

Variation and Timing of the Cranial Ossification Sequence of the Oriental Fire-Bellied Toad, *Bombina orientalis* (Amphibia, Discoglossidae)

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ABSTRACT The sequence of appearance of the 17 different skull bones in the oriental fire-bellied toad, *Bombina orientalis*, is described. Data are based primarily on samples of ten or 11 laboratory-reared specimens of each of 11 Gosner developmental stages (36-46) representing middle through late metamorphosis. Ossification commences as early as stage 37 (hind limb with all five toes distinct), but the full complement of adult bones is not attained until stage 46 (metamorphosis complete). Number of bones present at intermediate stages is poorly correlated with external morphology. As many as four Gosner developmental stages elapse before a given bone is present in all specimens following the stage at which it may first appear. The modal ossification sequence is frontoparietal, exoccipital, parasphenoid, septomaxilla, premaxilla, vomer, nasal, maxilla, angulosplenic, dentary, squamosal, quadratojugal, pterygoid, prootic, interfrontal, sphenethmoid, and mentomeckelian. Most specimens are consistent with this sequence, despite the poor correlation between cranial ossification and external development as assayed by Gosner stage.

The timing of cranial ossification in *Bombina orientalis* differs in many respects from that described for two other, distantly related anurans, the leopard frog (*Rana pipiens*) and the western toad (*Bufo boreas*). These include the total number and sequence of appearance of bones, and the timing of ossification relative to the development of external morphology. Interspecific variation may reflect differences in the timing of the tissue interactions known to underlie skeletal differentiation and evolution.

Development of a wide variety of vertebrate tissues is characterized by a precise sequence of events or interactions which control differentiation and morphogenesis. This is especially evident in the developing skull, in which carefully timed sequences of interactions underlie the formation of both bony and cartilaginous skeletal elements (Hall, '80, '82). Recent studies suggest that changes in the relative timing of developmental events underlie fundamental aspects of skull evolution in many vertebrate lineages (Alberch, '80; Hall, '84; Hanken, '84). Thus, knowledge of the timing of developmental events is vital to analysis of both the development and the evolution of cranial morphology. It is surprising, therefore, that the normal sequence of cranial ossification—a

basic feature of skull development—is known for relatively few taxa. This is particularly true for anuran amphibians, despite their long use as important research animals of developmental and evolutionary biologists.

We recently initiated experimental studies of the growth and development of the skull of the oriental fire-bellied toad, *Bombina orientalis*, an anuran species that is becoming a favored research animal of many developmental biologists (e.g., Carlson and Ellinger, '80; Ellinger, '78, '80; Ellinger and Carlson, '78; Kawamura et al., '72). An initial analysis involved establishment of the normal sequence of cranial ossification through metamorphosis in laboratory-reared animals. Here we present this information, along with 1) preliminary observations of individual

variation in cranial ossification at several developmental stages as determined by external morphological features, and 2) a limited comparison with other anurans. These data supplement published studies of the timing and sequence of early embryonic development in this species (Michael, '81; Sussman and Betz, '78) and permit detailed comparison with the timing and sequence of skull development in other anurans (references below).

Morphology of the larval chondrocranium in *B. orientalis* has been described previously (Sokol, '75), as has that of the adult skull in two related species, *B. variegata* (Slabbert, '45; Slabbert and Maree, '45) and *B. bombina* (Ramaswami, '42). Readers interested in details of cranial morphology are referred to these papers. Terminology of cranial elements follows that advocated by Trueb ('73, '84). A detailed description of the cranial morphology in *B. orientalis*, and results of experimental analyses of skull growth, will be presented in later papers.

MATERIALS AND METHODS

Cranial ossification was documented in laboratory-reared frogs obtained from crosses between adults of a breeding colony maintained at Dalhousie University. Breeding-colony adults were obtained from a variety of sources, including an animal supplier (Charles Sullivan Inc., Nashville, Tenn.) and a colony maintained by Dr. Mark Ellinger at Southern Illinois University, Carbondale, Ill. Breeding was induced by hormone injection, following the procedures of Ellinger and Carlson ('78).

Tadpoles were reared in shallow plastic trays (40 × 27 × 10 cm) containing dechlorinated tap water, and were maintained at room temperature which varied between 18° and 23°C over the several months of the experiment. They were fed boiled lettuce ad libitum. Approximately one-half of the rearing-tray water was replaced every other day. These rearing conditions were conducive to growth and survival; mortality following hatching and through metamorphosis was less than 5%. Metamorphosis usually was completed within 6 weeks following hatching.

Samples of tadpoles were removed periodically and preserved in 10% neutral-buffered formalin. Following preservation, specimens were measured (snout to posterior end of vent; SVL) and sorted according to the stag-

ing table of Gosner ('60). This table defines a series of 46 stages based on external morphology, including embryonic (1–25), larval (26–45), and postmetamorphic (46) development. Samples of ten or 11 specimens were chosen from each Gosner stage between 36 (developing hind limb with all five toes distinct) and 46 (tail fully resorbed). A typical sample comprised offspring from matings of several adults. These were supplemented by several tadpoles of earlier stages (25–35), and three adult frogs (stage 46) from the breeding colony. Specimens were differentially stained for bone and cartilage as whole mounts following the techniques of Dingerkus and Uhler ('77) and Wassersug ('76), as modified by Hanken and Wassersug ('81), from which observations of cranial ossification were made. A total of 137 specimens were examined.

