Evolutionary biology

Lamprey *Hox* genes and the evolution of jaws

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In vertebrates with jaws (gnathostomes), the jaws are formed from the first pharyngeal arch (PA1), which does not express homeobox (*Hox*) genes. Cohn[1] describes expression of the *HoxL6* gene in the PA1 of the lamprey *Lampetra fluviatilis*, a jawless (agnathan) vertebrate, and postulates that a retreat of *Hox* expression from PA1 might have favoured the evolution of jaws in the gnathostome lineage after the split from agnathans[1]. Here we examine the distribution of *Hox* genes in another lamprey species, *Lethenteron japonicum*, and find that none are expressed in the PA1. We conclude that Cohn's finding is not a general feature within the lamprey group and is therefore unlikely to be related to jawlessness.

In gnathostome embryos, *Hox* genes display rostral boundaries of expression that are collinear with their relative chromosomal positions[2,3]. Nested collinear *Hox* expression patterns display sharp anterior boundaries that map to distinct hindbrain segments (rhombomeres) and pharyngeal arches that give rise to the neural-crest-derived skeletal structures in the head and neck region[4]. Notable exceptions are the first rhombomere (r1) and the jaw-forming first pharyngeal arch (PA1), which do not express *Hox* genes. Analysis of *Hox* expression patterns in the jawless lamprey may therefore provide insight into how the gnathostome *Hox* code has been established and its relevance to the evolution of craniofacial structures in vertebrates.

We isolated 11 *Hox* complementary DNAs from embryos of the Japanese lamprey *L. japonicum (Lj)*. Alignment of the deduced amino-acid sequences of isolated cDNAs, as well as phylogenetic comparisons, revealed that they belong to paralogue groups (PG) 2 to 9, designated *LjHox2, -3d, -4x, -4w, -5i, -5w, -6w, -6/7m, -8p, -Q8* and *-9r*, respectively (GenBank accession numbers: AY497314, AB125269–AB125278; Fig. 1a). On the basis of comparison of the nucleotide sequences, *LjHox4x, LjHox4w, LjHox5w, LjHox6w* and *LjHoxQ8* are the orthologues of *Hox4x, Hox4w, Hox5w, Hox6w* and *HoxQ8*, respectively, in *Petromyzon marinus*[5–7], a lamprey species belonging to a different genus. *P. marinus* has three or four *Hox* clusters[5–7]. Our results from *L. japonicum* are in keeping with this, as we never isolated more than three *Hox* genes from any single cognate group.

All the *LjHox* genes are strongly expressed at the pharyngula stage. In the neural tube, *LjHox* PG2–8 genes display offset rostral expression borders, moving from anterior to posterior (Fig. 2a–i). The rostral boundary of *LjHox2* expression is in the rhombomere r2 (results not shown), which is similar to that occurring in gnathostomes, where *Hoxa2* is the only *Hox* gene expressed in r2 (refs 4, 8). The anterior border of *LjHox3d* expression occurs in the middle of r4 (ref. 9). More posteriorly, *LjHox4w, LjHox6w* and *LjHoxQ8*, which may be linked in the same cluster[6,7], display clear collinear expression patterns (Fig. 2d, f, i). This suggests that collinear *Hox* expression in the embryonic central nervous system represents an ancestral character for vertebrates. It is notable that partial *Hox* collinearity is already present in the neural tube of protochordates[10].

By contrast, in the pharyngeal arches only *LjHox2* and *LjHox3d* display conserved collinear expression patterns in the neural-crest-derived ectomesenchyme (Fig. 2j–m). As in gnathostomes, the rostral expression boundaries of *LjHox2* and *LjHox3d* are present in PA2 and PA3, respectively. *LjHox4x* is instead expressed at low levels, with no clear anterior boundary (Fig. 2c,n), whereas *LjHox4w* and *Hox* PG5–8 are not expressed in the pharyngeal arch ectomesenchyme (Fig. 2d–i), despite their collinear patterns in the neural tube. In the pharyngeal endoderm, *Hox* PG2 and PG3 genes are not expressed, whereas *LjHox4w, -5i, -6w* and *-Q8* are all co-expressed in the most caudal pouch (Fig. 2d–f, i–k, o).

