Limb development in vertebrates

- The developing limb has long been a pioneering model for understanding pattern formation: the process in which the spatial organization of differentiated cells and tissues is generated in the embryo.
- Pattern formation can be considered as a two-step process; first cells are informed of their position and, thus, acquire a positional value (specification); cells then remember and interpret this value to form the appropriate structures (differentiation) (Wolpert, 1969).

- The three main axes of the vertebrate limb are:
  - the proximodistal (PD), running in the human arm from shoulder to digits;
  - the anteroposterior (AP), from thumb to the little finger; and
  - dorsoventral (DV), from the back of the hand to the palm.

- Much of the classical work on vertebrate limb development has been carried out in chicken embryos because the developing wing and leg are easy to access.
- More recently, mice have emerged as powerful models in which to study limb patterning, owing to the ability to manipulate gene function in a spatially and temporally regulated manner in the limb (Logan et al., 2002).

- The main stages of chick wing and mouse forelimb development are similar, and it has been usual to extrapolate findings between these models (Martin, 1990; Fernandez-Teran et al., 2006); however, there are some differences as shown in the figure:

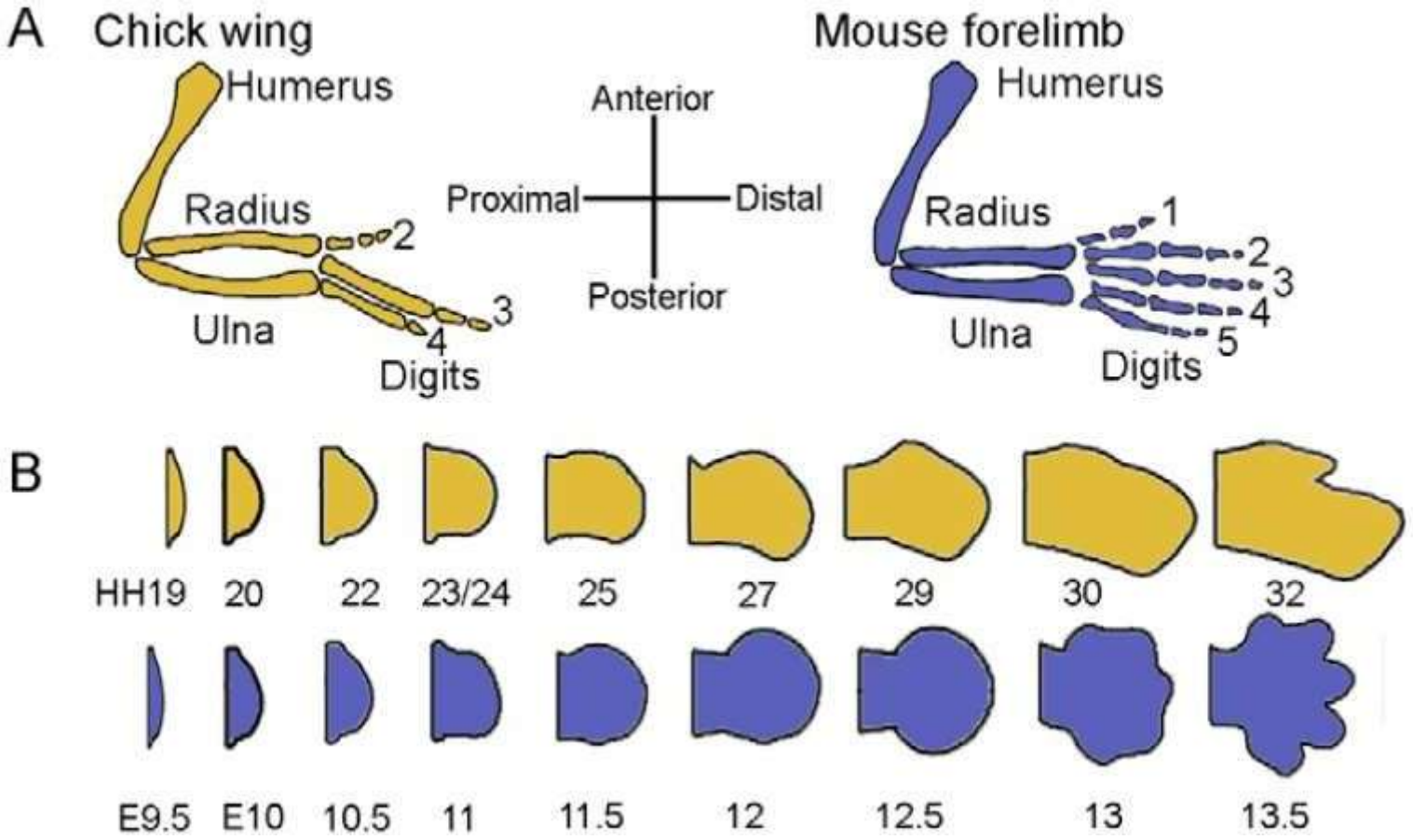

Fig. Chick wing and mouse forelimb development. (**A**) A schematic of fully developed chick wing (yellow) and mouse forelimb (blue) skeletons with anteroposterior (AP) and proximodistal (PD) axes shown (as applied to all elements except the humerus). (**B**) Schematics of equivalently staged chick wing (Hamburger-Hamilton stages, HH) and mouse forelimb buds (embryonic day, E), from early stages to hand plate development. Note, mouse hindlimb development is delayed by about half a day relative to the forelimb (Martin, 1990; Fernandez-Teran et al., 2006).

- The chick wing and the mouse forelimb skeleton have the typical vertebrate plan with three main regions along the PD axis, humerus, radius/ulna and digits together with a variable number of wrist elements (not shown). In the chick wing, there are only three digits across the AP axis, rather than five digits, as in the mouse forelimb.