All specimens were scored for the presence of each of the following bones: 13 intramembranous centers—frontoparietal, parasphenoid, septomaxilla, premaxilla, maxilla, vomer, angulosphenial, dentary, pterygoid, nasal, squamosal, quadratojugal, and interfrontal; and four endochondral centers—prootic, sphenethmoid, mentomeckelian, and exoccipital. The interfrontal was reported in *B. orientalis* and *B. bombina* (Tschugunova, '81), but it has not been observed in other anurans (Trueb, '73, pers. comm.). In adults, the interfrontal comprises a single, median, intramembranous ossification that articulates broadly with the posterior border of the sphenethmoid between the anterior portions of the paired frontoparietals. We observed the bone to form from as many as four irregularly shaped ossification centers that appear in the frontoparietal fontanelle and presumably coalesce to form the single, adult element. Right and left sides were scored separately for paired bones (e.g., nasal). An index of ossification was calculated for each specimen, in which the presence of a given bone (both sides if normally paired) counted as one point, summed over all bones. Presence of a given bone on one side only counted as one-half point; the maximum ossification index value possible was 17. Frequency of each element at a given stage was computed by summing the individual ossification scores for that bone (one point if present on both sides, one-half point if present on one side only) over all animals and dividing this sum by the number of specimens, expressed as a percentage.

RESULTS

General observations

Calcified deposits are present within the otic capsule of all specimens of stage 36 examined, as well as several specimens of earlier stages. All of these specimens, however, lack distinct, ossified cranial elements, and the deposits are absent from more advanced stages. The first detectable cranial ossification is present in tadpoles of stage 37 (Fig. 1A, Table 1). Paired frontoparietals, exoccipitals, and parasphenoids appear first, typically in that order (see below); they are present in all specimens of stage 38 and greater (Fig. 2). This initial burst of appearance of ossification centers then subsides; on average, less than one new bone appears between stages 39 and 41. When present, this bone is the septomaxilla (Fig. 2).

Rate of appearance of ossification centers increases again at stage 42 (protrusion of forelimbs) and is particularly rapid between stages 44 and 46; an average of one or two ossification centers appear between stages 41 and 44 and the remaining 11 bones appear during stages 44–46. Only one of 10 recently metamorphosed specimens (stage 46) exhibits the full complement of adult skull bones (ossification index equals 17). Remaining specimens of this sample lack the prootic, interfrontal, mentomeckelian, and/or the sphenethmoid. All of these specimens were preserved within a few months of having attained stage 46 and had shown modest growth in overall body size during that period (Table 1, Fig. 1B). All three adult specimens possess the full complement of adult skull bones, although in each specimen the mentomeckelian is united synostotically with the dentary.

Two additional ossifications, variably present in other anurans, are absent from all specimens of *B. orientalis*: 1) the palatine, which is also absent from *B. variegata* (Slabbert, '45) and *B. bombina* (Ramaswami, '42), and 2) the columella, which according to Stadtmuller ('31; summarized by Ramaswami, '42) is variably present in the genus *Bombina*.

Variation

Despite the general trend toward increased ossification with increased (Gosner) developmental stage, there is extensive variation in individual ossification at several stages. Indeed, many specimens possess fewer bones than specimens preserved at an earlier stage

(Fig. 1A). Thus developmental stage as aduced from external morphology correlates poorly with that measured by cranial ossification. This is particularly true for stages 42–45. The range of ossification index values for stages 43 and 44 overlap almost completely, and there is substantial overlap between stages 42 and 45. This variation, however, decreases sharply by stage 46, in which all specimens possess at least 13 of the full complement of 17 bones (Fig. 1A).

Variation in ossification index at a given Gosner stage may be traced to variation in the stage at which several bones first appear (Fig. 2, Table 2). Only four bones—sphenethmoid, interfrontal, mentomeckelian, and prootic—attain 100% frequency from 0% in the course of a single stage (Fig. 2). All other

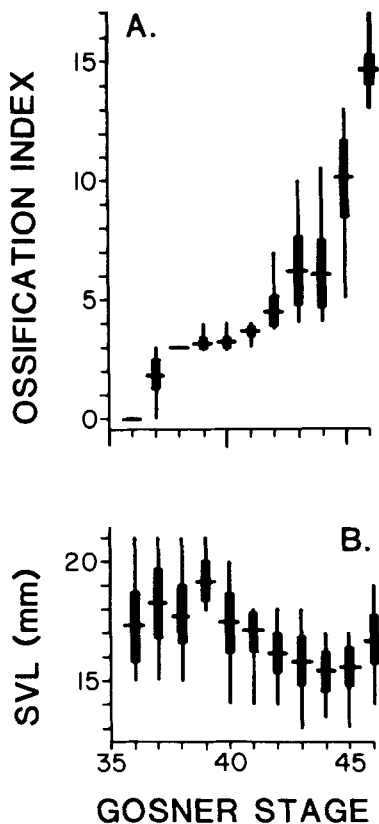


Fig. 1. Cranial ossification (A) and body size (B) in *Bombina orientalis* according to Gosner stage. Horizontal line, sample mean; vertical line, range; solid rectangle, 95% confidence interval. The stage 46 sample includes recently metamorphosed specimens. All three adult specimens have the maximum ossification index value of 17.