None of the *LjHox* genes was expressed in the PA1 at any of the stages that we analysed (Fig. 2, and data not shown), in contrast to the findings of Cohn in *Lampetra fluviatilis*[1]. In gnathostomes, the ectopic expression of *Hoxa2* in the *Hox*-free PA1 suppresses jaw development, whereas *Hoxa2* inactivation results in the transformation of PA2 into jaw-related structures[11–13]. The expression pattern of *LjHox2* is strictly conserved, which is consistent with lamprey PA1 and PA2 being patterned by molecular mechanisms that are similar to those in gnathostomes[14]. In agreement with this, *Fgf8*, which is expressed

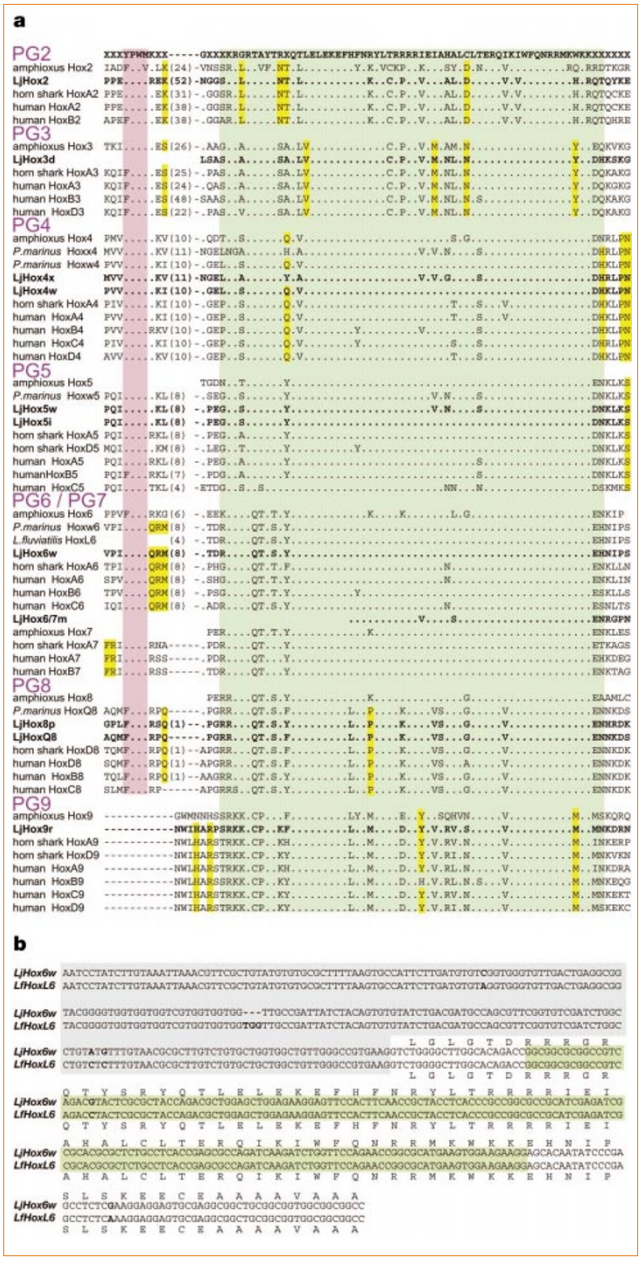

Figure 1 Identification of *Lethenteron japonicum (Lj) Hox* genes. **a,** Alignment of deduced amino-acid sequences of *LjHox* genes with those of other chordates and the assignment of paralogue groups (PGs) are shown. Homeodomains are indicated in green and the YPWM motif, which is unique to *Hox* PG1-8 genes, in pink. *LjHox* sequences are shown in bold. Amino acids characteristic of each paralogue group are in yellow. **b,** Alignment of *LjHox6w* and *Lampetra fluviatilis (Lf) HoxL6* nucleotide sequences with their deduced amino-acid sequences. Intronic regions are shaded; homeodomains are in green. Substituted nucleotide sites between the two sequences are in bold. Note that the intronic regions have accumulated a few changes and that the only two substitutions in the exonic region are synonymous.

