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Hox gene collinearity may be related to Noether theory on Symmetry and its linked conserved quantity

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Abstract

Hox Gene Collinearity (HGC) is a fundamental property that determines the development of many animal clades including Vertebrates. In the Hox gene clusters the genes are located in a sequence Hox1, Hox2, Hox3,… along the 3' to 5' direction of the cluster in the chromosome. During Hox cluster activation the Hox genes are expressed sequentially in the ontogenetic units D1, D2, D3,… along the anterior (A)- Posterior (P) axis of the early embryo. This collinearity, first observed by E.B. Lewis, is surprising because the spatial extent of these structures (Hox clusters and embryos) differ by about 4 orders of magnitude. Biomolecular mechanisms alone cannot explain this correlation. Long range physical interactions like diffusion or electric attractions should be involved. A biophysical model (BM) has been formulated which cooperates with the biomolecular processes and describes the data successfully.

Hundred years ago E. Noether made a fundamental discovery in Mathematics and Physics. She proved rigorously that a physical system obeying a symmetry law (e.g.rotations or self similarity) is linked to a conserved physical quantity. It is argued here that HGC obeys a 'primitive' self similarity symmetry of the genes of a Hox cluster along a finite straight line. In the case of Vertebrates, the associated partially conserved quantity is the ever increasing 'ratchet'- like gene ordering where some Hox genes are missing. Another application of Noether's Theory is performed to rotationally symmetric embryos like the sea urchin.

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1 Introduction

In 2018 the anniversaries of two major scientific discoveries were celebrated. One in Biology for the forty years of Hox Gene Collinearity that was formulated by E.B. Lewis in 1978 [1- 2] . The other was the centenary of the far reaching theory in Mathematics and Physics as introduced by Emmy Noether [3 - 4]. In the present paper I will try to show how these two groundbreaking discoveries may be linked.

In 1918 Emmy Noether proposed a rigorous and elegant corelation between two fundamental concepts: Symmetry and Conservation laws. In simple terms Noether proved that, under some well founded conditions in Classical Mechanics, a physical system obeying a symmetry law is necessarily associated to a conserved physical quantity [3 – 4]. For instance, the physical laws which are symmetrical under translations in space (freedom to choose the origin of the coordinate system) lead to the conservation of momentum. Similarly, energy conservation is a consequence of the symmetry under time translations [3, 5]. Symmetry is a very broad concept with many facets covering esthetic, philosophical, physical and mathematical aspects. Hermann Weyl gave a broad definition as follows: A body, a spatial configuration, is symmetric with respect to a given plane E if it is carried into itself by reflection (mirror image) in E [6]. Similarly, the operation of rotations on physical bodies may define rotational symmetry: e.g. a rotation of a body around a given axis, say by 30^0, carries every point of the body to another point which belongs to the body. Thus, according to Pythagoras, the circle and the sphere are completely rotationally symmetric (invariant) in two and three dimensional spaces respectively [6] .

Many operations (not only geometric) can be applied on physical bodies or entities which may lead to an invariance (symmetry) related to this particular operation. Noether's theory (NT) proved very helpful in the solution of Physical problems even in the most modern fields of elementary particles and quarks. Given a symmetry, the search is directed to discover the corresponding conserved quantity (particle) and vice versa. For instance, testing the validity of some Supersymmetry theories applied to elementary particles, a corresponding conserved quantity (particle) was expected [4, 7]. The lasting search for this particle led to the discovery of the Higgs boson in the CERN Laboratory in 2012.

2 Hox Gene Collinearity on a straight line

Hox genes are a group of genes playing an essential role in the development of most animals including the vertebrates (hence also the humans). Some of these genes are grouped in clusters and denoted Hox1, Hox2, Hox3,…… Numbering starts at the 3' end of the telomeric side of the chromosome and it is directed toward the 5' end of the centromeric side of the DNA sequence.

