JOURNAL OF EXPERIMENTAL ZOOLOGY (MOL DEV EVOL) 291:169–174 (2001)

Current Problems With the Zootype and the Early Evolution of Hox Genes

BERND SCHIERWATER[1,2] AND ROB DESALLE[2]
[1]*Tierärztliche Hochschule–ITZ, Division of Ecology and Evolution, 30599 Hannover, Germany*
[2]*American Museum of Natural History, Division of Invertebrate Zoology, New York, New York 10024*

ABSTRACT "*Hox* cluster type" genes have sparked intriguing attempts to unite all metazoan animals by a shared pattern of expression and genomic organization of a specific set of regulatory genes. The basic idea, the zootype concept, claims the conservation of a specific set of "*Hox* cluster type genes" in all metazoan animals, i.e., in the basal diploblasts as well as in the derived triploblastic animals. Depending on the data used and the type of analysis performed, different opposing views have been taken on this idea. We review here the sum of data currently available in a total evidence analysis, which includes morphological and the most recent molecular data. This analysis highlights several problems with the idea of a simple "*Hox* cluster type" synapomorphy between the diploblastic and triploblastic animals and suggests that the "zootype differentiation" of the *Hox* cluster most likely is an invention of the triploblasts. The view presented is compatible with the idea that early *Hox* gene evolution started with a single proto-Hox (possibly a paraHox) gene. *J. Exp. Zool. (Mol. Dev. Evol.) 291:169–174, 2001.*

Hox genes, like no other class of genes, have sparked intriguing discussions on the developmental mechanisms underlying the diversity and evolution of metazoan animals. These transcription factors (Gehring, '93), which can specify regional identity in animal bauplans, are believed to have played a major role in the diversity of form and function of developmental programs and ultimately the radiation of animal phyla (Li and McGinnis, '99; Carrol et al., 2000). Attempts to simplify biological complexity (cf. Blackstone, '97) have led to different views of how these genes function in development and evolution. The widely cited "animal zootype" hypothesis was proposed (Cohen, '93; Hughes et al., '93; Slack et al., '93) as a means of uniting all animal body plans using the shared pattern of regulatory gene expression. This hypothesis was based upon the possession of a specific set of "*Hox* cluster type" genes as a synapomorphy defining the kingdom Animalia. Within the vertebrates, several authors have translated this early embryonic similarity into the "hourglass model" (Duboule, '94; Collins, '98), which is based on the classical observation of similarity of embryos in a wide array of organisms as first proposed by Haeckel (1874). The invocation of this generalization about vertebrate body plans implies a consistency with the concept of universal morphogenetic fields (Richardson et al., '97). Acceptance of the "animal zootype" would imply consistency with an even more universal basal morphogenetic field and further would imply a fundamental genetic and developmental basis for how body plans develop and evolve.

In an interesting recantation, Slack ('98) rejected the general notion of an "animal zootype." Several other authors (Finnerty, '98; Finnerty and Martindale, '98; Martinez et al., '98; Adoutte et al., '99, 2000; de Rosa et al., '99) suggest that the "animal zootype" is flawed on the basis of examination of the first occurrence of the "*Hox* cluster" in animal evolution. Part of this argument is the suggestion that the metazoan radiation started with a duplication of a hypothetical *protoHox* gene cluster into two clusters, a *Hox* and a *paraHox* cluster (Brooke et al., '98; Akam; 2000; Kappen, 2000; Pollard and Holland, 2000; Shimeld and Holland, 2000). In this respect it is also instructive to examine the recent critique of the "verte-

Grant sponsor: German Science Foundation; Grant number: DFG Schi 277/10-2.
*Correspondence to: Bernd Schierwater, Bünteweg 17d, Institut für Tierökologie & Zellbiologie, Division of Ecology and Evolution, 30559 Hannover, Germany. E-mail: bernd.schierwater@ecolevol.de
Received 6 March 2000; Accepted 29 November 2000

brate zootype" where authors have rejected the classical notion of the existence of generalized body plans in vertebrate evolution. Richardson et al. ('97, '98) suggest that there "is no highly conserved embryonic stage in the vertebrates" contrary to classical notions. They base their statement on careful phylogenetic analyses of the relevant morphological and molecular information. In essence, the raw similarities first observed by Haekel (1874) are used as a hypothesis and rigorous testing of the hypothesis is accomplished in a phylogenetic context (Richardson et al., '97, '98). Because of the importance of these generalized statements about animal body plans (Davidson and Ruvkun, 2000) and some confusion as to the utility of the zootype concept, a rigorous review and modification of the "animal zootype" concept are warranted.

