Molecular development of fibular reduction in birds and its evolution from dinosaurs

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Pharmacological inhibition of Ihh pathway. (A) Late application of Cyclopamine disrupts Ihh signaling during endochondral ossification, as confirmed by the compared expression of one of its downstream targets, Ptc1, between control and experimental embryos; (B) Application of Cyclopamine at HH29 does not avoid distal fibular reduction in spite of reduced length of tibia and fibula.
Supplementary Figure 2:

The fibula is as large as the tibia at early development in species with relatively short fibulae: Larger fibulae at early development of zebra finches and budgerigars indicate their reduction is ensuing at later stages.
The fibulare develops closer to the distal fibula in birds than in other extant reptiles. (A) Bearded dragon (*Pogona vitticeps*) leg cartilages showing the fibula and the fibulare develop well separated; (B) Zeugopod development in other extant reptilia indicates that the proximity of the fibulare to the distal fibula at early development is a derived condition of birds (Drawings based on Müller and Alberch 1990; Rieppel 1993c; Rieppel 1993a; Rieppel 1993b; Rieppel 1994; Fabrezi et al. 2007).
Supplementary Figure 4:

**Fibular reduction in species with different adult fibulo-tibial ratios.** In all species, essentially adult fibulo-tibial proportions have already been attained around stage HH36.
Fibular reduction depends on time and rate of differentiation. In species with lower fibulo-tibial ratios (duck, zebrafinch, budgerigar), ossification begins first in the tibia, and then in the fibula. Scale 300 µm.
Supplementary Figure 6:

Post-morphogenetic Cyclopamine treatment causes the fusion of the fibulare to the fibula (A) Whole mount immunofluorescence showing the fusion of the fibulare to the distal fibula 24hr after Cyclopamine treatment (see figure 1B for comparison); (B) Whole mount double immunofluorescence shows that the fused fibula-fibulare has a molecular profile like that of a single element, with IHH expression at the center, while COL-II is expressed in the proximal epiphysis and the distal fibulare (white arrowheads indicate the fusion of fibulare to the fibula).
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