LETTERS

Changes in *Hox* genes' structure and function during the evolution of the squamate body plan

Nicolas Di-Poï[1], Juan I. Montoya-Burgos[1], Hilary Miller[2], Olivier Pourquié[3,4]†, Michel C. Milinkovitch[1] & Denis Duboule[1,5]

***Hox* genes are central to the specification of structures along the anterior–posterior body axis[1,2], and modifications in their expression have paralleled the emergence of diversity in vertebrate body plans[3,4]. Here we describe the genomic organization of *Hox* clusters in different reptiles and show that squamates have accumulated unusually large numbers of transposable elements at these loci[5], reflecting extensive genomic rearrangements of coding and non-coding regulatory regions. Comparative expression analyses between two species showing different axial skeletons, the corn snake and the whiptail lizard, revealed major alterations in *Hox13* and *Hox10* expression features during snake somitogenesis, in line with the expansion of both caudal and thoracic regions. Variations in both protein sequences and regulatory modalities of posterior *Hox* genes suggest how this genetic system has dealt with its intrinsic collinear constraint to accompany the substantial morphological radiation observed in this group.**

In many animal species, *Hox* genes are clustered, and their expression domains, in both time and space, reflect their respective genomic order[1]. Although this genetic system has been used as a paradigm in the study of the evolution of body plans[6], recent studies have highlighted an unexpected diversity in *Hox* gene number, genomic organization and expression patterns[7,8]. In tetrapods, these genes are classified into 13 groups of paralogy and are tightly clustered at four loci: *HoxA* to *HoxD*. A clear correspondence between particular *Hox* groups and defined morphological boundaries along the antero-posterior axis has been documented, either by comparing expression profiles between various vertebrates or by genetic experiments in the mouse[1,3,4,9].

Vertebrate species have highly variable number of vertebrae, ranging from fewer than ten to several hundreds[10–12], a parameter that is probably dependent on the speed of the segmentation clock relative to axial growth, as proposed for snakes[13]. Within reptiles, squamates (that is, lizards and snakes) have a large realm of morphologies, suggesting that *Hox* genes were modified, either in their structure or in their regulation. Previous expression analyses in snakes showed an expansion of anterior *Hox* gene expression along the body axis, in parallel with body plan elongation[9], and revealed that collinearity was fully respected[14]. However, these studies involved selected genes, in the absence of genomic information. Here we describe how structural and regulatory adaptations in this gene family may have accompanied the transition towards such a body plan and suggest that the unexpected invasion of all Squamata *Hox* clusters by transposons might have facilitated such adaptations.

We characterized the genomic organization of posterior *Hox* loci in the corn snake (*Pantherophis guttatus*) and other reptiles, including the turtle, tuatara and several lizards, with a particular focus on repeated elements that are generally excluded from these loci in tetrapods but are abundantly present in the green anole lizard[5] (*Anolis carolinensis*). We sequenced the posterior *HoxA* (from *Hoxa13* to *Hoxa10*), *HoxC* (*Hoxc13* to *Hoxc12*) and *HoxD* (*Evx2* to *Hoxd10*) clusters in the corn snake (Fig. 1 and Supplementary Fig. 1), as well as the posterior *HoxD* cluster in other reptiles including the gecko (*Gekko ulikovski*) and the slow-worm (*Anguis fragilis*). Two non-Squamata reptile species, the turtle (*Trachemys scripta*) and the tuatara (*Sphenodon punctatus*), were used as outgroups (Fig. 1). We annotated both coding and non-coding regulatory sequences and compared them with *Hox* clusters from birds, mammals and amphibians (Supplementary Fig. 2).

A rather generic distribution of *Hox* genes was found, except in the corn snake, which lacks *Hoxd12*; this is similar to *Xenopus* (Fig. 1 and Supplementary Fig. 2a). However, we scored important modifications in conserved non-coding elements (CNEs) located at the posteriormost part of both *HoxA* and *HoxD* clusters, with the loss in all squamates (but not in non-Squamata reptiles or in other amniotes) of region XII[15] located between *Evx2* and *Hoxd13*, and the loss in corn snakes only of both region XI[16], between *Hoxd13* and *Hoxd12*, and another CNE between *Hoxa13* and *Hoxa11* (Fig. 1 and Supplementary Figs 1 and 2). As for the loss of *Hoxd12*, CNEs lost in the corn snake are also absent from *Xenopus Hox* clusters (Fig. 1 and Supplementary Fig. 2).

