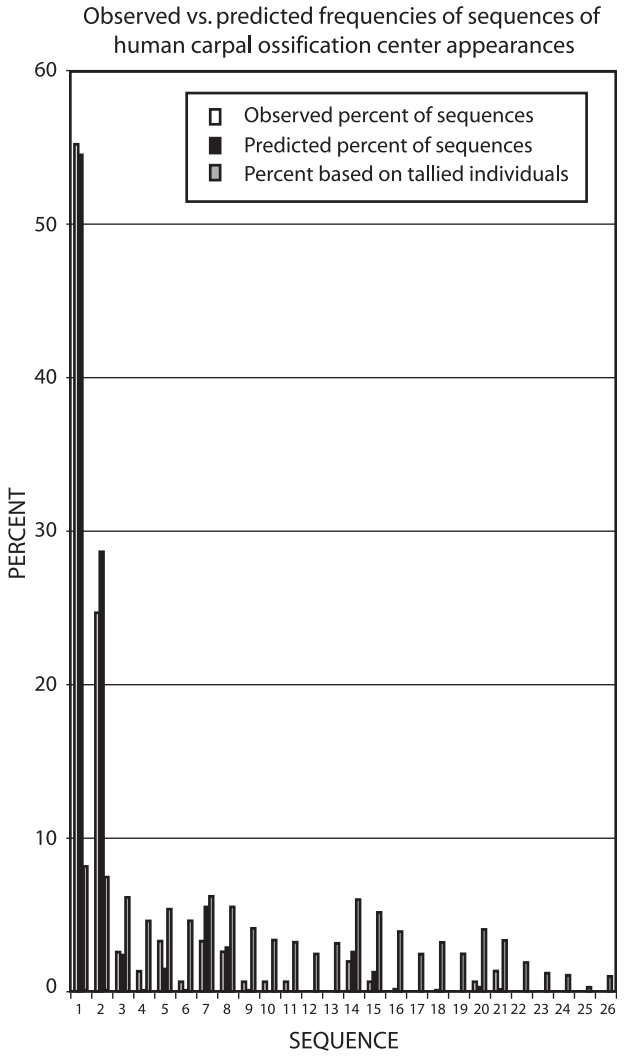

Fig. 6. Observed versus predicted sequence frequencies for sequences of human carpal ossification center appearances. See discussion in text.

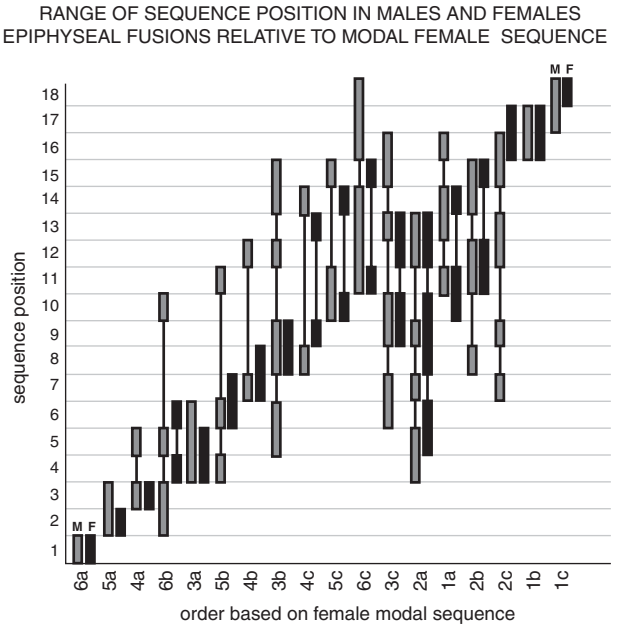

Fig. 7. Predicted range of character-state transformations in male and female hand and wrist epiphyseal unions based on OSA sequence maps. Vertical axis represents maturity score (see discussion in text). Order of characters on horizontal axis based on inferred female modal sequence. Based on cross-sectional observations of Pryor ('25).