TABLE 1. Cranial ossification and body size of *Bombina orientalis* according to Gosner stage

Gosner stage	Ossification index ¹			SVL (mm)		N	
	Mean ± SE	Range	Mean ± SE	Range			
36	0	–	0	17.3	0.7	15–21	10
37	1.9	0.3	0–3	18.3	0.7	15–21	11 ²
38	3.0	–	3	17.8	0.6	15–21	10
39	3.2	0.1	3–4	19.2	0.4	18–21	10
40	3.2	0.1	3–4	17.5	0.6	14–20	10
41	3.7	0.1	3–4	17.1	0.4	14–18	10
42	4.5	0.3	4–7	16.2	0.4	14–18	10
43	6.2	0.7	4–10	15.8	0.5	13–18	10
44	6.1	0.7	4–10.5	15.4	0.4	13.5–17	10
45	10.1	0.8	5–13	15.6	0.4	13–17	10
46	14.8	0.4	13–17	16.7	0.5	14–19	10
(postmetamorphic) ³							
46 (adults)	17	–	17	41.2	1.1	39–43	3

¹Ossification index equals the number of different bones present (paired bones count as 1/2 point for each side).

²N = 10 for SVL.

³Preserved within a few months of attaining stage 46.

TABLE 2. Variation in the timing of cranial osteogenesis in *Bombina orientalis*

Cranial element	Latest Gosner stage with frequency of 0%	First Gosner stage with frequency of 100%	Duration (No. of stages)
Frontoparietal	36	38	2
Exoccipital	36	38	2
Parasphenoid	36	38	2
Septomaxilla	38	42	4
Premaxilla	41	45	4
Vomer	41	46	5
Nasal	41	46	5
Maxilla	42	46	4
Angulosplenic	42	46	4
Squamosal	42	46	4
Dentary	43	46	3
Quadratojugal	44	46	2
Pterygoid	44	46	2
Prootic	45	46 ¹	1
Interfrontal	45	46 ²	1
Sphenethmoid	45	46 ³	1
Mentomeckelian	45	46 ³	1

¹Frequency equals 85% in postmetamorphic specimens preserved within months of reaching stage 46, but equals 100% in all three adult specimens.

²Frequency equals 50% in postmetamorphic specimens preserved within months of reaching stage 46, but equals 100% in all three adult specimens.

³Frequency equals 20% in postmetamorphic specimens preserved within months of reaching stage 46, but equals 100% in all three adult specimens.

bones "require" at least one, and as many as four, additional Gosner stages following that of their earliest appearance before each is present in all specimens (Table 2).

Variation in the timing of ossification relative to external morphology is particularly common to late-appearing bones (e.g., maxilla, angulosplenic, squamosal; see below), but it is not limited to them. For example, the septomaxilla may appear as early as stage 39 but it does not attain a frequency of 100% until stage 42, a span of four Gosner stages.

Ossification sequence

The varied complements of skull bones present in specimens of intermediate ossification index values allow identification of the complete sequence of cranial ossification. No single ossification sequence is followed by all specimens. The distribution of bones in most specimens, though, is consistent with the following sequence of appearance: frontoparietal, exoccipital, parasphenoid, septomaxilla, premaxilla, vomer, nasal, maxilla, angulosplenic, dentary, squamosal, quadratojugal, pterygoid, prootic, interfrontal, sphenethmoid, mentomeckelian.