Although vertebrate *Hox* clusters, including those of turtle and tuatara, are relatively similar in size, all Squamata clusters were found to be substantially larger, mostly as a result of increased intronic and intergenic sizes (Fig. 1 and Supplementary Fig. 1). Such increases in length were correlated with the accumulation of transposable and other interspersed repeats, with the green anole showing the highest number. The predominant type of interspersed repeats found in anole *Hox* clusters consists of Penelope-like retrotransposons (PLEs)[5], whereas repeats in other Squamata species are more degenerated and include short (SINE) and long (LINE) non-LTR retrotransposons, as well as DNA transposons (Fig. 1 and Supplementary Fig. 1). This atypical structure for *Hox* gene clusters suggests that a strong constraint was lost within this order of animals, permitting repeats to invade loci that are otherwise resistant. In turn, such repeats may have impacted significantly on both the rearrangement of coding and non-coding regulatory *Hox* regions and the direct regulation of *Hox* gene transcription in Squamata, for example through epigenetic modifications around their insertion sites[17].

In amniotes, *Hox* expression boundaries along the rostro-caudal axis shift together with the displacement of morphological transitions between vertebral types[3,4]. We examined whether this correspondence was respected in snakes, despite the large expansion of

[1]National Research Center 'Frontiers in Genetics', Department of Zoology and Animal Biology, University of Geneva, Sciences III, 1211 Geneva 4, Switzerland. [2]School of Biological Sciences, Victoria University of Wellington, Wellington 6140, New Zealand. [3]Howard Hughes Medical Institute, [4]Stowers Institute for Medical Research, Kansas City, Missouri 64110, USA. [5]School of Life Sciences, Federal Institute of Technology (EPFL), 1015 Lausanne, Switzerland. †Present address: Institute de Génétique et de Biologie Moléculaire et Cellulaire, CNRS (UMR 7104), Inserm U964, Université de Strasbourg, Illkirch, F-67400, France.

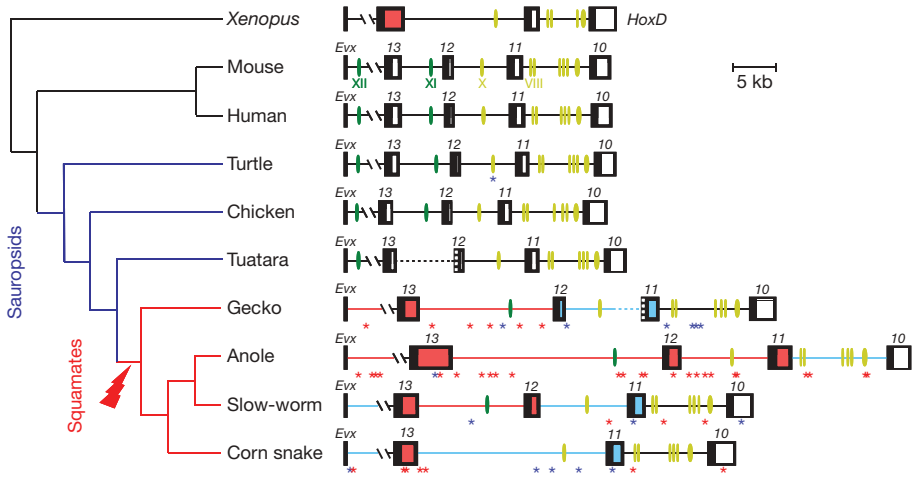

Figure 1 | Genomic organization of the posterior *HoxD* cluster. Schematic representation of the posterior *HoxD* cluster (from *Evx2* to *Hoxd10*) in various vertebrate species. A currently accepted phylogenetic tree is shown on the left. The correct relative sizes of predicted exons (black boxes), introns (white or coloured boxes) and intergenic regions (horizontal thick lines) permit direct comparisons (right). Gene names are shown above each box. Colours indicate either a 1.5-fold to 2.0-fold (blue) or a more than 2.0-fold (red) increase in the size of intronic (coloured boxes) or intergenic (coloured lines) regions, in comparison with the chicken reference. Major CNEs are represented by green vertical lines: light green, CNEs conserved in both mammals and sauropsids; dark green, CNEs lost in the corn snake. Gaps in the genomic sequences are indicated by dotted lines. Transposable elements are indicated with asterisks of different colours (blue for DNA transposons; red for retrotransposons).

their thoracic and caudal regions and also the transformation of the lumbo-sacral region into four cloacal vertebrae with forked ribs[13,14]. We investigated the expression patterns of *Hox* group 10 to group 13 genes by *in situ* hybridization in corn snake embryos at various stages. For comparison we used embryos from the whiptail lizard, which has a comparatively short body plan, with clearly identifiable regions in the pre-caudal vertebral column including 17 vertebrae bearing ribs, a single rudimentary lumbar vertebra and two sacral vertebrae. Although *Hoxc13* and *Hoxc12* were, as expected, expressed in the snake tail bud[4,18] and subsequently in caudal mesoderm at the level of the first future caudal vertebra, neither *Hoxa13* nor *Hoxd13* was expressed at a detectable level in mesodermal layers during the whole process of somitogenesis in the corn snake, except for a weak and transient signal (Fig. 2 and Supplementary Figs 3 and 4). These two genes were nevertheless transcribed in the developing cloacal region at early stages, as well as in the hemipenes (Fig. 2 and Supplementary Fig. 5). In contrast, all *Hox13* genes and *Hoxd12* were expressed in the lizard tail bud (Fig. 2 and Supplementary Fig. 4).