Testing two assumptions of the "animal zootype"

We suggest that notions of the "animal zootype" are based on attempts to accommodate the transformation (Bang et al., 2000), in a straight line, from basal (diploblastic) to higher (triploblastic) animals. Two assumptions need to be met in order for a simple (zootype related) transformational interpretation (Bang et al., 2000) to be valid as an explanation for the evolution of *Hox* genes and body plans from diplo- to triploblastic animals. First, the presence of a shared main body axis must exist in all metazoan animals, and second, all Metazoa must possess *Hox* type genes whose expression imparts positional information that is involved in the spatial specification of the anterior–posterior (a-p) body axis of all metazoan animals. Both of these assumptions of the zootype concept can be examined by first recognizing that the relevant morphological and developmental data need to be examined from different functional levels (Abhouief, '97). We suggest that the relevant morphological and developmental attributes of diploblastic and triploblastic animals need to be scored as character data and analyzed in a phylogenetic context to test the validity of the animal zootype, much in the same way the vertebrate data were used to examine the validity of the "vertebrate zootype" (Richardson et al., '97, '98). In order to accomplish this task we reexamine the evidence for (1) a homologous anterior–posterior (a-p) axis across all metazoans, and (2) the presence of a structurally and functionally conserved *Hox* gene cluster in diploblastic animals.

Homologous a–p axis in all metazoans?

While current knowledge of the function of *Hox* genes in development is too sparse to draw a conclusion about a-p axis homology in bilaterians and cnidarians, the morphological and ontogenetic information is illuminating. The oral–aboral body axis of cnidarians is functionally reminiscent of the a-p (head–trunk) axis in triploblasts, and different authors have speculated that the oral–aboral body axis in diploblastic cnidarians might be related to, and hence homologous to, the a-p axis in triploblasts (Brusca and Brusca, '90; Gruner, '93; Ax, '95). Corroborating evidence supporting this hypothesis, however, has not been reported. Furthermore, there is no main body axis at all in the two most basal diploblast phyla, the Placozoa and the Porifera (Fig. 1). The Placozoa change body form and movement direction constantly, and no axis of symmetry can be seen at any developmental stage. Some primitive Porifera might develop a functional foot–osculum axis, which from a developmental and morphological point of view is neither comparable to the oral–aboral body axis of the cnidarians nor to the anterior–posterior axis of triploblastic animals (Brusca and Brusca, '90; Gruner, '93; Ax, '95). We suggest that the "animal zootype" concept has from its inception been in conflict with the existing morphological data.

Are hox *gene clusters in diploblastic animals structurally and functionally conserved?*

Since the detection of *Hox* genes in diploblastic animals (Murtha et al., '91; Schierwater et al., '91), some 20 *Hox* genes have been isolated from these basal metazoans (Kuhn et al., '96, '99; Finnerty, '98). Observations made on these genes have been the source of broad speculation about putative homology statements concerning head, trunk, and tail genes from higher animals. Oddly, one and the same *Hox* genes have been assigned as a "head" homolog in one paper and a "tail" homolog in another (e.g., for *Cnox-1* genes see Schierwater et al., '91; Shenk et al., '93; Finnerty, '98; Martinez et al., '98). Accordingly, some authors have claimed a lack of "tail" genes while others claim a lack of "head" genes in diploblasts. Interestingly, no one has yet clearly claimed homology of any diploblast *Hox* gene to a "trunk" gene (cf. Finnerty, '98, however). Using analysis of leech head and trunk development, Bruce and Shankland ('98) and Shankland and Seaver (2000) have proposed that the trunk is a synapomorphic development of bilaterian (triploblastic) animals.