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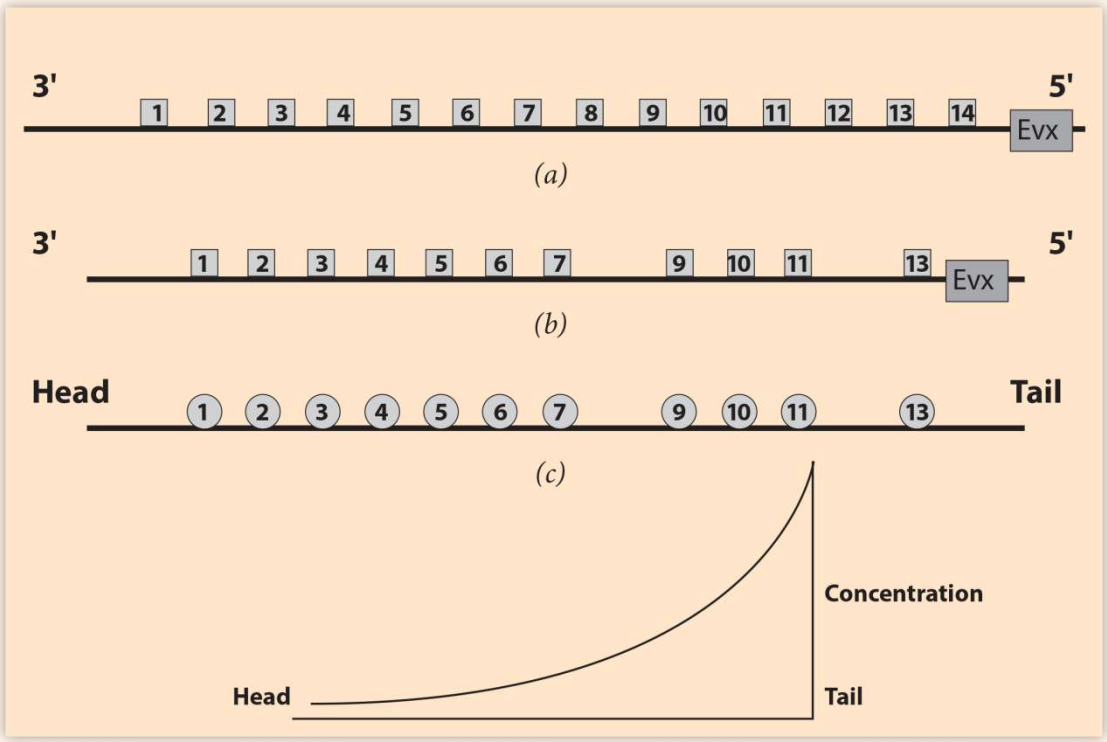
In Fig.1a, a (theoretical) common ancestor Hox cluster is depicted together with a prototypic animal clade, the mouse HoxA cluster [8]. In Fig.1a,b the distances between the Hox genes are not faithfully represented.



[Figure 1]

Orderings of Hox genes and ontogenetic units. (a) Hox gene ordering of a (theoretical) common ancestor. Gene **Evx** is close to the 5'end of the Hox cluster. (b) Ordering of the mouse HoxA cluster. Hox8 and Hox12 are missing. (c) The corresponding ontogenetic units along the A/P axis. (d) The observed monotonic concentration gradient of a morphogen. The peak is at the tail region.

The Even Skipped Gene (Evx) lies close to the centromeric side of the mouse HoxA cluster. It is believed that the tandem duplication of an ancestral ur-Hox gene followed by several evolutionary modifications may be the origin of these organized Hox gene arrays [9]. Many other hypotheses have been proposed based on new data on gene duplication and gene ordering [10].