Hox11 gene expression in the snake paraxial mesoderm concerned mostly the cloacal region, in a similar manner to the sacral region in chicken[4]. *Hoxa11* labelled the first cloacal vertebra, whereas *Hoxd11* expression matched the first caudal vertebra and *Hoxc11* expression started in between. Similarly, *Hoxa11* and *Hoxd11* somitic boundaries in lizard were located at the level of the first sacral and first caudal pre-vertebrae (Supplementary Fig. 4). Finally, we looked at the expression boundaries of all three *Hox10* paralogous genes. In other amniotes, *Hox10* genes function to repress rib formation and hence they determine the thoraco-lumbar transition[4,19]. In snakes this morphological transition does not exist, because ribs are found until the start of the caudal region. Accordingly, *Hoxd10* expression showed a sharp boundary at the level of the first caudal pre-vertebrae (Fig. 2 and Supplementary Fig. 5), similarly to *Hoxd11*.

In contrast, *Hoxa10*, which was activated much earlier in both the snake tail bud and presomitic mesoderm, showed an expression boundary well within future rib-bearing thoracic vertebrae (Fig. 2 and Supplementary Figs 3 and 5). Similarly, the expression of *Hoxc10* was shifted into thoracic somitic mesoderm[14] (Fig. 2 and Supplementary Fig. 5), as several *Hox* expression boundaries, which were shifted anteriorly at late stages of snake development and were thus quite variable throughout somitogenesis (Fig. 2 and Supplementary Fig. 4). In contrast, the expression of both lizard *Hoxa10* and *Hoxc10* precisely matched the unique lumbar-like pre-vertebra (Fig. 2). However, lizard *Hoxd10* labelled the first caudal pre-vertebra, as in snake embryos (Fig. 2); that is, more posteriorly than in other amniotes[4,19].

We compared *Hox* coding sequences of non-Squamata (turtles, alligator and tuatara) and Squamata (lizards and snakes) reptiles, to identify patterns of divergence and constraints across the different paralogous groups of *Hox* genes, which could correlate with the important morphological differences observed within these lineages[20]. We used likelihood models that estimated separately the rate of nucleotide substitutions either affecting (non-synonymous substitution, d_n) or not affecting (synonymous substitution, d_s) the amino-acid sequence for each group stem-lineage. The estimates of d_n/d_s ratios (or ω) are used as indicators of selective pressure acting on protein-coding sequences in the different stem-lineages, their generally low values (ω typically less than 0.2; Table 1) thus indicating an active purifying selection throughout the evolution of vertebrate *Hox* genes. However, a marked increase in ω ratios was observed for several posterior *Hox* genes in the stem-lineage of squamates (*Hoxd12* and *Hoxa10*), snakes (*Hoxd11*) or sauropsids (*Hoxc10*). With the exception of *Hoxd11*, for which three positively selected sites were identified in the stem-lineage of snakes, the higher ω ratios observed for *Hoxd12*, *Hoxa10* and *Hoxc10* are due to the relaxation of purifying selection rather than the presence of positively selected residues (Table 1).

The examination of branch lengths in the vertebrate phylogenetic tree also provides evidence for a significant relaxation of purifying selection acting on *Hoxd12* in the stem lineage of squamates, as indicated by a sevenfold increase in non-synonymous substitutions (relative-rate test, $P < 10^{-7}$), as well as within individual lizard species, leading to the loss of the gene in snakes (Supplementary Fig. 7). In addition, whereas the levels of non-synonymous substitutions are relatively low in sauropsid *Hox13* genes (Supplementary Fig. 6), a relaxed selection of both *Hoxa10* and *Hoxc10*, but not *Hoxd10*, is observed in the stem-lineage of squamates and sauropsids, respectively (Table 1 and Fig. 3), which is indicative of a release in functional constraints. The alignment of all *Hox10* coding sequences thus revealed three strongly conserved motifs among vertebrates, and series of functionally non-equivalent amino-acid substitutions were specifically observed for *Hoxa10* and *Hoxc10* in snakes, whereas the *Hoxd10* sequence remained well conserved (Fig. 3 and Supplementary