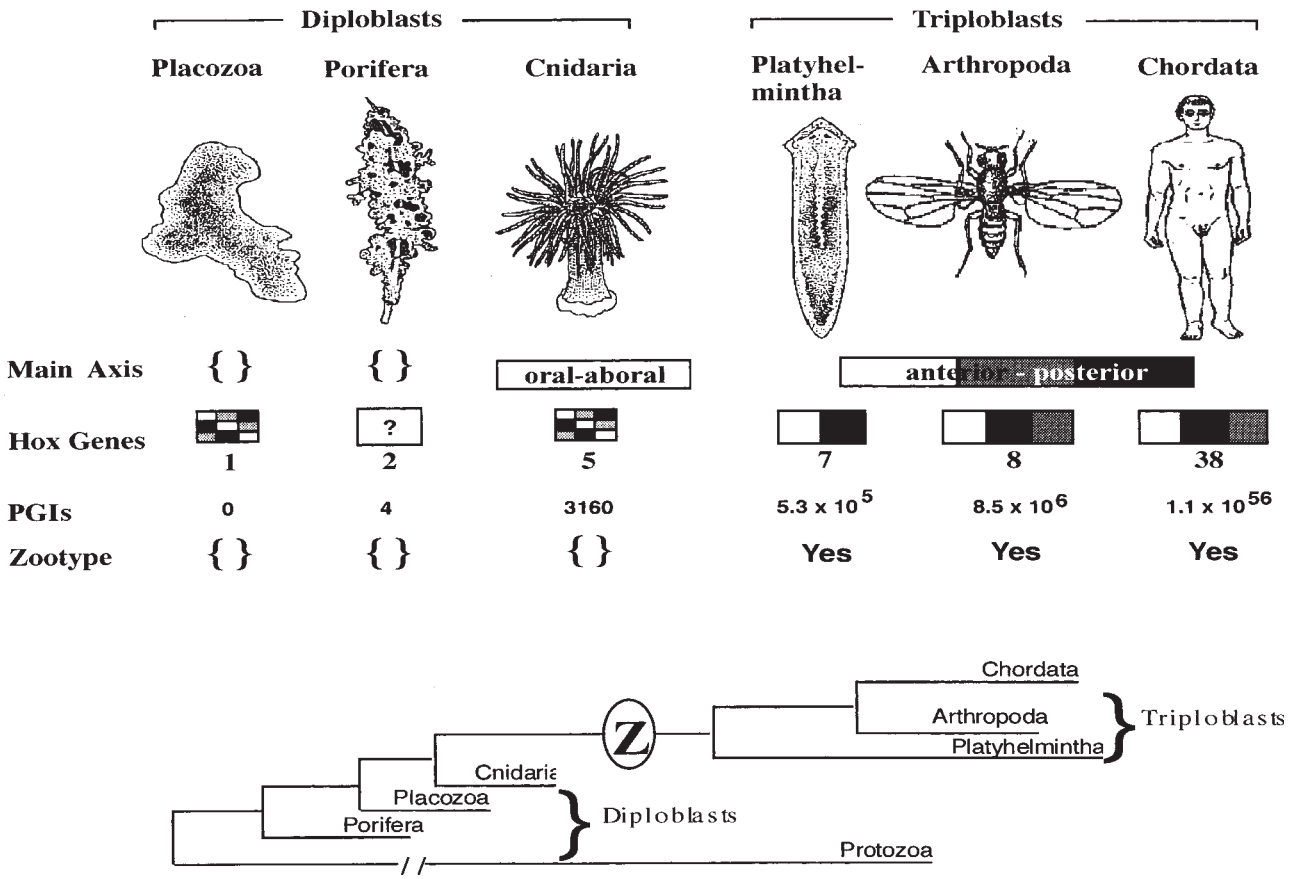

Fig. 1. Anagenetic evolution from the presumably most basal Placozoa or Porifera to the most derived Chordata mirrors an increase in the complexity of bauplan patterns. The coelenterates, which include the phylum Cnidaria, are the first diploblastic animals, which developed a main body axis, the "oral–aboral" axis (but see also Knoll and Caroll, '99). All higher, triploblastic animals are characterized by an "anterior–posterior" body axis. The development of the latter is specified by a set of *Hox* genes encoding positional information for the anterior (head genes; white), medial (trunk genes; grey), and posterior region (tail genes; black) in triploblasts. In contrast, *Hox* genes in diploblastic animals are characterized by diagnostic amino acid residues from different *Hox* gene families within a single gene (shown as a white/grey/black mosaic; only partial sequence information is available for the Porifera *Hox* genes (Degnan et al., '95), which could thus not be analyzed (it should also be noted, however, that some authors question these sequences as Porifera *Hox* sequences). The number of *Hox* genes present in one organism to some degree reflects the complexity of its body plan (e.g., Knoll and Caroll, '99); given here are conservative estimates for the first four phyla (Shenk et al., '93; Degnan et al., '95; Balavoine, '96; Kuhn et al., '96, '99; Finnerty et al., '98; Martinez et al., '98; Schierwater and Kuhn, '98) and exact numbers for the fruit fly and humans (Duboule, '94). The finding of the presence of exactly one *Hox* gene in Placozoa and five in Cnidaria seems to be well supported, however (Kuhn et al., '96, '99; Finnerty et al., '98; Schierwater and Kuhn, '98; it should be noted, however, that some authors regard the placozoan *Trox-2* gene as a *paraHox* gene). Since *Hox* genes are transcription factors that can also interact with other *Hox* genes, the increase in possible "regulatory gene interactions" (RGIs) within a given set of Hox genes can theoretically grow faster than exponentially. If only consecutive gene interactions were considered, the number of possible RGIs could grow as fast as · (2**(k – 1) * (G(G – 1) ... (G – k + 1)) for k = 2 to G (G = number of regulatory genes); e.g., for 13 genes the number of RGIs would already exceed the number of cells in a human body. The number of *Hox* genes and the first appearance of a