In the early stages of embryogenesis, one can ontologically divide the embryo into body Domains D1, D2, D3,… following the Anterior/Posterior axis from Head to Tail (Fig.1). E.B. Lewis noticed that some Drosophila Hox genes are activated in a collinear manner [1]. Schematically, Hox1 is activated in D1, Hox2 in D2, Hox3 in D3 … For instance, in the cells of D1 only Hox1 gene is transcribed. In the cells of D2, Hox2 gene is transcribed … The clear cut expressions of the Hox genes in the ontogenetic domains D1, D2… of the vertebrates are only schematic. In reality these expressions are partially overlapping and nested gradually toward the

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tail end of the embryo or the limb bud tip e.g. [2, 11]. This nested pattern denoted as Hox Gene Collinearity (HGC) can be easily incorporated in the present representation. In addition the spatial and temporal features of HGC are still intensively studied in several animal species [12]. The above particular local transcription of the Hox genes is a very surprising event that proved later to be a universal property widespread to most animal clades where the Hox genes are grouped in clusters [8].

The linear size of the Hox clusters extends up to some hundreds of nanometers (nm) whereas the linear size of an early embryo (Head to Tail) is about 1 millimeter (mm) (Fig.1). The sizes of these two entities (gene cluster and early embryo) differ by more than four orders of magnitude. In HGC the unexpected one-to-one correspondence of the two sequences constitutes an enigma of Developmental Biology. Several explanatory models were proposed which can correctly reproduce many of the performed genetic engineering experiments [e.g.11, 13]. All these models are based solely on well established biomolecular processes responsible for gene activation or inhibition. Nevertheless the collinearity phenomenon is still an enigma [2]. Notice that the multiscale nature of HGC hints at the action of long range forces which may cooperate with the biomolecular mechanisms. Physical processes like Diffusion or Electric attraction can trigger such forces [14].

As early as 2001 I started formulating a Biophysical Models (BM) which combines long range gradients based mainly on passive Diffusion causing pulling forces at the level of the Hox clusters [14 -17]. For many years this model was poorly endorsed until novel experimental techniques enabled the observation of geometric deformations of the Hox clusters (e.g. elongations) during Hox gene activation [18 – 20]. These novel observations can be explained by the action of physical forces [15]. According to BM, a monotonic morphogen gradient is established along the Anterior-Posterior axis of the embryo (Fig.1) [21, 22]. In the following, the morphogen signals are transduced inside the cell and produce specific molecules which are allocated in particular positions inside the cell nucleus. The existence and action of such molecules is thoroughly studied [23- 24]. In analogy it is here assumed that similar molecules P are produced and fixed facing the Hox cluster (Fig. 2). It is further assumed that the P molecules are positively charged while the Hox gene cluster is negatively charged (N). An attractive force F acting on the Hox cluster is created resembling a truncated Coulomb force [15, 16].

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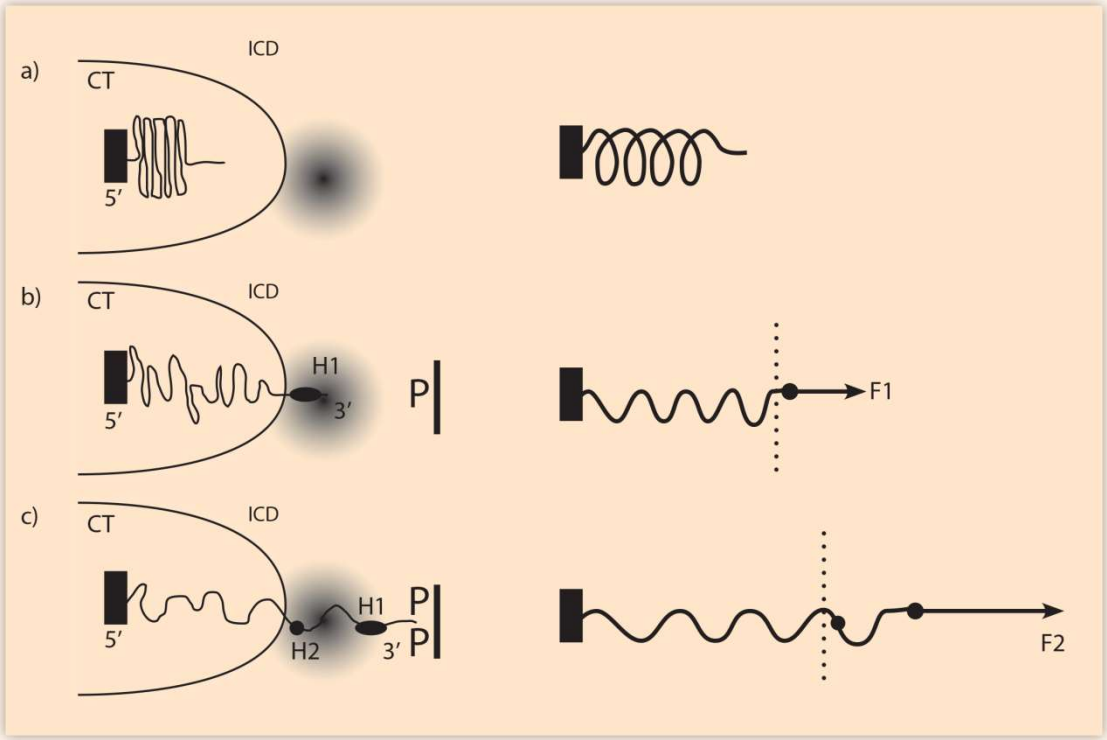