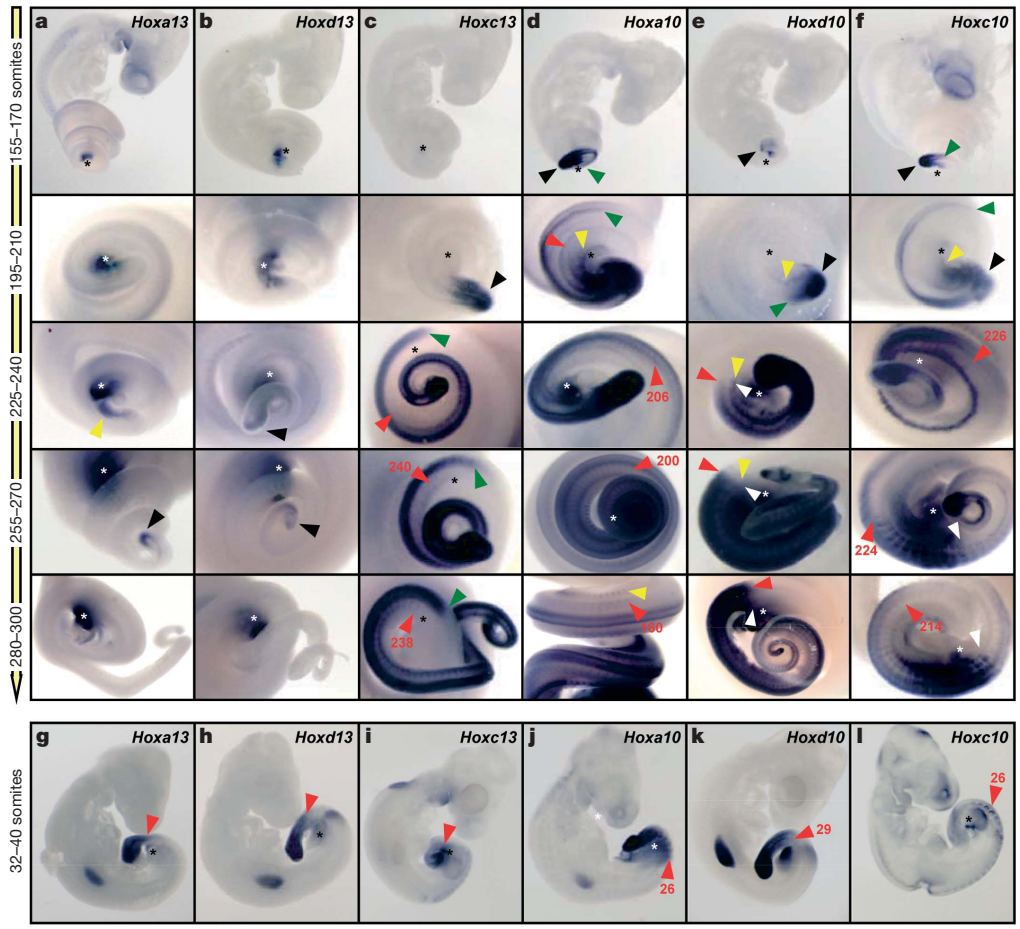

Figure 2 | Expression patterns of *Hox13* and *Hox10* genes in snake and lizard embryos. a–f, Whole-mount *in situ* hybridizations showing the expression of *Hoxa13* (**a**), *Hoxd13* (**b**), *Hoxc13* (**c**), *Hoxa10* (**d**), *Hoxd10* (**e**) and *Hoxc10* (**f**) during corn snake development (from 155 to 300 somites). Each panel shows a lateral view of the embryo (155–170 somites) or a posterior view of the somitic expression boundary at higher magnification (195–300 somites). Anterior expression boundaries are indicated with arrowheads of different colours, corresponding to distinct tissues: red, somitic mesoderm; yellow, lateral plate mesoderm; green, neural tissue; black, tail bud. The positions of the cloaca and of the fourth cloacal pre-vertebra are indicated with asterisks and white arrowheads, respectively. Numbers of somites refer to the pre-vertebral level. **g–l**, Expression of *Hoxa13* (**g**), *Hoxd13* (**h**), *Hoxc13* (**i**), *Hoxa10* (**j**), *Hoxd10* (**k**) and *Hoxc10* (**l**) in whiptail lizard embryos at 32–40 somites.

Fig. 8). We also scored strong variations in both the presence and the length of monotonic amino-acid repeats within specific coding regions of posterior *Hox* genes, especially between mammals and squamates (not shown). Because repeat-length mutations in *Hox13* genes induce various morphological phenotypes in amniotes[20,21], such natural variations, not considered by our phylogenetic analyses, may also represent a signature of adaptive changes in the coding sequence of these genes.

The importance of specific *Hox* paralogous groups in labelling anatomical transitions in the axial skeleton has largely been documented[22]. Whereas group 10 genes specify lumbar identities by preventing the development of ribs posterior to the thorax, *Hox11* activity is required for the genesis of sacral vertebrae[22]. *Hox12* genes seem dispensable in this context[16,23], whereas *Hox13* genes promote the termination of posterior axial structures[24]. Accordingly, group 11 genes in both snakes and lizards label the cloacal region, either within the region itself (*Hoxc11*) or at one of its ends (*Hoxa11* and *Hoxd11*). The evolution of positively selected sites in *Hox11* genes may be associated with the evolution of snake-specific functions accompanying the appearance of truncated ribs over the cloacal region (Fig. 4), in a similar manner to that proposed for *Hoxa11* and the evolution of pregnancy in mammals[25].