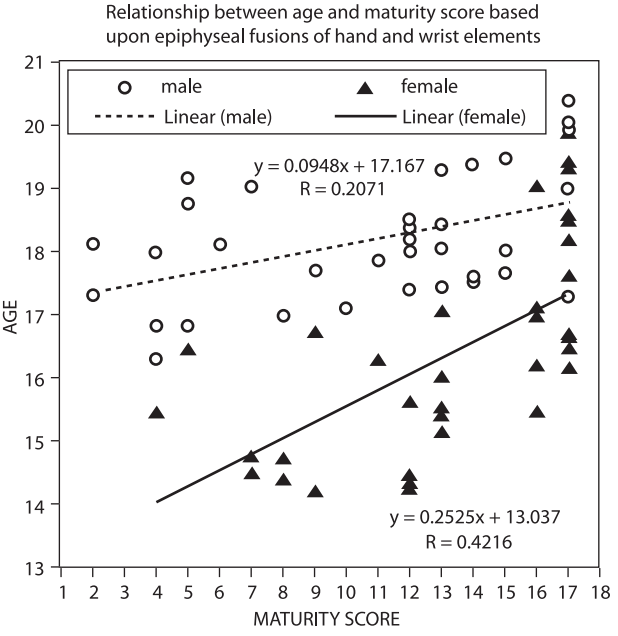

Fig. 8. Human hand and wrist semaphoront maturity score based on epiphyseal closures in hand and wrist elements compared with age of semaphoronts.

ossification of the capitate and hamate and occupy most of the possible sequence pathways (Fig. 9B). The several predicted sequences that were not observed after capitate and hamate ossification likely reflect inadequate sample sizes and undersampling of the actual diversity of semaphoront phenotypes. However, the existence of a strong modal sequence potentially reflects a heritable bias in the production of variant sequences (see Garn et al., '66).

Given Garn et al.'s ('66) data, which calls for six binary coded characters, there are 720 possible sequences (Fig. 9A). Thus, if the sequence order were random, then the probability for sampling any particular sequence is 0.13% (i.e., 1/720). The probability for the distribution of sequences actually observed in this longitudinal data set is exceedingly small (1.397×10^{-742}). If one only considers the series of sequences possible after the ossification of the capitate and the hamate,

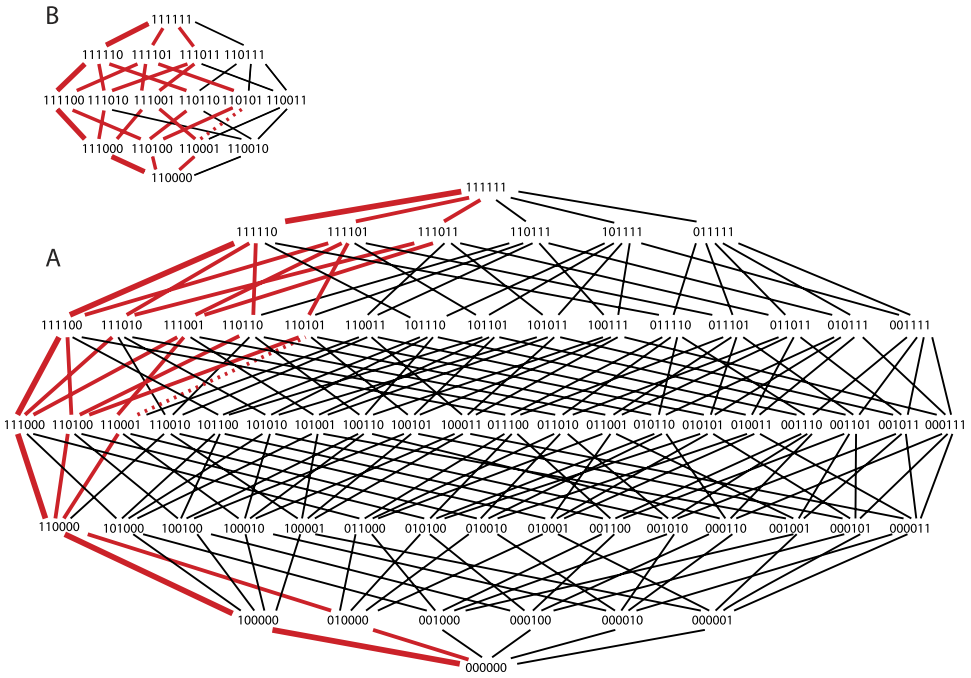

Fig. 9. Map of all possible developmental sequences based on OSA analysis of human carpal ossification center data. (**A**) Map assuming six binary character transformations. (**B**) Map of possibilities assuming earlier ossification of capitate and hamate. In both (A) and (B), the darkest line is the modal sequence; other bold lines represent observed sequences, and the dashed line represents the predicted sequence that was not observed (see Fig. 2). Data were developed from Garn et al. ('66).