- The first visible signs of limb development are small bulges, called limb buds, which grow out of either side of the body wall at appropriate levels.
- The early bud consists of a meshwork of apparently homogeneous undifferentiated mesenchymal cells covered with ectoderm.
- Chick wing buds have a translucent rim due to the thickened ectoderm known as the apical ectodermal ridge (AER).

- This thickened AER is required for bud outgrowth, and develops about a day later in the mouse forelimb.
- As the bud elongates, the mouse limb forms a relatively broader hand plate than the chick wing, and cells near the body wall begin to differentiate into various specialised tissues, while cells at the bud tip remain undifferentiated.
- It takes 7 days after wing buds first appear (about 5 days in the mouse forelimb) for the complete skeleton to been laid down, with the humerus forming first and the digits last.

- Detailed cell-marking experiments in chick wing buds have shown that, in addition to the pronounced outgrowth that occurs along the PD axis, there is also considerable expansion of the posterior region of the bud across the AP axis.
- Thus, the posterior-distal region of the early wing bud forms the digits, whereas the anterior-distal half contributes to more proximal structures.
- In the chick wing, there is also non-uniform expansion of the AER, with the posterior part expanding more than the anterior part.

- Fate-mapping of the mouse forelimb bud also shows that the posterior part contributes more to digit development than does the anterior part.
- These localised differences in chick and mouse limb bud expansion cannot readily be related to cellular behaviour because most cells are proliferating. There are, however, indications that cell cycle times may be slower in the anterior region of the chick wing than in the posterior region, thus potentially contributing to differential expansion.
- Apoptosis is not thought to influence overall limb bud growth in either mouse or chick, to any large extent, and, where present, is concentrated in restricted areas.

- In the early chick wing bud, cell death occurs in the anterior and posterior necrotic zones, and might be associated with the relatively narrow hand plate of the chick wing compared with the mouseforelimb.
- In mouse forelimb buds, there is also a region of cell death at the anterior margin but no posterior necrotic zone.