In agreement with the generic tetrapod situation, both lizard *Hoxa10* and *Hoxc10* label the unique vertebra without ribs, which is located immediately anterior to the sacrum (Fig. 4). However, such a situation would be problematic in snakes, in which group 11 genes are expressed immediately adjacent to ribs bearing thoracic vertebrae; that is, in structures that should normally not tolerate the rib-suppressing function of group 10 proteins. Spatial collinearity is nevertheless respected, because both *Hoxa10* and *Hoxc10* are expressed at more anterior levels than *Hox11* genes, in areas corresponding to future rib-bearing thoracic vertebrae[14] (Fig. 4). The marked relaxation in the coding sequences of these two genes suggests that the encoded proteins have lost this rib-suppressing activity in snakes to cope with the strict constraint imposed by collinearity. *Hoxd10*, which did not show this relaxation in protein sequence, is

Table 1 | Summary of ω ratios for posterior *Hox* genes in different vertebrate stem-lineages

Gene	Amniotes	Non-eutherian mammals	Eutherian mammals	Sauropsids	Squamates	Snakes
Hoxa13	0.0718	0.0677	0.0097	0.0231	0.0596	0.0607
Hoxc13	0.0769	n.d.	0.0084	0.0716	0.0500	0.0616
Hoxd13	0.0635	0.0154	0.0135	0.0293	0.0459	0.0462
Hoxc12	0.0893	n.d.	0.0031	0.0302	0.1762 (2 sites)	0.0606
Hoxd12	0.3433	0.0931	0.1347	0.1507 (5 sites)	**0.2537**	–
Hoxa11	0.0674	0.0912	0.1854	0.0714	0.0446	0.0848 (2 sites)
Hoxc11	0.1084	0.0395	0.0808 (2 sites)	0.1305	0.0182	n.d.
Hoxd11	0.1147	0.0431	0.0458	0.0895	0.0346 (1 site)	**0.2230** (3 sites)
Hoxa10	0.0348	0.0804 (2 sites)	0.1179	0.1491	**0.3831**	0.1251
Hoxc10	**0.2068**	n.d.	0.1611	**0.2389**	0.0345	0.1031
Hoxd10	0.0896	0.1402	0.0955	0.0308	0.0887	0.1019

Amino-acid sites under positive selection are indicated within brackets; ω ratios more than 0.2 are in bold. n.d., not determined.

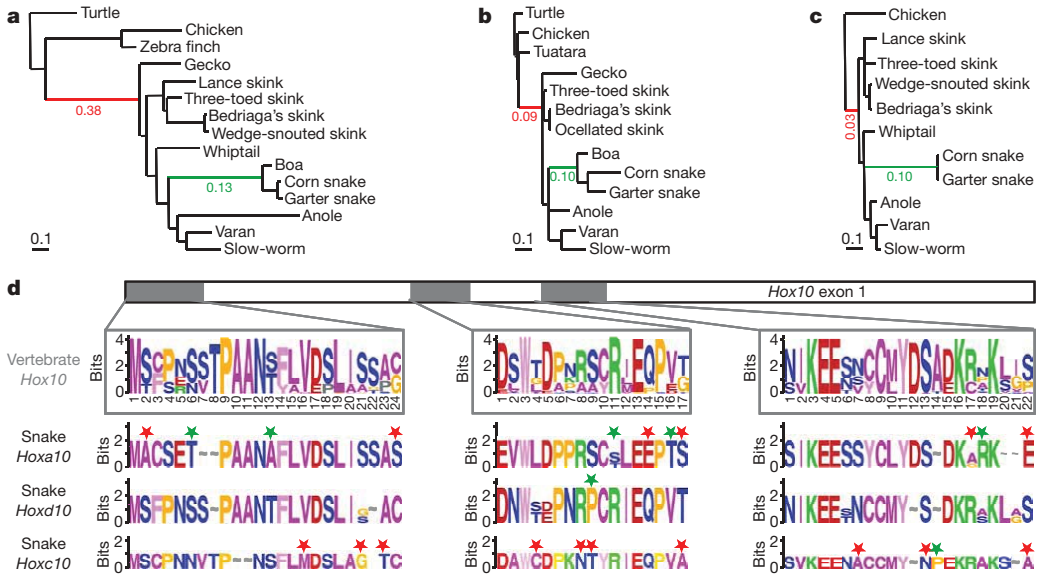

Figure 3 | Comparison of vertebrate HOX10 protein sequences. **a–c**, Phylogenetic trees of sauropsid *Hoxa10* (**a**), *Hoxd10* (**b**) and *Hoxc10* (**c**) exon 1. Branch lengths are proportional to the number of non-synonymous substitutions per site. Values for ω are indicated for the stem-lineage of squamates (red) and snakes (green). **d**, Amino-acid substitutions in the first exon of Squamata *Hox10* paralogous genes. Grey boxes indicate the locations of strongly conserved amino-acid motifs among all tested vertebrate *Hox10* genes. The three snake sequence logos show variations within individual *Hoxa10*, *Hoxd10* and *Hoxc10* genes. Amino acids are colour-coded by physicochemical properties. The size of the letters is proportional to the number of species with the given amino acid. Stars indicate the locations of sites substituted either in all squamates (red stars) or in snakes only (green stars).