[Figure 2]

Hox cluster elongation and its mechanical analogue (adapted from S. Papageorgiou (2012) Curr.Genomics 13: 254-251). (a) (Left) Before activation the Hox cluster is condensed inside the chromatin territory (CT). (Right) Mechanical analogue of an uncharged elastic spring.fixed at its left left end. (b) (Left) The fixed pole P extrudes Hox1 (H1) outside the cluster in the interchromosome domain (ICD) towards the transcription factory region (shadowed). (Right) A small force F1 expands the spring. (c) (Left) The attracting pole P increases (PP) and Hox2 is also extruded. (Right) A stronger force F2 elongates the spring further.

A simple heuristic form of the forces is:

$$\mathbf{F = P \times N} \qquad (1)$$

The factor **P** in (1) varies according to the cell position along the morphogen gradient in the anterior-posterior axis. **P** is high at the tail and low at the head. The physical force **F** pulls gradually the Hox genes to a region where gene activation is possible (Fig. 2). In this model the forces depend on both macro and micro influences and the Hox cluster behaves like an expanding elastic spring [25, 26]. In the Evolutionary process, each Hox gene of the cluster is endowed with specific features and a functional role. In this course, some of these genes are even lost (deleted) (see Fig. 1a). Even in the case of missing genes the ordering remains ever increasing like a 'ratchet'. For instance the ordering (1, 2, . , 4 , 5,…) may be observed but the ordering(1, 2, . , 5 , 4,…) is not allowed. (Fig.1a). This Hox gene ordering and the ontogenetic units of the embryo are located on a finite line with discrete ends (Fig.1b,c).

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A phenomenon or object is self-similar if the image of the part is similar to the image of the whole. E.g. the image of a coastline is the same, independently of the scale of the image. Similarly, the branching picture of a neuron is the same, independently of the magnification of the drawing. These phenomena or objects were coined 'fractals' by Mandelbrot who was the first who studied them consistently [27]. In this analysis, self similarity is a continuous symmetry applying to all dimensions (scale invariance). Next step is to incorporate the fractals in NT: self similarity is the symmetry of fractals and the conserved quantity is the generic pattern (e.g. the scale invariant coastline).

The genes of a Hox cluster and the embryonic body elements of the vertebrates are somehow 'self similar' objects embedded in the same space, namely the one dimensional finite line with distinct ends. The Hox gene collinearity is a 'primitive' self similarity on a straight line, since it applies to only two discrete dimensional scales (genetic and embryonic). In contrast, the proper self similarity is a continuous symmetry applying to all dimensions.