main body axis in the Cnidaria would theoretically allow the invention of a zootype situation to be dated back to the Cnidaria. Overall evidence, including development, morphology, and the number and type of *Hox* genes present in diploblastic animals, however, suggests that the zootype is an invention of the triploblasts (circled "Z" in the phylogram). Any phylogram based on morphological, developmental, and molecular data (including 18S rDNA sequences; data matrix available from the authors) supports this view and the clear separation and monophyletic origin of the triploblasts. Obtaining a basal position for either the Placozoa or Porifera widely depends on the outgroup, the species, and the algorithm used and is not important to the discussion here; the PAUP phylogram shown uses the protozoan *Apusomonas proboscidea*, the sponge *Axinella polypoides*, the placozoan *Trichoplax adhaerens*, the anthozoan *Bellonella rigida*, the flatworm *Dugesia mediterranea*, the insect *Aeshna cyanea*, and the cephalochordate *Branchiosteum lanceolatum* and is based on a total of 1,791 characters (single tree, exhaustive search; CI = 0.70, RI = 0.58); all nodes are supported by bootstrap values of > 86% and Bremer supports of > 5 (the full data matrix can be requested from the authors and will be available on the internet).

Such a hypothesis suggests that the bilaterian and prebilaterian body plans share no homology of underlying a-p determination. Rather, the triploblast ancestral genome would have co-opted the *Hox* gene cluster to establish a-p axis determination after divergence from the diploblastic–triploblastic split. This hypothesis is readily tested given recent studies on *Hox* genes and their function in cnidarian and more basal diploblastic animals (Martinez et al., '98; Schierwater and Kuhn, '98; Finnerty and Martindale, '99). A phylogenetic analysis of the homeobox sequences of anterior, medial, and posterior group *Hox* genes from cnidarians, fruit flies, and mice suggests that any medial group *Hox* genes might be missing in cnidarians (Martinez et al., '98). The authors conclude that these genes derived later through duplication of a single precursor after the origin of *Cnidaria*. A recent study implicating WNT signaling molecules in establishing axis formation in Hydra (Hobmayer et al., 2000) suggests that the axis in these diploblastic metazoa may be under the control of non-*Hox* systems.