expressed in snake at the same antero-posterior level as *Hoxd11*, that is, the only possible position allowed by the combined constraints of keeping in register with spatial collinearity and maintaining a rib-suppressing activity.

In contrast to all other amniotes analysed so far[3,4], including the whiptail lizard, neither *Hoxa13* nor *Hoxd13* is expressed within the post-cloacal tail bud and somitic mesoderm in the snake. Because *Hox13* genes are the most posterior group in tetrapods and thus determine structures located at the extremities of body axes, it has been proposed that they may function to terminate the patterning process[24]. Consequently, it is tempting to associate the absence of *Hoxa13* and *Hoxd13* expression with the elongation of the caudal region in snakes, perhaps by delaying the progressive shrinking of presomitic mesoderm, which seems to control the termination of somitogenesis[13]. Accordingly, a larger number of cell generations is estimated to be required to build the snake axis than in the chicken[13]. For both *Hoxa13* and *Hoxd13*, modifying the transcriptional regulation was probably a more parsimonious solution than relaxing protein sequences, because of their additional functions in the development of cloaca and hemipenes[26]. In contrast, *Hoxc13* was expressed in the tail bud along with *Hoxc12*, as expected from patterns in other tetrapods, which may be the minimum required for a difference to emerge between cloacal and post-cloacal vertebral morphologies in snakes.

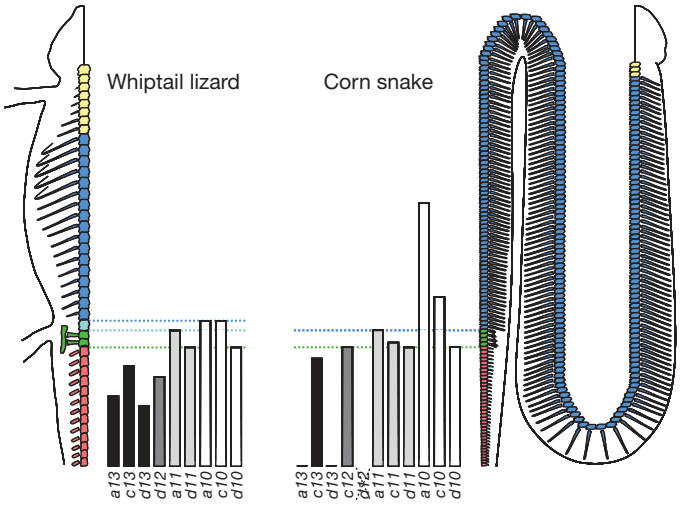

Figure 4 | Evolutionary modifications of the posterior *Hox* system in the whiptail lizard and corn snake. The positions of *Hox* expression domains along the paraxial mesoderm of whiptail lizard (32–40 somites, left) and corn snake (255–270 somites, right) are represented by black (*Hox13*), dark grey (*Hox12*), light grey (*Hox11*) and white (*Hox10*) bars, aligned with coloured schemes of the future vertebral column. Colours indicate the different vertebral regions: yellow, cervical; dark blue, thoracic; light blue, lumbar; green, sacral (in lizard) or cloacal (in snake); red, caudal. *Hoxc11* and *Hoxc12* were not analysed in the whiptail lizard. Note the absence of *Hoxa13* and *Hoxd13* from the corn snake mesoderm and the absence of *Hoxd12* from the snake genome.

Our results suggest that snakes, like mammals, use the full realm of group 11 genes to determine the cloacal region, which is critical for both vertebrate axial patterning and physiology. However, anteriorly and posteriorly to this region, a simplified *Hox* code seems to be at work: first, only *Hoxc13* (not *Hoxa13* or *Hoxd13*) and *Hoxc12* (not *Hoxd12*) may help to terminate body elongation, and second, only *Hoxd10* may have conserved the rib-suppressing activity. Snakes may therefore implement a smaller number of *Hox* gene functions in these posterior areas. Such a relaxation in functional redundancy, when compared with that in other tetrapods (Fig. 4), either at the level of transcription or at that of coding sequences, may have been a pre-requisite for the marked modifications that occurred in the snake axial skeleton. Anterior shifts in the expression patterns of *Hoxa10* and *Hoxc10* may still correlate with the presence of distinct structures and/or various size of ribs or vertebral processes along the antero-posterior axis, despite the apparent homogeneity of the snake thoracic region[11].