then there are 24 possible sequences (Fig. 9B), each having a random probability of 4.17%. Eleven of the 24 possible sequences are observed to occur, whereas OSA predicts 13. Eighty-eight of the 154 sampled individuals (57%) fall on the modal sequence (indicated by the bold line in Figure 9B). Although several hundred orders of magnitude more likely than the distribution based on six binary characters, the random chance of obtaining the observed distribution of sequences is still highly improbable (7.663×10^{-132}), indicating great constraint in the production of variant sequences.

Maturity scores and ontogenetic staging: Although sequence polymorphism is indicated by different phenotypes having identical maturity scores, staging individuals into ''classes'' based on these maturity scores assumes that all character transformations have equal weight. The expression of certain elements during development is much more labile than others (see Garn et al., '66), however, contributing to an increased incidence of sequence polymorphism. For example, in the human wrist, the triquetral is much more variable in its timing of expression than other wrist elements, and its use would potentially obscure age estimates. (Garn et al., '66). Accordingly, they recommend using more stable elements for maturity estimation. Staging based on maturity scores should similarly account for character variability.

This widespread polymorphism limits stable nodes in consensus analyses of recovered trees to be used to formulate stages (Brochu, '96). Attempts to generate consensus nodes based on the data presented here indicate that nodes are not necessarily correlated with a consistent sequence, reducing their theoretical potential as stage arbiters. Alberch ('85), who noted the arbitrariness of circumscribing ontogenetic stages for the entire organism, has also criticized the logic of staging. Although his criticism is generally supported here because of the complications arising from sequence polymorphisms, it is clear that stable stages may be developed for restricted character components (e.g., the dental series, carpal ossification center appearances, etc.) of the entire organism. Indeed, current computer resources are not capable of OSA treatment of the entirety of an organism's development, and it is unlikely that entire organism stages will be available in the foreseeable future.

Sequence characterization and ontogenetic trajectories: Kluge ('88) describes a method of ontogenetic characterization based on Alberch et al.'s ('79) formalization of heterochronic developmental patterns. The method characterizes developmental change as a curve that plots shape changes against either size or age. This curve, or ontogenetic trajectory, is then described as a function of onset of growth, cessation of growth, and growth rate—which are all potentially comparable parameters between taxa (e.g., Creighton and Strauss, '86; see their Fig. 2). Kluge ('88), following Creighton and Strauss ('86), uses multivariate methods for size and shape description, in conjunction with simplifying quantitative growth models (e.g., the generalized negative exponential Bertalanffy growth curve used by Creighton and Strauss, '86), to expand the initial abstract formalizations of Alberch et al. ('79). For example, general size is represented as a ''vector summarizing the joint size increase in all morphometric traits'' (Creighton and Strauss, '86; p 97). Kluge ('88) further asserts that a single such curve, or more reasonably, a series of non-overlapping curves, can then describe ontogenetic changes in shape over an organism's entire life cycle. Heterochronic changes between taxa can be assessed by comparing the descriptive curve parameters (e.g., Creighton and Strauss, '86).

Ontogenetic trajectories are powerful tools for recognizing and formalizing heterochronic evolutionary phenomena; however, they may not adequately represent the event sequence of differentiation. Efforts to map differentiation events onto ontogenetic trajectories (Kluge, '88) can obscure sequence information because of both operational and theoretical considerations.

