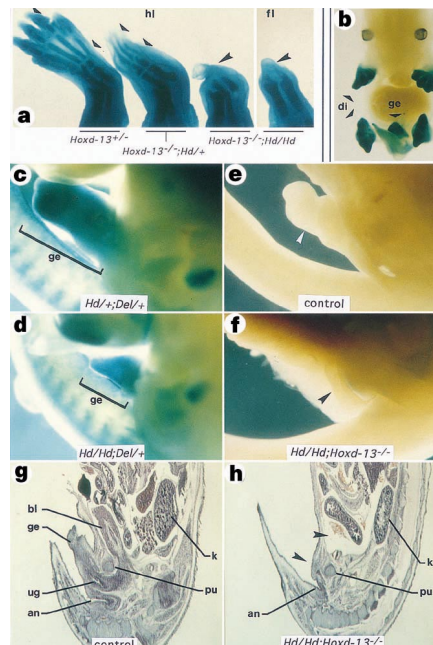# Of fingers, toes and penises

Vertebrate *Hox* genes are essential for limb development. The posterior-most *Hoxd* and *Hoxa* genes are required for growth and patterning of digits and are also strongly expressed in the genital bud, which gives rise to the urogenital system, including the penis. Here, we show that removal of posterior *Hox* gene function results in a concomitant loss of digits and genital bud-derivatives, illustrating that similar developmental mechanisms are at work in these different buds.

We raised compound mutant mice carrying the *hypodactyly* (*Hd*) allele[1] and null alleles of the *HoxD* complex inactivating either *Hoxd-13* (ref. 2), or *Hoxd-11*, *Hoxd-12* and *Hoxd-13* in *cis* configuration $HoxD^{Del}$ (ref. 3). *Hd* mice have a deletion in *Hoxa-13* (ref. 1), so these combinations removed most *Hox* gene function in the digits. The effect was comparable to that observed when using a *Hoxa-13* loss-of-function allele[4]: a total digit agenesis (Fig. 1a). Double-mutant limbs were truncated right after the zeugopods (Fig. 1a; large arrowheads) with only traces of proximal tarsal condensations (Fig. 1a).

Expression of *Hoxd* was visualized using a *lacZ* reporter gene inserted either in *Hoxd-13* (Fig. 1b) or at the $HoxD^{Del}$ locus[3]. β-gal staining revealed a sizeable blue eminence in control *Hd*/+;*Del*/+ mice (Fig. 1c) which showed considerable digit development. But a second *Hd* allele (*Hd/Hd*; *Del*/+ mice) induced a severe truncation of the eminence (Fig. 1d). Removing group-13 function (*Hd/Hd*;*Hoxd-13*$^{-/-}$) led to complete agenesis of the bud (Fig. 1e, f). This progressive reduction in genital bud size with reductions in *Hox* gene dose was accompanied by proportional reductions in digit size (compare Fig. 1a with c, d). Mild anatomical defects in these two structures also occur in the human hand-foot-genital syndrome which involves a mutation within *Hoxa-13* (ref. 5).

Histological analyses of *Hd/Hd*; *Hoxd-13*$^{-/-}$ fetuses confirmed the complete absence of external genitalia (Fig. 1g, h). In addition, the lower urinary system, particularly the bladder and urethra, were missing, as well as derivatives of the urogenital sinus. Kidneys, which do not express *Hoxd-13* (ref. 6), were present in compound mutant animals. *Hd/Hd*;*Hoxd-13*$^{-/-}$ fetuses died *in utero* before embryonic day 16 like most *Hd/Hd* mice[1].

The development of the distal limbs and genital eminence share important features. Both structures are regions of apical growth and involve epithelial–mesenchymal interactions. Further, digits and genitalia represent 'morphogenetic ends' of the body, digits

at the distal end of the limbs and genitalia at the trunk posterior. Molecular data support this analogy, as most genes expressed during digit development are also functional in the genital eminence. *Hox* genes are particularly relevant as 5′-located *Hoxa* and *Hoxd* genes are involved in organizing the terminal parts of axial structures[5–7].

There is other evidence to suggest a phylogenetic link between digits and external genitalia. In fish, which lack digits and external genitalia, posterior *Hox* genes are expressed in areas from which these structures emerged (the cloacal region and paired fins). Also, *Hoxd* gene expression in developing murine digits and genital eminence is controlled by the same transcriptional enhancer element[8–10]. Finally, *Hoxc-13* and *Hoxb-13,* the other group-13 paralogues, are expressed neither in digits nor in the genital bud[11,12].

During evolution, the acquisition of the distal *Hoxd* expression domain in paired fin buds may have been linked to the emergence of digits by extending the terminal phase of proliferation[13]. Related phylogenetic histories between digits and the penis/clitoris thus seem plausible and may reflect combined adaptive or preadaptive solutions to the transition towards a terrestrial environment. Palaeontological observations do not exclude the possibility that ancestral tetrapods had protruding external genitalia. As such animals were aquatic[14], a genital organ may not have been linked to terrestrial life but instead might have reflected the requirement for elaborate internal fertilization.

Digits and an ancestral genital organ could have emerged from the same genetic

Figure 1 Absence of digits and genitalia in mice lacking posterior *Hox* function. **a,** Dissected hindlimbs (hl) and forelimbs (fl) from control mice and mice lacking most or all group-13 function. Small arrowheads, digits; large arrowheads, absence of digit. **b,** Expression of *Hoxd-13/lacZ* reporter gene in digits (di) and genital eminence (ge) of a 12.5-day-old fetus (E12.5). **c,** Expression of *Hoxd/lacZ* reporter in E13.5 developing genital eminence of fetuses with a phenotype close to normal (*Hd*/+;*Del*/+). **d,** Removal of one dose of *Hoxa-13* (*Hd/Hd*;*Del*/+) led to truncation of the genital eminence. Left hindlimbs were removed to view the eminence. Other blue domains are sites of posterior *HoxD* locus expression. **e,** Control E15.5 fetus with genital eminence (arrow). **f,** Same-aged fetus in absence of group-13 function. **g,h,** Mid- to parasaggital sections through fetuses shown in **e** and **f,** respectively. ug, urogenital sinus; an, anus; k, kidneys; pu, pubis; bl, bladder. In the absence of group-13 function, the eminence is absent as well as the bladder (arrows). The rectum and anus are also abnormal (arrow).

regulatory innovation. It is nevertheless equally possible that the underlying molecular mechanisms were first deployed in forming an external genital organ and subsequently used to produce digits, or vice versa. In any event, the combined improvement of apical growth mechanisms along these axes produced the adaptative potentials necessary for efficient locomotion and internal fertilization in the terrestrial environment.

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