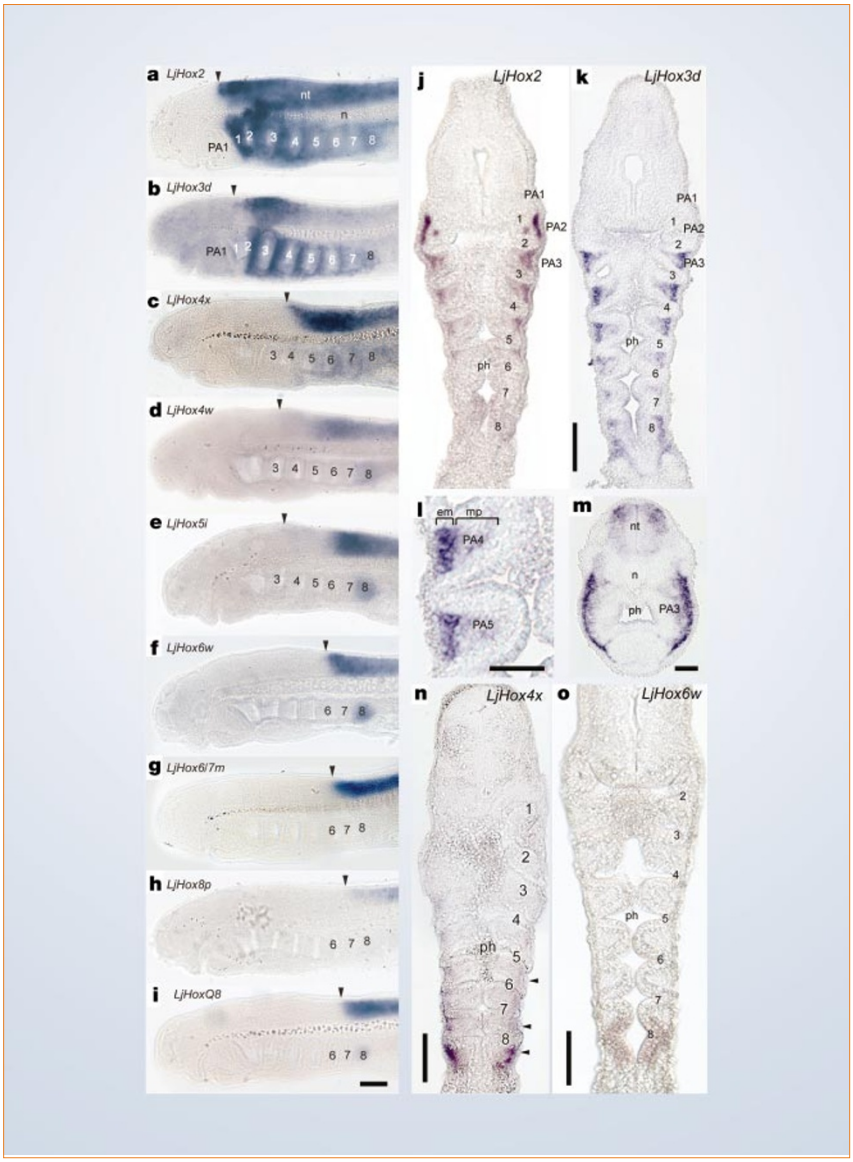

Figure 2 Expression patterns of *Hox* genes in *Lethenteron japonicum (Lj)* as revealed by *in situ* hybridization and whole-mount immunostaining. **a–i,** Expression of *LjHox* genes in stage-26 larvae. Arrowheads indicate rostral expression boundaries. **j, k,** Expression of *LjHox2* (**j**) and *LjHox3d* (**k**) in horizontal sections of stage-25 embryos. **l,** High-magnification micrograph of pharyngeal arches (PAs). Positively staining cells correspond to the neural-crest-derived ectomesenchyme (em). **m,** *LjHox3d*-positive neural crest cells populate PA3. **n, o,** Expression of *LjHox4x* (**n**) in the posterior pharyngeal ectomesenchyme (arrowheads) and *LjHox6w* (**o**) in the endodermal pharyngeal pouch 8. Key: mp, muscle plate; n, notochord; nt, neural tube; ph, pharynx; 1–8, pharyngeal pouches. Scale bars: a-i, 0.1 mm; j,k, 0.1 mm; l,m, 0.05 mm; n,o, 0.1 mm. Full methodological details are available from the authors.

at the mid–hindbrain boundary in gnathostomes and prevents *Hox* expression in PA1 (ref. 15), is also expressed at the lamprey mid–hindbrain boundary[14]. Moreover, the nucleotide sequences of *LjHox6w* and *HoxL6* (ref. 1) display a high degree of homology, indicating that these genes are orthologous (Fig. 1b).

The discrepancy between Cohn's analysis and ours remains unclear. One possibility is that it might be due to genus- or species-specific regulatory differences in lamprey *Hox6* expression patterns. In any event, the *Hox6* expression in PA1 observed in *L. fluviatilis* does not appear to be a general feature within the lamprey group and is therefore not functionally relevant to jawlessness. Rather, the lack of *Hox* expression in the lamprey PA1 may reflect the fact that in both lampreys and gnathostomes the rostral-most pharyngeal arch forms highly specialized structures that are morphologically distinct from those of more posterior arches.

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