Operationally, a major obstacle to developing ontogenetic trajectories is the lack of absolute age data for specimens of most animal species. Exceptions typically comprise laboratory animals (most often representing species of medical significance or those that reproduce and grow rapidly), whose growth conditions may not reflect the degree of variation experienced by natural populations (they could prove invaluable, however, in helping to address issues of developmental canalization). This lamentable lack of age data has led to the widespread use of size as a proxy for age. Unfortunately, not only is size variable with respect to age, but maturity is also variable with respect to size and/or age. Ectotherms present special complications in that growth rates, and thus size, may be contingent on environmental temperature as well as age. The error margins resulting from the imperfect correlation of maturity, size, and age prohibit accurate placement of discrete differentiation events onto ontogenetic trajectories and potentially mask sequence information. For example, in the above analysis of epiphyseal union data the earliest complete epiphyseal union of the distal radius of the 61 analyzed male semaphoronts was at 17 years and 4 months of age, whereas a condition of incomplete union was observed as late as 19 years, a significant discrepancy (1 year, 8 months) in the context of human growth patterns. Whether differentiation events are more closely correlated with size or age has not been clearly demonstrated. Indeed, it could be that the timing of certain maturational events is inversely proportional to size. For example, the early fusion of epiphyseal elements could cause early truncation of growth and correspondingly small size. The representation of sequence(s) on an ontogenetic trajectory is thus confounded not only by the lack of strong age and size correlation, but also by the potential that sequences may not correlate well with either.

Regardless of the imprecision of age or size as calibrating standards for particular developmental events, the generation of curves simplifying an ontogenetic trajectory does not provide an explicit graphic representation of sequence polymorphism (i.e., multiple sequences). In essence, the development of a single ontogenetic trajectory for a species relies on calculating normative values for growth and differentiation, which emphasizes median or modal values at the expense of sequence polymorphism.

These difficulties in obtaining and analyzing sequence with ontogenetic trajectories do not diminish their potential to elucidate heterochronic evolutionary patterns. Indeed, ontogenetic trajectories play a vital role in assessing the contribution of age- and size-related heterochronic differences in normative data sets, particularly regarding the issue of rate. It is equally clear, however, that sequence comprises another category of biological variation that may not be adequately represented by normative trajectories. The ability of OSA to clearly characterize sequence, together with the widespread availability of data amenable to OSA methodology (but lacking the requisite specimen age information for formulating ontogenetic trajectories) underscores the utility of this new method.

CONCLUSION

Development can be characterized in a number of ways. Most typically, absolute age is related to shape, size, or weight changes. Sequence represents another aspect of ontogeny related to but not necessarily well correlated with age or size. Most ontogenetic sequence characterizations assume character independence and provide normative data for age-at-appearance (or size or weight proxies for age-at-appearance; Garn et al., '66). These studies generally do not indicate the presence or absence of sequence polymorphism. Fewer studies actually indicate sequence by either longitudinal observation of individual organisms over time (e.g., Garn et al., '66) or by estimation of these sequences from non-longitudinal (cross-sectional) semaphoront samples (e.g., Roth, '84; Brochu, '96). OSA is designed to enable analysis of the most parsimonious distribution of ontogenetic sequences when adequate longitudinal samples are not available.

Kluge ('88) and Alberch ('85) allege that qualitative description of ontogenetic transformations (which they consider continuous) can cause significant distortion and loss of information. In contrast to these opinions, it is clear that the characterization of sequence data, in particular the determination of sequence polymorphisms, is an important element in developmental studies that may have evolutionary implications. Such sequence data may not be adequately captured in ontogenetic characterizations based solely on comparison with age or size. This does not deny the importance of such curve-fitting methods to the characterization of developmental rate, but rather provides a complementary, explicit characterization of sequence. The OSA method of sequence characterization is particularly useful when size or age is not consistent with character maturation.

OSA also indicates independence (or lack of constraint) in ontogenetic sequence data by providing estimates of sequence consistency for both individual characters and suites of characters. If character appearances are not causally related during ontogeny, then their hierarchic consistency can decrease. The relative consistency of different partitions of the data may be useful in refined interpretations of transformation order, allowing highly resolved portions of the data to be discovered. OSA-derived sequences can be compared both intra- and interspecifically and indicate the nature of variation between compared samples. Heterochronic patterns can be formalized, including estimation of variability and constraint in compared samples.

Longitudinal samples that document individual growth and development are not available for most taxa such that ontogenetic pathways must be hypothesized rather than directly observed. Traditional methods for sequence characterization or establishing developmental stages generally do not consider sequence consistency or polymorphism. OSA's use of a parsimony standard introduces rigor into such hypotheses, minimizing the character conflicts indicative of ontogenetic sequence polymorphisms. It is hoped that this tool will prove of value not only for discovering ontogenetic sequences and polymorphisms but also for its potential to establish predictive and comparable hypotheses of sequence in ontogeny.

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