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A natural deletion of the *HoxC* cluster in elasmobranch fishes*

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Abstract

Hox proteins are a metazoan-specific family of transcription factors that are required for developmental patterning. The genomic arrangement of *Hox* genes into four paralogous clusters is a primitive feature of jawed vertebrates. Using high-throughput sequencing, we demonstrate the absence of all *HoxC* transcripts from embryos of the shark, *Scyliorhinus canicula*, and the skate, *Leucoraja erinacea*, and the absence of all *HoxC* genes and two *HoxC*-associated miRNAs from the genome of *L. erinacea*. These data suggest a loss of the entire *HoxC* cluster in elasmobranch fishes, and represent the evidence for natural deletion of an entire *Hox* cluster in vertebrates.

Jawed vertebrates typically possess four *Hox* clusters with up to fourteen genes each that arose from an ancestral cluster by genome duplication events. An additional round of whole genome duplication in teleost fishes permitted the *Hox* genes to diversify both structurally and functionally – teleosts possess seven or more clusters, with variable loss or retention of *Hox* paralogs. The retention of the four *Hox* clusters appears to be required for viability since deletion of all copies of *HoxA, B, C*, or *D* clusters has not been reported in vertebrates (1).

Developmental expression profiling in the three lineages of chondrichthyan fishes (Fig. 1) – holocephalans (*Callorhinchus milii*), batoids (*Leucoraja erinacea*), and sharks (*Scyliorhinus canicula*) – revealed expression of all forty-five members of the *HoxA–D* clusters in *C. milii* (average contig length (ACL) = 1214 +/− 565 (SD) base pairs (bp), average coverage (AC) = 25.9 +/− 18.5), but expression of only thirty-four *Hox* genes encoded by the *HoxA, B*, and D clusters in *L. erinacea* and *S. canicula* (ACL = 1294 +/− 578 bp and 1236 +/− 710 bp, AC = 25.1 +/− 15.1 and 21.0 +/− 21.5, respectively, Table S1). Expression of the eleven *HoxC* genes was undetectable in *S. canicula* (consistent with (2)) or *L. erinacea* ($p = 2.60 \times 10^{-7}$).

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Fig. S1
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To distinguish between genomic deletion and transcriptional silencing of the *HoxC* cluster in elasmobranchs (batoids + sharks), we analyzed a 26x coverage assembly of the *L. erinacea* genome. Contigs encoding thirty-four skate *HoxA, B,* and *D* genes were identified (ACL = 5179 +/− 2850 bp, AC = 25.3 +/− 2.8), but no contig or singleton sequence encoding any of the eleven members of the *HoxC* complex was detectable ($p = 6.51 \times 10^{-7}$). Vertebrate *Hox* clusters also encode the *miR-10* and *miR-196* microRNA families (3). The *C. milii Hox* clusters encode three *miR-10* and three *miR-196* genes, with *miR-10c* and *miR-196a-2* mapping within the *HoxC* cluster (Figs. 1 and S1). The *L. erinacea* genome encodes two members each of the *miR-10* and *miR-196* families (Fig. S1; ACL = 3008 +/− 1778 bp, AC = 20.5 +/− 1.0). Pre-miRNA sequences for *miR-10c* and *miR-196a-2* were undetectable ($p = 6.51 \times 10^{-7}$). Similarly, of the non-coding sequence elements conserved between orthologous *C. milii* and human *HoxA, B,* and *D* clusters (4), 51% of the 249 elements were identified in the *L. erinacea* genome, but none of the 25 elements from within the *HoxC* cluster were detected ($p = 1.01 \times 10^{-7}$, Table S2).

