

### Variability of Mandibular Form: Variance, Fluctuating Asymmetry and Integration of Deer Mouse Jaws

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One of the central aims of evolutionary developmental biology is to explain the variability of complex morphologies. Variability refers to the ability to vary and is an outcome of interactions between developmental and evolutionary processes. The mandible has emerged as a favored model system for studies of variability because this bone comprises multiple developmental units of both structure and process. It is particularly useful for dissecting the relationship between embryonic and variational modularity, which often differ. To explore the structure of variability we examine variation among individuals, fluctuating asymmetry (FA) and integration among parts in second generation deer mice (*Peromyscus maniculatus bairdii*) reared in the laboratory. We measured mandibles using landmarks plus semilandmarks that capture the curving shape of mandibular processes and alveolar regions. The dominant component of variation spans the entire mandible, relating changes in orientation of the condylar and angular processes to those of the incisor alveolus. Similarly the major component of variation in FA spans jaw processes and incisor alveolus. The spatial patterning of among individual variation and FA are significantly similar, although patterns of covariation are weakly correlated. Not surprisingly, variation shows a higher level of integration than does FA, but not in the alveolar region, where the two are equal in degree of integration. Our preliminary results thus indicate a complex hierarchical structure of variability, with highly integrated dimensions that span the entire jaw and other highly localized, weakly canalized regions. Both patterns are evident in among-individual variation and FA.

### Reproductive Patterns in Amniotes with Special Reference to Mammals

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The different extinction and diversification of marsupial and placental mammals at the K/T-boundary can possibly be explained by the differences in their strategies of reproduction evolved in the late Mesozoic. Marsupial neonates are born after a short gestation with immature lungs, which allow only low metabolic abilities in the first weeks. In contrast, the placental neonates have well developed lungs, allowing a high metabolic rate and thermoregulatory capacity early in postnatal development. Hence, placental young are more resistant against environmental stress which means an evolutionary advantage under harsh conditions, e.g., at the K/T-boundary. Because neonates are the result of a fetomaternal metabolism, it is essential to understand which differences between the placental and marsupial type of placentation correspond to the developmental degrees of the neonates. The marsupial morphotype includes a superficial and diffuse placenta formed by the yolk sac. Until shell coat rupture the embryo depends on histiotrophic nutrition by uterine secretion. In late gestation the placenta invades the uterine mucosa in many taxa and hemotrophes become the main source of nutrients. In placentals, however, the stem species had a well developed compact, chorionic-lantoic, endotheliochorial, definitive placenta with labyrinthine fetomaternal interdigitation (Carter and Mess, 2006, pers. obs.). This type of placentation permits a more intimate contact between fetal and maternal blood system and an effective, hemotrophic nourishment of the embryo throughout pregnancy, which might be accountable for the advanced developmental stage of placental neonates.

### Histone Deacetylase Inhibitor, Trichostatin A, Affects Gene Expression Patterns During Morphogenesis of Chick Limb Buds *in vivo*

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Epigenetic controlling and chromatin remodelling are involved in embryonic genesis and tumorigenesis. Acetylation is one of the key modifications to control gene transcription. HDACs inhibitor are considered to

be among the most promising targets in drug development for cancer therapy. However, the basic aspects of what kinds of genes are more sensitive to the modification of acetylation *in vivo* are not fully understood. Spatiotemporal expression patterns of developmental genes are implicated in embryonic development. Taking chicken embryonic limb as an experimental model, we have investigated the reaction of a batch of genes in the limbs treated with Trichostatin A (TSA), a histone deacetylase (HDAC)-inhibitor. The results show that TSA (75  $\mu$ M) changes the expression levels of the genes, which have important functions during limb development. Among them, *BMP4*, *SF/HGF* and *Twist1* were up-regulated; *BMP2*, *FGF8*, *Shh*, *Scleraxis*, *Myf5* and *MyoD* were down-regulated. In contrast to that, the *Pax3*, *Paraxis*, *Mxx1*, *CREB*, and *PCNA* were still expressed at the same levels as controls. Increasing the concentration of TSA (>750  $\mu$ M) can induce apoptosis and embryonic limb malformations. Our results indicate that chicken limb development can serve as a convenient *in vivo* model for studying the effect of HDAC inhibitors on gene expression. It may be useful for improving our understanding of the role of chromatin remodelling and epigenetic control of gene expression patterns and developing the drugs against cancer.

### Sequence Heterochronies in Lissamphibia

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Variations in life history traits of lissamphibians is often correlated with modifications of developmental sequences. The larvae of anuran amphibians have undergone major adaptive changes (e.g., in feeding mode) in relation to many aspects of their life history. We analyzed the timing of cranial muscle development in larval lissamphibians from a comparative, phylogenetic perspective to investigate the role of sequence heterochrony in lissamphibian evolution. Three outgroup taxa (*Neoceratodus forsteri* and two salamanders) and seven ingroup taxa were investigated. The developmental timing data was analyzed quantitatively using the Parsimov technique. Parsimov is an event-pair and parsimov based method that can be used to interpret developmental timing data in a phylogenetic framework, and to establish the minimum number of heterochronic events. Our results corroborated previous observations that cranial muscles generally tend to develop from anterior to posterior. Especially the development of eye muscles is characterized by an unexpectedly high degree of heterochrony. Tentative results indicate that anlagen of the eye muscles in anurans develop simultaneously with the anlagen of the mandibular and hyoid arch muscles. However, interesting differences in the timing of eye muscle development occur within the pipidae. In *Xenopus laevis* these muscle anlagen develop earlier and in the closely related *Hymenochirus boettgeri* they develop later than the anlagen of the mandibular and hyoid arch muscles. In general, our results show more heterochronic events than expected, also in cranial muscles other than eye muscles.

### On the Unique Deep Plantar Tendons Arrangement in the Foot of Piciformes (Aves): Its Possible Origin and Evolutionary Implications

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The monophyly of Piciformes sensu Wetmore (1960) has often been questioned. A strong argument for monophyly of the Piciformes is a unique arrangement of the deep plantar tendons of m. flexor digitorum longus (FDL) and m. f. hallucis longus (FHL) which occurs in Galbulidae, Bucconidae, Capitonidae, Indicatoridae, Picidae. In this arrangement the tendon of the second muscle supplies digits I, II and IV, while that of the first supplies only digit III. Scenario of the origin of such an important character of piciforms has not been revealed prior to our studies. We show that the best supported hypothesis is a derivation of an arrangement in question from an ancestral one, confined to the pool of ancient birds, ancestors of trogons, coraciiforms and piciforms. The unique piciform arrangement of deep plantar tendons is only partially explained by the acquisition of zygodactyly. It bears ancestral prezygodactyl features and cannot be functionally explained by climbing adaptations.

### Characterization of Cow Long Bone Tissue

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