Consistent with previous phylogenetic analyses[27] is our demonstration that the transition from short lizard-like to elongated snake-like body forms involved progressive changes in the Squamata body plan, which can be somewhat prefigured in lizards. For example, the expression of *Hoxd10* in the whiptail lizard does not correspond to

the lumbo-sacral transition, as in other amniotes[4,19], yet it coincides with that found in snakes (Fig. 4), as if the function of *Hoxd10* at the caudo-cloacal transition (the end of rib-bearing vertebrae) was already implemented in lizards. Similarly, although the loss of the CNE XII in the lizard *HoxD* loci may not have drastically modified posterior *Hoxd* gene expression, the additional loss of region XI in the snake may have elicited significant effects[15].

Elongated body shapes have evolved independently in different vertebrate groups such as eels, salamanders, caecilians, lizards and snakes[11,12,14], and preliminary observations suggest that these various species use similar processes both for segmentation and for segmental identity[13,28]. Future studies integrating the period of the somitic clock with *Hox* gene expression in vertebrates with a specific elongation of either their thorax (for example burrowing snakes and caecilians) or their post-sacral region (for example varanoids and salamanders) will confirm whether natural selection has indeed exploited the flexibility of similar developmental mechanisms. The fact that limb reduction is commonly found along with trunk elongation suggests that posterior *Hox* genes may have had major functions in coordinating the emergence of these novel forms[9,14], because extensive genetic analyses in mice have demonstrated the critical importance of these genes in limb development and evolution[29].

METHODS SUMMARY

Hox genes were cloned after PCR amplification from reptile genomic DNA, using various combinations of degenerated primers corresponding to sequences that are highly conserved in vertebrates. Full posterior *Hox* clusters were amplified by long-range PCRs with the Expand Long Template PCR System (Roche) and LA Taq DNA polymerase (TaKaRa Bio), and were sequenced to high coverage (more than 20-fold) with the 454 FLX sequencing technology (Roche). Annotation of vertebrate *Hox* clusters was generated from sequence alignments and nucleotide BLAST searches at the University of California Santa Cruz and the National Centre for Biotechnology Information. To further validate exon boundaries, putative coding regions were predicted with GenScan. Interspersed repeats were identified and classified with both Censor and Repeat-Masker, using nucleotide sequences and default parameters.

In all phylogenetic analyses, exonic sequences were aligned using ClustalW and manually rearranged using the Bioedit alignment software. Estimation of ω was performed with the maximum-likelihood (ML) method as implemented in CODEML in PAML v.4.2 (ref. 30) and using branch-specific evolutionary models. The imposed species tree is in accord with current knowledge on vertebrate phylogeny. Selective pressures acting on sites along specific branches of the phylogenetic tree were assessed with branch-site models, and amino-acid sites under positive selection were identified with the Bayes Empirical Bayes (BEB) method (threshold probability of more than 0.90 under model A).

Whole-mount *in situ* hybridizations were performed with standard procedures at a hybridization temperature of 68 °C and digoxigenin-labelled riboprobes (Supplementary Table 1). Snake and lizard embryos were carefully staged by somite counting as described[6,13], using standard or three-dimensional optical projection tomography imaging, and gene expression boundaries reported here correspond to the pre-vertebral level. For histology, embryos after whole-mount *in situ* hybridization were fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose, embedded in OCT compound and cryosectioned at 20 μm.

Received 9 October; accepted 18 December 2009.

