

## **Determining the onset of ossification and reconstructing ontogeny in vertebrates: A comparison of clearing and staining, histological and computed tomography methods.**

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Analyses of skeletal development that focus on comparisons among a variety of taxa can illuminate the evolution of ontogenies within particular clades. Many recent analyses, particularly of mammals, comprise data about the onset of ossification derived from cleared and stained specimens, histological sections, or computed tomography scanning (CT). It has been noted anecdotally that histological sections may show the appearance of ossification earlier in absolute age than cleared and stained specimens, but comparisons to CT datasets have not been previously made. My study assesses whether systematic biases exist among these three methods using the development of the skull in the marsupial *Monodelphis domestica*. A collection of 80 specimens of known age spanning birth to Day 24, with each day represented by 2-4 specimens, was used to make comparisons among the three methods throughout skeletal development. To account for underlying variation in the onset of ossification among individuals of the same age, 10 cleared and stained specimens were also CT scanned so that comparisons between techniques could be made using the same individual. The onset of ossification was generally observable on the same day using all three methods; however, with CT and histological methods the onset of ossification was often apparent days earlier than with clearing and staining. This disagreement was particularly obvious with small elements. In addition to methodological differences, I also found considerable variation in the estimated ossification sequence when only one method was used. Future comparative studies of ontogeny must consider how methodological variation may potentially skew results if ontogenies are not based on comparable datasets, especially in light of real variation in developmental sequence.

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