While genealogical analyses of short (and often only partial) homeobox sequences from cnidarians have led to a variety of contradictory trees (Finnerty et al., '98; Kuhn et al., '99), further evidence against the existence of a head, trunk, and tail *Hox* gene cluster in diploblastic animals comes from the analysis of diagnostic residues within the homeodomain. It has been proposed that simple homologs to head, trunk, and tail genes do not exist in recent diploblasts (Schierwater and Kuhn, '98). Data exist on a fair number of full-length homeobox sequences (plus flanking regions) from different cnidarians (Kuhn et al., '96, '99) as well as from the presumably most basal metazoan animal, the placozoan *Trichoplax adhaerens* (Schierwater and Kuhn, '98). In all cases, analyses of the deduced amino acid sequences of the *full-length* homeodomains suggest that the diploblast *Hox* genes do not represent direct homologs of *Hox* genes belonging to any of the anterior, medial, or posterior *Hox* families known from triploblastic animals (Kuhn et al., '96; Schierwater and Kuhn, '98). Instead, diploblast *Hox* genes appear to be genetic chimeras, harboring diagnostic residues from different *Hox* gene families in the same homeodomain (Fig. 1). Different studies aimed at demonstrating axial expression of multiple *Hox* genes in cnidarians have been conducted, but no data were found to support homology of the cnidarian oral–aboral and the triploblast a-p axis (Finnerty and Martindale, '98). Data for one gene, *Cnox-2*, are available that suggest a role in the maintenance (but not in the development) of the head region (Finnerty, '98; Cartwright et al., '99).

The presumably most primitive metazoan animal, the placozoan *Trichoplax adhaerens*, might possess only a single *Hox* gene (Schierwater and Kuhn, '98; note, however, that some authors regard this so called *Trox-2* gene as a *paraHox* gene). The observation of a single *Hox* type gene in Placozoa is in conflict with the "animal zootype" hypothesis and supports the view that the number of *Hox* genes correlates with the amount of regional specialization of an animal body plan (Knoll and Carroll, '99). Such correlation is likely to be observed in higher taxonomic level comparisons only, i.e., between phylogenetically and developmentally very distant taxa. To complicate interpretation of observed correlation further, we note that from a gene regulatory view such correlation can be hyperexponential (Fig. 1). For example, if the difference between the triploblastic Platyhelmintha and the diploblastic Cnidaria was two *Hox* genes (7–5 genes, respectively), this could theoretically result in the Platyhelmintha having more than 100 times as many options for regulatory gene interactions than the Cnidaria (Fig. 1). Thus slightly increasing the number of interacting *Hox* genes could be very efficient at increasing the complexity of a gene regulatory network and possibly similarly influence the complexity of animal body plans.

Invention of a zootype

It appears to us that the "animal zootype" was at first proposed because of the commonality among all animals of possessing *Hox* genes and the further suggestion that these *Hox* genes are responsible for the same morphological phenomena in all metazoan animals. However, to equate the existence of genes, gene clusters, and even gene expression with a final morphology, are mistaken (Abhouief, '97; Janies and DeSalle, '99). An analysis of all relevant data (Fig. 1) allows us to test the hypothesis that the "animal zootype" is a metazoan invention. Not supporting this notion, our analysis clearly demonstrates that the "animal zootype" (as defined as an involvement of *Hox* genes in a-p axis determination; see above) is an invention of triploblastic animals. The suggestion that the animal zootype is an invention of triploblastic animals is further supported by some recent data from the hydrozoan *Hydra*. In this diploblastic animal no two of the *Cnox Hox* genes

(*Cnox-1*, *Cnox2*, or *Cnox3*) are chromosomally linked within a range of at least 150 kilobases (Gauchat et al., 2000).