1. Kmita, M. & Duboule, D. Organizing axes in time and space; 25 years of colinear tinkering. *Science* **301,** 331–333 (2003).
2. Deschamps, J. & van Nes, J. Developmental regulation of the Hox genes during axial morphogenesis in the mouse. *Development* **132,** 2931–2942 (2005).
3. Gaunt, S. J. Conservation in the Hox code during morphological evolution. *Int. J. Dev. Biol.* **38,** 549–552 (1994).
4. Burke, A. C., Nelson, C. E., Morgan, B. A. & Tabin, C. *Hox* genes and the evolution of vertebrate axial morphology. *Development* **121,** 333–346 (1995).
5. Di Poi, N., Montoya-Burgos, J. I. & Duboule, D. Atypical relaxation of structural constraints in *Hox* gene clusters of the green anole lizard. *Genome Res.* **19,** 602–610 (2009).
6. Duboule, D. The rise and fall of Hox gene clusters. *Development* **134,** 2549–2560 (2007).
7. Garcia-Fernandez, J. The genesis and evolution of homeobox gene clusters. *Nature Rev. Genet.* **6,** 881–892 (2005).
8. Lemons, D. & McGinnis, W. Genomic evolution of Hox gene clusters. *Science* **313,** 1918–1922 (2006).
9. Cohn, M. J. & Tickle, C. Developmental basis of limblessness and axial patterning in snakes. *Nature* **399,** 474–479 (1999).
10. Hoffstetter, R. & Gasc, J. P. in *Biology of the Rept*ilia (eds Gans, C., d'A. Bellair, A. & Parsons, T. S.) Vol. 1, 201–310 (Academic, 1969).
11. Romer, A. S. *Osteology of the Reptiles* (Krieger Publishing Co., 1997).
12. Duellman, W. E. & Trueb, L. *Biology of Amphibians* (Johns Hopkins University Press, 1994).
13. Gomez, C. *et al.* Control of segment number in vertebrate embryos. *Nature* **454,** 335–339 (2008).
14. Woltering, J. M. *et al.* Axial patterning in snakes and caecilians: evidence for an alternative interpretation of the *Hox* code. *Dev. Biol.* **332,** 82–89 (2009).
15. Kmita, M., Tarchini, B., Duboule, D. & Herault, Y. Evolutionary conserved sequences are required for the insulation of the vertebrate *Hoxd* complex in neural cells. *Development* **129,** 5521–5528 (2002).
16. Herault, Y., Beckers, J., Kondo, T., Fraudeau, N. & Duboule, D. Genetic analysis of a *Hoxd-12* regulatory element reveals global versus local modes of controls in the *HoxD* complex. *Development* **125,** 1669–1677 (1998).
17. Feschotte, C. & Pritham, E. J. DNA transposons and the evolution of eukaryotic genomes. *Annu. Rev. Genet.* **41,** 331–368 (2007).
18. Godwin, A. R. & Capecchi, M. R. *Hoxc13* mutant mice lack external hair. *Genes Dev.* **12,** 11–20 (1998).
19. Carapuço, M., Novoa, A., Bobola, N. & Mallo, M. *Hox* genes specify vertebral types in the presomitic mesoderm. *Genes Dev.* **19,** 2116–2121 (2005).
20. Kohlsdorf, T. *et al.* A molecular footprint of limb loss: sequence variation of the autopodial identity gene *Hoxa-13*. *J. Mol. Evol.* **67,** 581–593 (2008).
21. Muragaki, Y., Mundlos, S., Upton, J. & Olsen, B. Altered growth and branching patterns in synpolydactyly caused by mutations in *Hoxd13*. *Science* **272,** 548–551 (1996).
22. Wellik, D. M. & Capecchi, M. R. *Hox10* and *Hox11* genes are required to globally pattern the mammalian skeleton. *Science* **301,** 363–367 (2003).
23. Suemori, H. & Noguchi, S. *HoxC* cluster genes are dispensable for overall body plan of mouse embryonic development. *Dev. Biol.* **220,** 333–342 (2000).
24. Young, T. *et al. Cdx* and *Hox* genes differentially regulate posterior axial growth in mammalian embryos. *Dev. Cell* **17,** 516–526 (2009).
25. Lynch, V. J. *et al.* Adaptive changes in the transcription factor HoxA-11 are essential for the evolution of pregnancy in mammals. *Proc. Natl Acad. Sci. USA* **105,** 14928–14933 (2008).
26. Scott, V., Morgan, E. A. & Stadler, H. S. Genitourinary functions of Hoxa13 and Hoxd13. *J. Biochem.* **137,** 671–676 (2005).
27. Wiens, J. J. & Slingluff, J. L. How lizards turn into snakes: a phylogenetic analysis of body-form evolution in anguid lizards. *Evolution* **55,** 2303–2318 (2001).
28. Raynaud, A. Preliminary data on the body lenghthening and somitogenesis in young embryos of *Anguis fragilis* L. and of *Lacerta viridis* Laur. *Bull. Soc. Hist. Nat. Toulouse* **130,** 47–52 (1994).
29. Zakany, J. & Duboule, D. The role of *Hox* genes during vertebrate limb development. *Curr. Opin. Genet. Dev.* **17,** 359–366 (2007).
30. Yang, Z. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* **24,** 1586–1591 (2007).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank A. Schmitz for sharing squamate tissues; A. Debry and F. Chabaud for technical assistance; and members of the Duboule and Milinkovitch laboratories for discussions and reagents. This work was supported by funds from the University of Geneva and the Federal Institute of Technology in Lausanne, the Swiss National Research Fund, the National Research Center (NCCR) 'Frontiers in Genetics', the EU programme 'Crescendo' and the ERC grant Systems*Hox*.ch (to D.D.).

Author Contributions N.D.P. and D.D. designed the experiments and analysed the data. N.D.P. performed the experiments except those involving the tuatara, which were conducted by H.M. J.I.M.B. performed the phylogenetic analyses. M.C.M. produced and prepared snake embryos and O.P. provided snake and lizard embryos. N.D.P. and D.D. wrote the paper, and all co-authors contributed in the form of discussion and critical comments.

Author Information Sequences of genes described in this paper are deposited in GenBank under accession numbers GU320304 to GU320335. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.D. (denis.duboule@unige.ch or denis.duboule@epfl.ch).