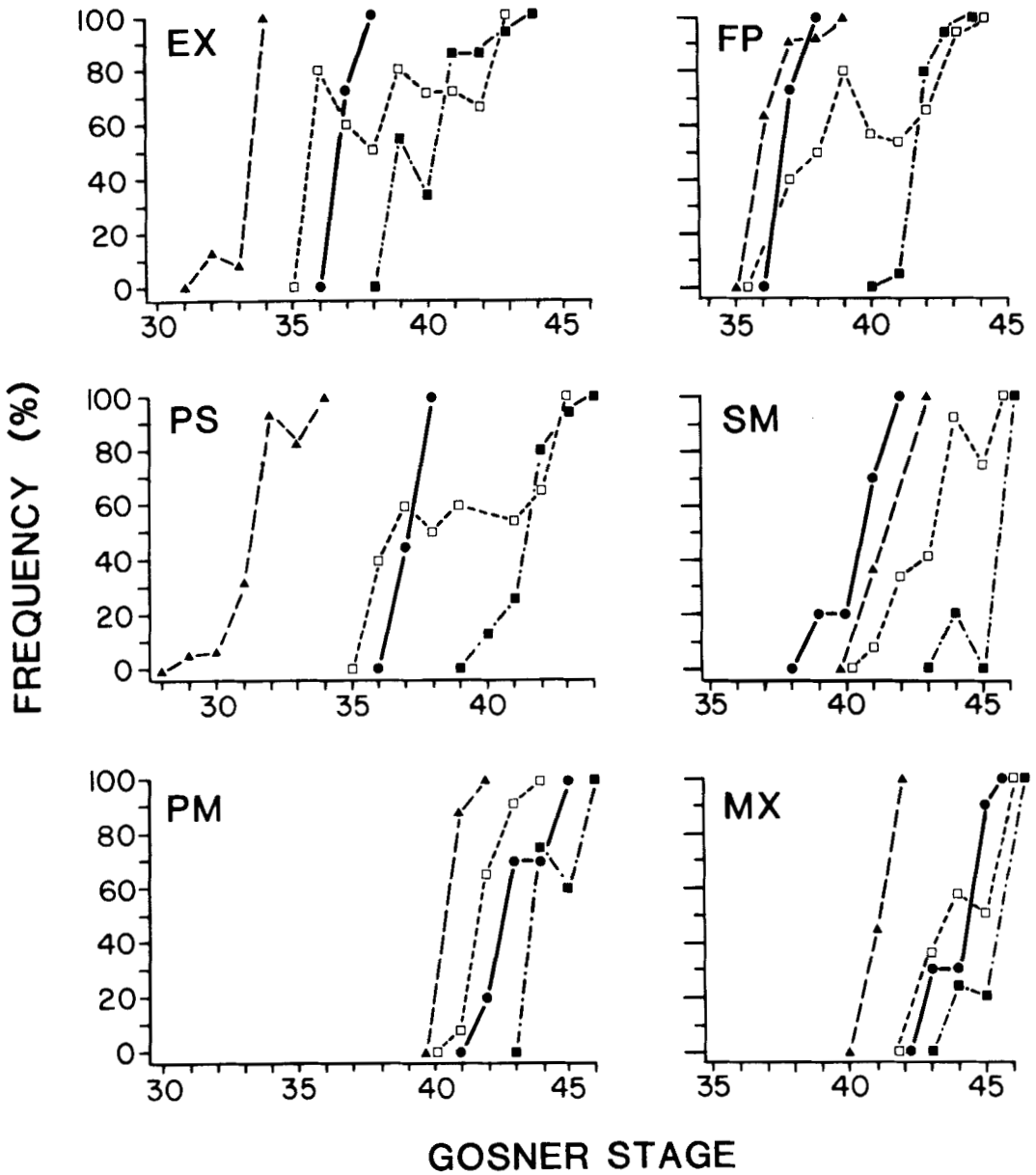
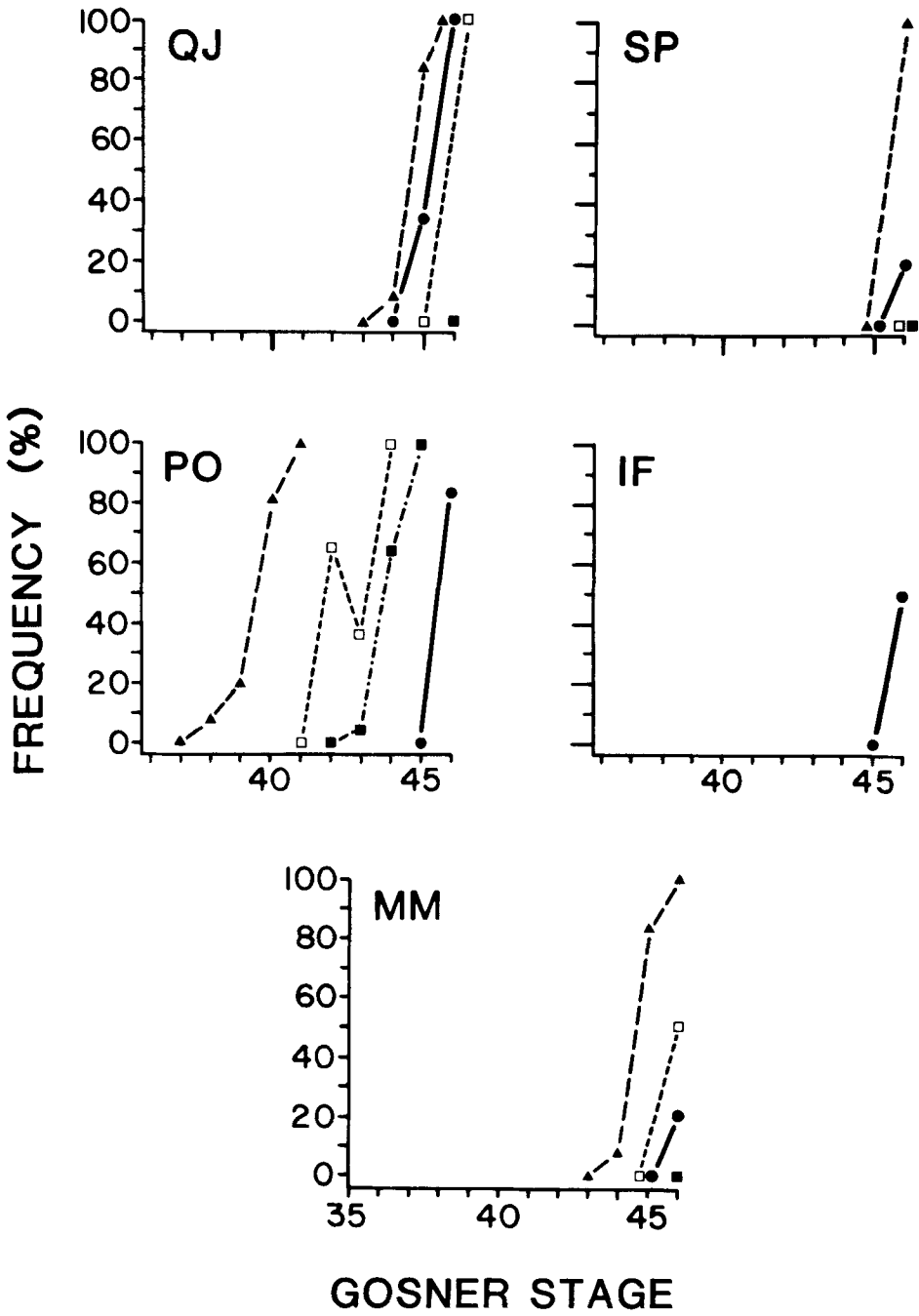


Fig. 2. Frequency of 17 individual skull bones at different Gosner stages in *Bombina orientalis* (●), *Rana pipiens* (▲), and *Bufo boreas* (■, laboratory-reared; □, field-collected). Both *Bufo* and *Rana* lack the interfrontal but have two additional bones—palatine and columella (not depicted)—which are absent from *Bombina*. All bones will attain 100% frequency in adults; stage 46 values less than 100% represent subadults. Data for *Rana* and

Bufo from Kemp and Hoyt ('69) and Gaudin ('73), respectively. Abbreviations: AN, angulosplenic; DN, dentary; EX, exoccipital; FP, frontoparietal; IF, interfrontal; MM, mentomeckelian; MX, maxilla; NA, nasal; PM, premaxilla; PO, prootic; PS, parasphenoid; PT, pterygoid; QJ, quadratojugal; SM, septomaxilla; SP, sphenethmoid; SQ, squamosal; V, vomer. Continued on pages 250 and 251.