3 HGC in Rotationally Symmetric Animals

The above primitive collinearity applies to 'directly' developing organisms like arthropods or vertebrates whose major body elements are formed at once during embryogenesis along the A/P axis [28]. However, besides these directly developing animals there is a large variety of invertebrates which, at the very early stages of embryogenesis, are 'indirectly' developing a larva, where the adult body shape is formed. This body plan differs from the initial A/P organization [28, 29]. The powerful mechanism of Evolution drives the creation of a new species by imposing a novel symmetry at the larva stage. For instance, twenty nine hours after fertilization in the larva stage of Echinoderms a rotational symmetry is superimposed on the linear symmetry (A/P) of the initial embryo [30]. In Fig. 3a(left) the larva of Holopneustes purpurescens is drawn with the morphology of five podia A-E located on a closed contour on top of the initial A/P axis [30].

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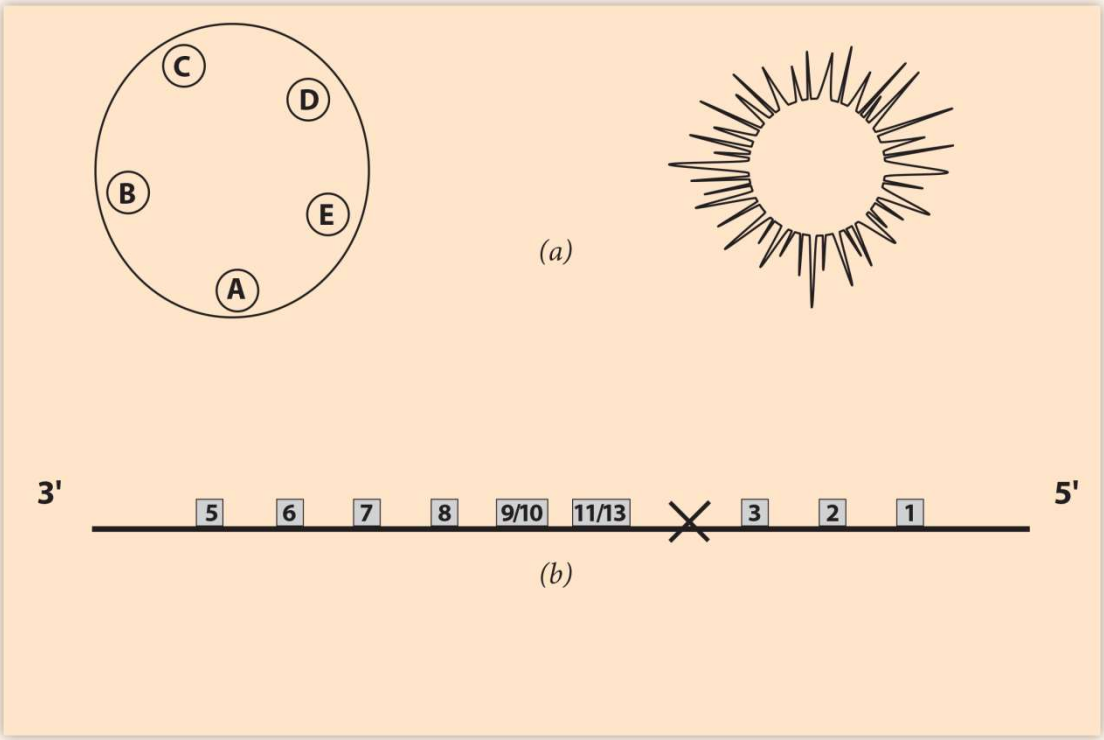

[Figure 3]

Developmental stages of the echinoderms. (a) (Left) Holopneustes purpuratus vestibula larva with A, B, C, D, E the podia locations [30]. (Right) Juvenile sea urchin. (b) The Hox gene cluster ordering of the sea urchin. Hox4 is lost.

The finite line with distinct ends is substituted by this rotationally symmetric contour. In Fig. 3a(right) an adult echinoderm (sea urchin) is depicted.