Origin of the Hox *cluster*

We suggest that there is no simple *Hox* gene cluster representing head, trunk, and tail genes in any diploblastic group. If an ancestral *Hox* cluster existed for diploblasts, possibly this gene would harbor diagnostic residues of more than one *Hox* gene family. The latter is the case for the *Trichoplax Trox-2* gene, which (i) harbors diagnostic residues from both head and tail genes, (ii) seems to be related to triploblast *Gsx paraHox* genes, and (iii) is possibly the only *Hox* type gene in the placozoan genome (Schierwater and Kuhn, '98). The phylogenetic scenario in Fig. 1 suggests that the metazoan radiation started with a single *protoHox* gene, which duplicated into a *protoHox*/*paraHox* gene cluster. Fig. 1 also implies that a second duplication event into separate *Hox* and *paraHox* gene clusters in derived diploblasts occurred. In this scenario, the differentiation of *Hox* clusters that control a-p axis determination is an invention of the triploblasts. The existence of an ancestral *protoHox*/*paraHox* gene in *Placozoa* fits our simultaneous analysis of the available data (Fig. 1) but is not readily supported by phylogenetic analysis based solely on 18S rDNA sequence data (see Collins, '99 for overview). The sum of empirical evidence available to date clearly does not support the existence of a zootype conform *Hox* type gene cluster in diploblastic animals and suggests that resolving the early evolution and differentiation of *Hox* genes will ultimately depend on (i) determining the complete *Hox* type gene repertoires in diploblastic animals, (ii) obtaining the yet hypothetical boxes of *Hox*, *protoHox*, and *paraHox* genes in diploblasts straight, and (iii) succeeding in resolving the phylogenetic relationships of the basal diploblast phyla.

LITERATURE CITED

Abhouief E. 1997. Developmental genetics and homology: a hierarchical approach. Trends Ecol Evol 12:405–408.

Adoutte A, Belavoine G, Lartilot N, de Rosa R. 1999. Animal evolution; the end of inter mediate taxa? Trends Genet 15:104–108.

Adoutte A, Belavoine G, Lartilot N, Lespinet O, Prud'homme B, de Rosa R. 2000. The new animal phylogeny: reliability and implications. Proc Natl Acad Sci USA 97:4453–4456.

Akam M. 2000. Arthropods: developmental diversity within a (super) phylum. Proc Natl Acad Sci USA 97:4438–4441.

Ax P. 1995. Das System der Metazoa I. Stuttgart: Gustav Fischer.

Balavoine G. 1996. Identification of members of several homeobox genes in a planarian using a ligation-mediated polymerase chain reaction technique. Nucleic Acids Res 24:1547–1553.

Bang R, DeSalle R, Wheeler W. 2000. Transformationalism, taxism and the role of developmental biology in systematics. Syst Biol 49:19–27.

Blackstone N. 1997. Argumentum ad ignorantium. O Rev Biol 72:445–447.

Brook NM, Garcia-Fernandez J, Holland PW. 1998. The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. Nature 392: 920–922.

Bruce AE, Shankland M. 1998. Dev Biol 201:101–112.

Brusca RC, Brusca GJ. 1990. Invertebrates. Sunderland, MA: Sinauer Associates, Inc.

Cartwright P, Bowsher J, Buss LW. 1999. Expression of a Hox gene, Cnox-2, and the division of labor in a colonial hydroid. Proc Natl Acad Sci USA 96:2183–2186.

Carroll RL. 2000. Towards a new evolutionary synthesis. TREE 15:27–32.

Cohen J. 1993. Development of the zootype. Nature 363:307.

Collins AG. 1998. Evaluating multiple alternative hypotheses for the origin of Bilateria: an analysis of 18S rRNA molecular evidence. Proc Natl Acad Sci USA 95:15458–15463.

Davidson EH, Ruvkun G. 1999. Themes from a NASA Workshop on Gene Regulatory Processes in Development and Evolution. J Exp Zool, Mol Dev Evol 285:104–115.

Degnan BM, Degnan SM, Giusti A, Morse DE. 1995. A hox/hom homeobox gene in sponges. Gene 155: 175–177.

de Rosa R, Grenier JK, Andreeva T, Cook CE, Adoutte A, Akam M, Carroll SB, Belavoine G. 1999. Nature 399:772–776.

Duboule D. 1994. Guidebook to the homeobox genes. Oxford: Oxford University Press.

Finnerty JR. 1998. The evolution of the Hox cluster: insights from outgroups. Curr Topics Dev Biol 40:211–253.

Finnerty JR, Martindale MQ. 1998. The evolution of the Hox cluster: insights from outgroups. Curr Opin Genet Dev 8:681–687.

Finnerty JR, Martindale MQ. 1999. Ancient origins of axial patterning genes: *Hox* genes and *paraHox* genes in Cnidaria. Evol Dev 1:16–23.