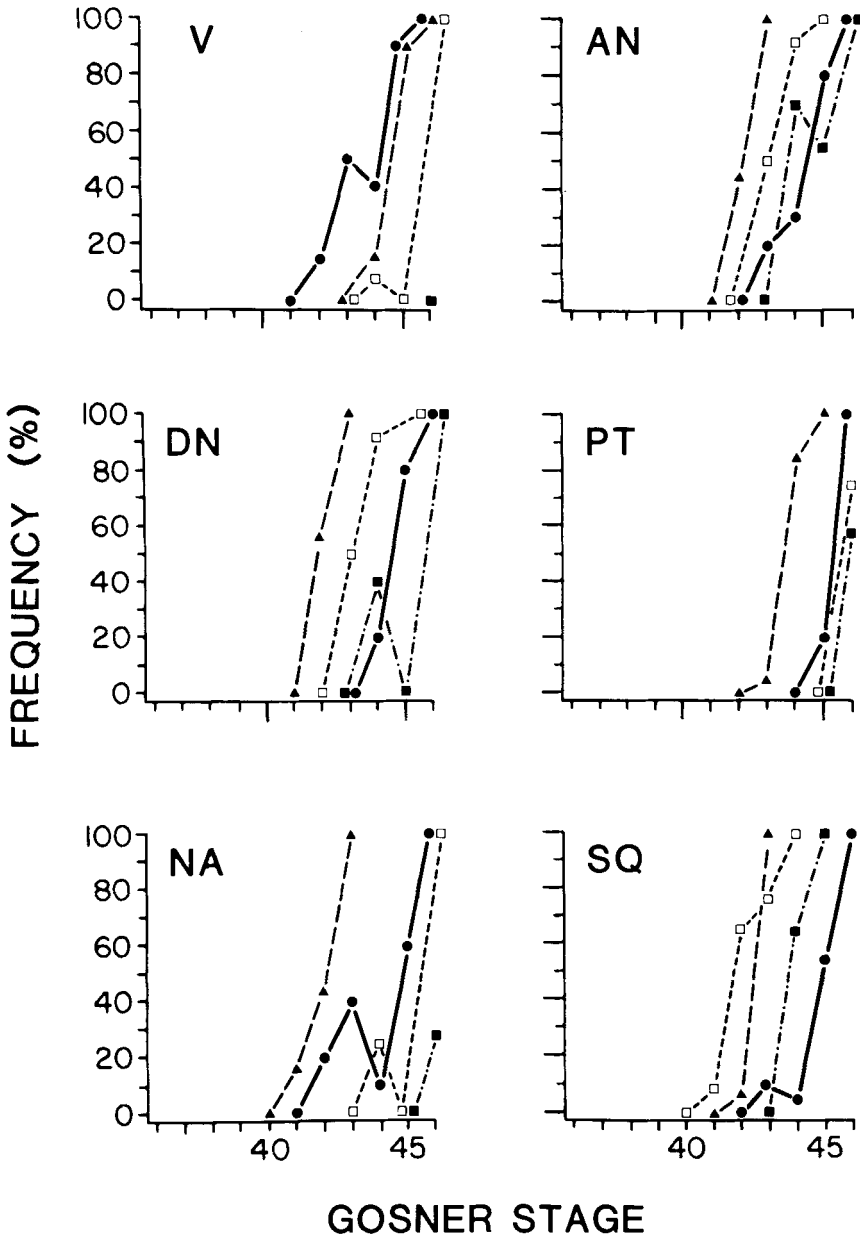


TABLE 3. Earliest appearance of skull bones in *Bombina orientalis*, arranged by Gosner stage

Gosner stage	Bone
37	Frontoparietal Exoccipital Parasphenoid
38	—
39	Septomaxilla
40	—
41	—
42	Premaxilla Vomer Nasal
43	Maxilla Angulosphenial Squamosal ¹
44	Dentary
45	Quadratojugal Pterygoid
46	Prootic Interfrontal Sphenethmoid Mentomeckelian

¹The squamosal was present in only one specimen of stage 43, which lacked either dentary. It typically appeared after the dentary which was first present no earlier than stage 44.

nethmoid, and mentomeckelian. The Gosner stages at which these bones may first appear are listed in Table 3.

The following exceptions to the modal sequence described above account for most of the observed variation in ossification sequence: 1) the parasphenoid is present in a single specimen (stage 37) which lacks both the frontoparietal and exoccipital. 2) Paired maxillae are present in one specimen (stage 43) which lacks either nasal. 3) A single specimen (stage 43) possesses both squamosals but neither dentary. 4) The mentomeckelian is present in one specimen (stage 46) which lacks both the sphenethmoid and interfrontal. Due to 3), the squamosal was first detected one stage earlier than the dentary, which it typically follows (Table 3).