Our data suggest that the lack of *HoxC* gene expression during *L. erinacea* and *S. canicula* development is attributable to a genomic deletion of the entire *HoxC* cluster in these taxa (Fig. 1). The most likely scenario is that the entire *HoxC* cluster was lost in a single genomic reduction event following the divergence of holocephalans and elasmobranchs, but prior to the divergence of batoids and sharks (Fig. 1); however, regulated chromosomal diminution cannot be excluded based on embryonic genomic DNA sequence (5). Homozygous mice lacking the *HoxC* complex exhibit only minor transformations of axial identity, but die perinatally due to pulmonary defects (6). The unique dispensability of the *HoxC* cluster for body plan development may have enabled elasmobranchs to survive the challenge of a genome reduction abrogating all *HoxC* cluster function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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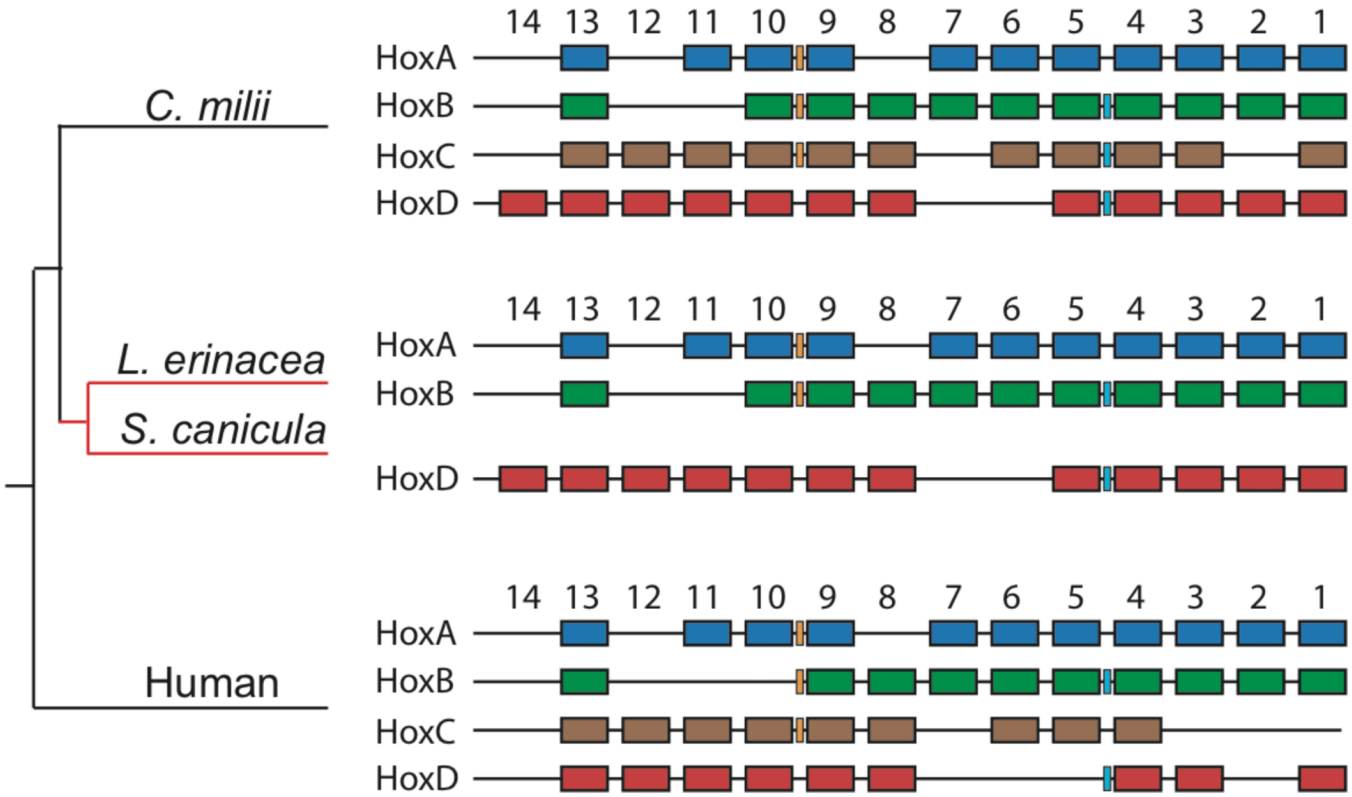

Figure 1.

Genomic deletion of the *HoxC* cluster in elasmobranchs. Jawed vertebrates typically possess four *Hox* clusters (*HoxA–D*, shown in blue, green, brown, and red, respectively), each encoding up to fourteen *Hox* transcription factors and microRNAs belonging to the *miR-10* (light blue) and *miR-196* (orange) families. Genomic and transcriptomic sequencing reveals the skate (*L. erinacea*) and shark (*S. canicula*) retain a full complement of *HoxA*, *B*, and *D* genes, but lack *Hox* genes and miRNAs encoded by the *HoxC* cluster. Loss of the *HoxC* cluster (denoted by red lines) occurred along the elasmobranch stem.