Finnerty JR, Master VA, Irvine S, Kourakis MJ, Warriner S, Martindale MQ. 1998. Homeobox genes in the Ctenophora: identification of paired type and Hox homologues in the atentaculate ctenophore *Beroe ovata*. Mol Mar Biol Biotechnol 5:249–258.

Gauchat D, Mazet F, Berney C, Schummer M, Kreger S, Pawlowski J, Galliot B. 2000. Evolution of Antp-class genes and differential expression of *Hydra Hox*/*paraHox* genes in anterior patterning. Proc Natl Acad Sci USA 97:4493–4498.

Gehring WJ. 1993. Exploring the homeobox. Gene 135:215–221.

Gruner HE. 1993. Wirbellose Tiere. 1. Teil. Stuttgart: Gustav Fischer.

Haeckel E. 1874. Die Gastrea-Theorie. Die phylogenetische Classifikation des Thierreichs und die Homologie der Keimblätter. Jena Z Naturw 8:1–55.

Hobmayer B, Rentzsch F, Kuhn K, Happel CM, Cramer von Laue C, Snyder P, Rothbacher U, Holstein TW. 2000. WNT signalling molecules act in axis formation in the diploblastic metazoan *Hydra*. Nature 407:186–190.

Hughes NC, Hughes SM. 1993. Development of the zootype. Nature 363:307.

Janies D, DeSalle R. 1999. Development, evolution and corroboration. New Anat 257:6–14.

Kappen C. 2000. Analysis of a complete homeobox repertoire: implications for the evolution of diversity. Proc Natl Acad Sci USA 97:4481–4486.

Knoll AH, Carroll SB. 1999. Early animal evolution: emerging views from comparative biology and geology. Science 284:2129–2137.

Kuhn K, Streit B, Schierwater B. 1996. Homeobox genes in the cniarian *Eleutheria dichotoma*: evolutionary implications for the origin of Antennapedia-class (HOM/Hox) genes. Mol Phyl Evol 5:30–38.

Kuhn K, Streit B, Schierwater B. 1999. Isolation of Hox genes from the scyphozoan *Cassiopeia xamachana:* implications for the early evolution of Hox genes. J Exp Zool 285:63–75.

Li X, McGinnis W. 1999. Activity regulation of Hox proteins, a mechanism for altering functional specificity in development and evolution. Proc Natl Acad Sci USA 96: 6802–6807.

Martinez DE, Bridge D, Masuda-Nakagawa LM, Cartwright P. 1998. Cnidarian homeoboxes and the zootype. Nature 393:748–749.

Miller DJ, Miles A. 1993. Homeobox genes and the zootype. Nature 365:215–216.

Murtha M, Leckman JF, Ruddle FH.1991. Detection of homeobox genes in development and evolution. Proc Natl Acad Sci 88:10711–10715.

Pollard S, Holland PWH. 2000. Evidence for 14 homeobox gene clusters in human genome ancestry. Curr Biol 10:1059–1062.

Richardson MK, Hanken J, Gooneratne ML, Pieau C, Reynaud A, Selwood L, Wright GM. 1997. There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development. Anat Embryol 196:91–106.

Richardson MK, Allen SP, Wright GM, Reynaud A, Hanken J. 1998. Somite number and vertebrate evolution. Development 125:151–160.

Schierwater B, Kuhn K. 1998. Homology of Hox genes and the zootype concept in early metazoan evolution. Mol Phyl Evol 9:375–381.

Schierwater B, Murtha M, Dick M, Ruddle FH, Buss LW. 1991. Homeoboxes in cnidarians. J Exp Zool 260:413–416.

Shankland M, Seaver EC. 2000. Evolution of the bilaterian body plan; what have we learned from annelids. Proc Natl Acad Sci USA 97:4434–4437.

Shimeld SM, Holland PW. 2000. Vertebrate innovations. Proc Natl Acad Sci USA 97:4449–4452.

Shenk MA, Bode HR, Steele RE. 1993. Expression of Cnox-2, a HOM/HOX homeobox gene in hydra, is correlated with axial pattern formation. Development 117:657–667.

Slack JM, Holland PWH, Graham CF. 1993. The zootype and the phylotypic stage. Nature 361:490–492.