DISCUSSION

Cranial ossification in Bombina orientalis

Cranial ossification in *Bombina orientalis* during metamorphosis is poorly correlated with the development of external morphology. This generalization holds both for the time of appearance of each of several bones (Table 2, Fig. 2) and for the total number of skull bones (i.e., ossification index) in a given specimen (Table 1, Fig. 1). Nevertheless, the sequence of appearance of successive elements is nearly invariant. In view of the extreme variation in ossification phenomena described above, it is in fact surprising that

there are not more exceptions to the modal sequence. Thus cranial ossification per se is well integrated, but it is only loosely correlated with the development of external, non-skeletal components.

Demonstration of a conservative ossification sequence begs the question of the nature of the developmental events that underlie the formation of these skeletal elements. Two alternative explanatory models are plausible. First, the ossification sequence represents a series of essentially independent events—formation of discrete ossification centers. These events typically occur in a prescribed order as a result of intracranial variation in certain developmental parameters, such as the threshold response of osteogenic sites to levels of circulating thyroid hormones. Second, formation of each ossification center is developmentally dependent on a prior ossification and is, in turn, a precondition for the appearance of subsequent centers. Kemp and Hoyt ('65a-c, '69) treated tadpoles of the leopard frog, *Rana pipiens*, with the growth hormone thyroxine, which induced precocious ossification of several skull bones, including the premaxilla, maxilla, and septomaxilla. The prootic, however, which in the leopard frog normally appears prior to any of these elements, failed to appear in thyroxine-treated animals. This evidence seems to support the former, "independent events" model, as opposed to the latter, "epigenetic cascade" model. Occasional exceptions to the normal ossification sequence of *B. orientalis* described in this study lend additional support to this view.

Comparison with other anurans

Cranial ossification sequence data presently are available for 17 species of anurans in addition to *B. orientalis* (see reviews in Gaudin, '73, '78; Trueb, '70, '84; Trueb and Alberch, '84). Few studies, however, provide either the complete ossification sequence or estimates of variation in ossification parameters, thus making detailed phylogenetic comparisons difficult. We will confine ourselves to comparison with two studies which present timing and variation data similar to those we have provided for *B. orientalis*.

Gaudin ('78) described cranial ossification in the western toad, *Bufo boreas* (Bufonidae), as adduced both from field-collected and from laboratory-reared specimens. Kemp and Hoyt ('69) described cranial ossification in laboratory-reared leopard frogs, *Rana pipiens* (Ranidae). Frequencies of individual skull bones

at different Gosner stages in these species are plotted alongside data for *B. orientalis* in Figure 2 (data for field-collected specimens of *Bufo* represent the San Fernando Valley group only). Data as presented in these papers do not allow calculation of ossification index values for individual specimens, but the stage of earliest appearance for each bone in both species is given. From this we calculated a modified ossification index, which equals the maximum number of different bones which may be present at a given stage (Fig. 3A,B). A similar, modified ossification index was calculated from our data for *B. orientalis* to facilitate direct comparisons among these three species in overall degree of cranial ossification at a given stage of external development. Lastly, timing of cranial ossification in these three species is compared in Figure 4 (A,B), which plots the stage of first appearance for each bone in each species.

There are several differences in the pattern of cranial ossification among the three species. Foremost is the adult complement of skull bones. *Bombina* has a maximum of 17 different bones as adults, including one, the interfrontal, that is absent from all other amphibians. Both *Bufo* and *Rana* have 18 different adult skull bones, including two—palatine and columella—commonly found in other frogs but not present in *Bombina*. These two bones are among the last to appear during anuran ontogeny (Trueb and Alberch, '84; Fig. 4). If the larger, adult complement of *Bufo* and *Rana* more closely resembles the primitive, or ancestral, configuration from which all recent anurans are derived, then overall skull development in *Bombina* is truncated precociously relative to that of the other two genera, and the palatine and columella have been lost. Thus, the interfrontal would represent a neomorph, a novel ossification that has evolved since the divergence of the lineages leading to *Bombina* and advanced anurans such as *Rana* and *Bufo*. (Additional examples of neomorphs are cited in Trueb, '70, '73).

Duration of cranial ossification, as measured by the number of stages elapsed between (and including) the earliest appearance of the first bone and the earliest appearance of the final bone, is greatest in *Rana* (18), versus *Bombina* (ten) and *Bufo* (11 and eight for wild-caught and laboratory-reared specimens, respectively; Fig. 3). In all species, the full, adult complement of skull bones is not attained until stage 46. Thus, interspecific

differences in the duration of cranial ossification as defined above mainly represent differences in the onset of cranial osteogenesis. Ossification in *Rana* begins at stage 29, eight stages earlier than in *Bombina* and at least seven stages earlier than in *Bufo*. However, all but one of the adult bones are present in *Rana* at stage 45, whereas at this stage in *Bombina* at least four bones are yet to appear, and in *Bufo* at least six; appearance of the full complement of skull bones likely is attained sooner following metamorphosis in *Rana* than in either of the other two species.

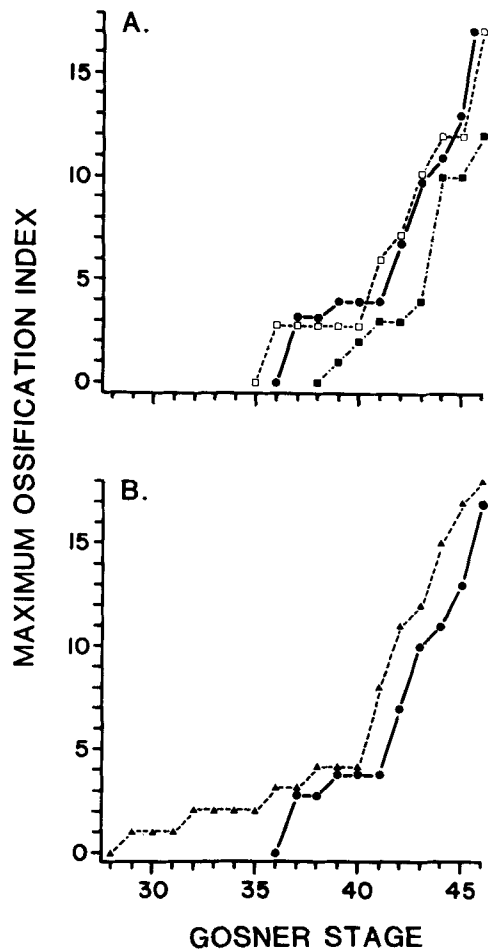


Fig. 3. Cranial ossification as a function of Gosner stage in *Bombina orientalis* compared to *Bufo boreas* (A) and *Rana pipiens* (B). Values equal maximum number of bones present, based on the stage of earliest appearance of each bone. Maximum, adult values equal 17 for *Bombina* and 18 for *Bufo* and *Rana*. Stage 46 values for *Bufo* represent subadults. Symbols and citations as in Figure 2.

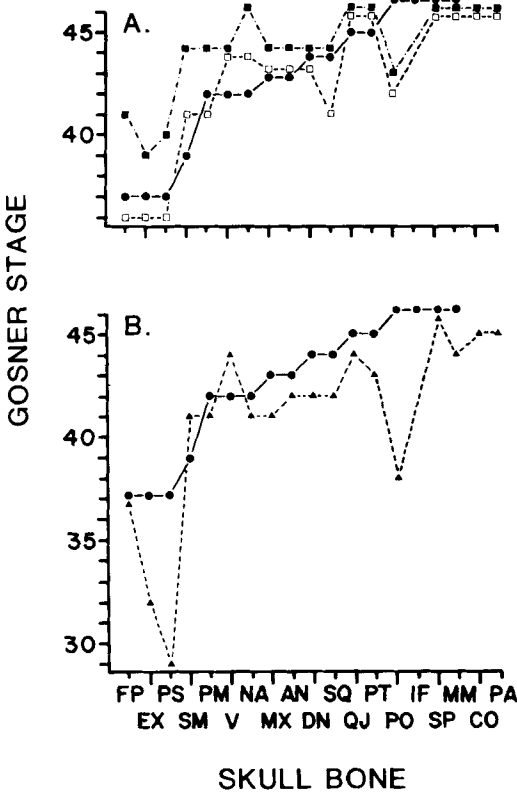


Fig. 4. Cranial ossification sequence of *Bombina orientalis* compared to those of *Bufo boreas* (A) and *Rana pipiens* (B). Sequences are based on the stage of earliest appearance of each bone. Symbols and citations as in Figure 2, also: CO, columella; PA, palatine.

Skull bones consistently appear earlier in field-collected specimens of *Bufo* than in laboratory-reared conspecific specimens (Gaudin, '73). Consequently, these two groups exhibit a significant difference in the timing of cranial ossification overall (Fig. 3A). In fact, differences in timing are so great that the field-collected sample apparently resembles laboratory-reared *Bombina* more closely than laboratory-reared *Bufo*. This is only a superficial resemblance, however, as revealed by detailed examination of the timing and sequence of appearance of individual bones in the two species (see below).

Cranial ossification sequences in the three species, as judged by the earliest appearance of each element, are compared in Figure 4. Bones are arranged in the order in which they appear in *Bombina*, which thus provides a "baseline" to evaluate differences with respect to the timing in *Rana* and *Bufo*

(cf. Fig. 2). In this manner, the precocious initiation of cranial ossification in *Rana* is seen to comprise the early appearance of only two bones—the parasphenoid and, later, the exoccipital. A third bone, the prootic, appears somewhat later when ossification is well under way. Most remaining bones appear only one or sometimes two stages earlier in *Rana* than in *Bombina*, whereas one bone (sphenethmoid) first appears at the same stage, and two bones (septomaxilla, vomer) first appear at a later stage, than in *Bombina*.

The differences between *Bombina* and *Bufo* are less conspicuous than those between *Bombina* and *Rana*. For example, both the vomer and nasal appear before the maxilla and angulosphenial in *Bombina*, but they follow these elements in field-collected *Bufo*. The prootic, which is among the last bones to appear in *Bombina*, precedes 11 bones (including three not present in *Bombina*) in field-collected *Bufo*. These same general features hold for laboratory-reared populations of *Bufo*, although the timing of ossification of most bones with respect to external morphology is retarded in comparison to the field-collected sample.

Based on these comparisons, skull evolution in these groups apparently has involved changes in the timing and sequence of appearance of different bones. However, presence of calcified tissue, the criterion most frequently used to compare the timing of cranial ossification phenomena, is only the culmination of a series of prior inductive tissue interactions relating to skeletal differentiation (Hall, '84). These interactions regulate the formation of cellular, osteogenic condensations which precede calcification, and thus they may determine the onset and rate of skeletal growth. The degree to which differences in the relative timing of cranial ossification among species are correlated with differences in the timing of prior inductive interactions is largely unknown. Information such as this likely will prove invaluable to a greater understanding of the mechanism of skull development and evolution.

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LITERATURE CITED

- Alberch, P. (1980) Ontogenesis and morphological diversification. *Am. Zool.* 20:653-667.
- Carlson, J.T., and M.S. Ellinger (1980) The reproductive biology of *Bombina orientalis*, with notes on care. *Herpetol. Rev.* 11:11-12.
- Dingerkus, G., and L.D. Uhler (1977) Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technol.* 52:229-232.
- Ellinger, M.S. (1978) The cell cycle and transplantation of blastula nuclei in *Bombina orientalis*. *Devel. Biol.* 65:81-89.
- Ellinger, M.S. (1980) Genetic and experimental studies on a pigment mutation, pale (P^a), in the frog, *Bombina orientalis*. *J. Embryol. Exp. Morphol.* 56:125-137.
- Ellinger, M.S., and J.T. Carlson (1978) Nuclear transplantation in *Bombina orientalis* and utilization of the pale mutation as a nuclear marker. *J. Exp. Zool.* 205:353-360.
- Gaudin, A.J. (1973) The development of the skull in the Pacific tree frog, *Hyla regilla*. *Herpetologica* 29:205-218.
- Gaudin, A.J. (1978) The sequence of cranial ossification in the California toad, *Bufo boreas* (Amphibia, Anura, Bufonidae). *J. Herpetol.* 12:309-318.
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190.
- Hall, B.K. (1980) Tissue interactions and the initiation of osteogenesis and chondrogenesis in the neural crest-derived mandibular skeleton of the embryonic mouse as seen in isolated murine tissues and in recombinations of murine and avian tissues. *J. Embryol. Exp. Morphol.* 58:251-264.
- Hall, B.K. (1982) The role of tissue interactions in the growth of bone. In A.D. Dixon and B.G. Sarnat (eds): *Factors and Mechanisms Influencing Bone Growth*. New York: Alan R. Liss, pp. 205-215.
- Hall, B.K. (1984) Developmental processes underlying heterochrony as an evolutionary mechanism. *Can. J. Zool.* 62:1-6.
- Hanken, J. (1984) Miniaturization and its effects on cranial morphology in plethodontid salamanders, genus *Thorius* (Amphibia: Plethodontidae). I. Osteological variation. *Biol. J. Linn. Soc.* In press.
- Hanken, J., and R.J. Wassersug (1981) The visible skeleton. *Funct. Photo.* 16(4):22-26, 44.
- Kawamura, T., M. Nishioka, and H. Ueda (1972) Reproduction of the Oriental fire-bellied toad, *Bombina orientalis*, with special reference to the superiority of this species as a laboratory animal. *Sci. Rep. Lab. Amphib. Biol. Hiroshima Univ.* 1:303-317.
- Kemp, N.E., and J.A. Hoyt (1965a) Influence of thyroxine on ossification of the parasphenoid bone in the skull of *Rana pipiens*. *Am. Zool.* 5:710.
- Kemp, N.E., and J.A. Hoyt (1965b) Influence of thyroxine on order of ossification of bones of the skull of *Rana pipiens*. *Am. Zool.* 5:719.
- Kemp, N.E., and J.A. Hoyt (1965c) Influence of thyroxine on ossification of the femur in *Rana pipiens*. *J. Cell. Biol.* 27:51A.
- Kemp, N.E., and J.A. Hoyt (1969) Sequence of ossification in the skeleton of growing and metamorphosing tadpoles of *Rana pipiens*. *J. Morphol.* 129:415-444.
- Michael, P. (1981) A normal table of early development in *Bombina orientalis* (Boulenger), in relation to rearing temperature. *Dev. Growth Differ.* 23:149-155.
- Ramaswami, L.S. (1942) The discoglossid skull. *Proc. Indian Acad. Sci.* 16B:10-24.
- Slabbert, G.K. (1945) Contributions to the cranial morphology of the European anuran *Bombina variegata* (Linne). *Ann. Univ. Stellenbosch* 23A:67-89.
- Slabbert, G.K., and W.A. Maree (1945) The cranial morphology of the Discoglossidae and its bearing upon the phylogeny of the primitive anura. *Ann. Univ. Stellenbosch* 23A:91-97.
- Sokol, O.M. (1975) The phylogeny of anuran larvae: A new look. *Copeia* 1975:1-24.
- Stadtmüller, F. (1931) Über eine Cartilago pararticularis am Kopfskelet von *Bombinator* und die schmalhausensche Theorie zum Problem der Gehörknöchelchen. *Z. Anat. Entwickl.* 94:792.
- Sussman, P., and T.W. Betz (1978) Embryonic stages: Morphology, timing, and variance in the toad *Bombina orientalis*. *Can. J. Zool.* 56:1540-1545.
- Trueb, L. (1970) Evolutionary relationships of casque-headed tree frogs with co-ossified skulls (family Hylidae). *Univ. Kansas Publ. Mus. Nat. Hist.* 18:547-716.
- Trueb, L. (1973) Bones, frogs, and evolution, in J.L. Vial (ed): *Evolutionary Biology of the Anurans*, Columbia: Missouri, pp. 65-132.
- Trueb, L. (1984) A summary of osteocranial development in anurans with notes on the sequence of cranial ossification in *Rhinophrynus dorsalis* (Anura: Pipidae: Rhinophrynidae). *Proc. Fifth Symp. African Anurans*. In press.
- Trueb, L., and P. Alberch (1984) Miniaturization and the anuran skull: case study of heterochrony, in H.R. Duncker and G. Fleischer (eds): *International Symposium on Vertebrate Morphology*. In press.
- Tschugunova, T.J. (1981) Interfrontalia in *Bombina orientalis* (Blgr.) and *Bombina bombina* (L.). In L.J. Borokin (ed): *Herpetological Investigations in Siberia and the Far East*, Moscow: Acad. Sci. USSR, Zool. Inst., pp. 117-121.
- Wassersug, R.J. (1976) A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol.* 51:131-134.