After the powerful new sequencing techniques developed in the last decade or so, many unexpected data were discovered concerning the Hox gene ordering of the rotationally symmetric animals. For instance, the Hox gene cluster of the sea urchin was surprising (Fig.3b). The normally anterior class of genes (Hox1, Hox2, Hox3) are ‘ translocated and inverted’ to the 5’ end of the cluster as shown in Fig.3b [31, 32]. This reorganization is coined TAI and many hypotheses have been put forward to explain its cause [32, 33].

Vertebrates and the major deuterostome clades (Echiniderms included) have a common ancestor [33]. The Vertebrates retain the ancestor’s Hox gene ordering while the Echinoderms bifurcate from this common ancestor’s Hox gene ordering [32, 33]. Below, as a typical example, only the case of the Vertebrates will be compared to the Echinoderms.

According to the self similar hypothesis proposed here, without breaking the relative Hox gene ordering, a bending of the linear arrangement brings Hox1 close to Hox13 (Fig.4, left).

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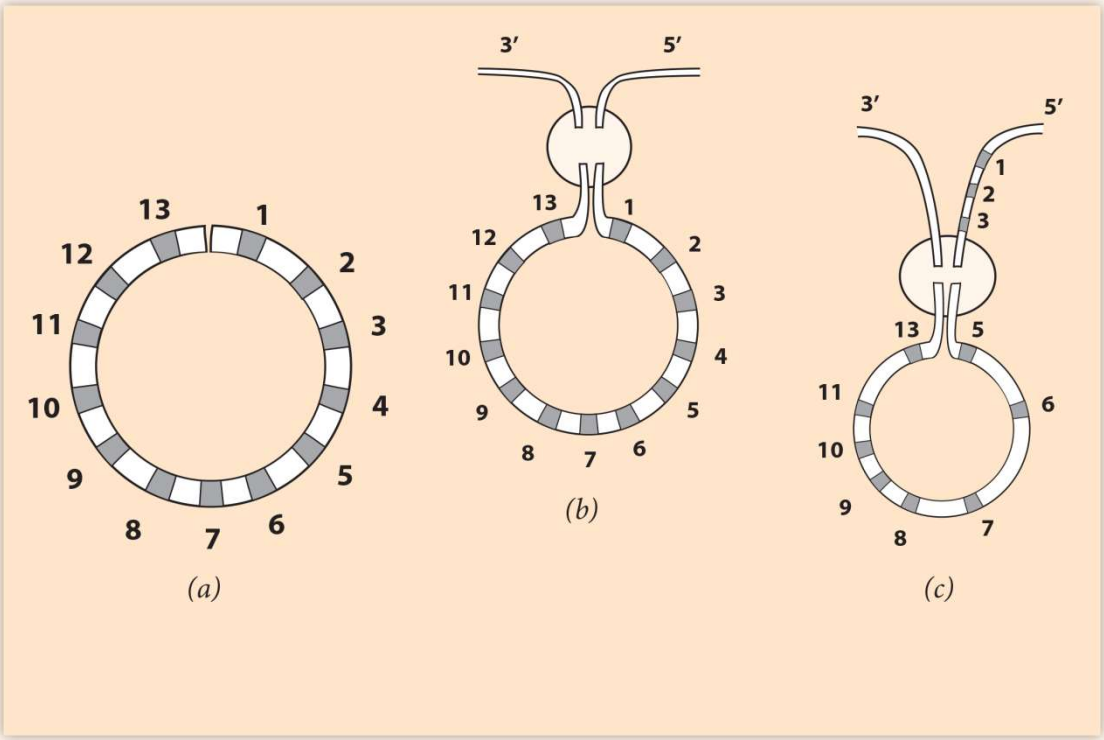

[Figure 4]

Circular ordering of Hox genes. (Adapted from S. Papageorgiou (2016) Current Genomics 17: 444-449). (a) The linear DNA is twisted and the two ends of the Hox cluster come close together. (b) In the encircled domain the ends Hox1 and Hox13 of the cluster are connected to to the 3' and 5' end of the flanking chromosome. If Hox1 is is attached to the 3' end and Hox13 to the 5' end, the linear arrangement represents produces the A. planci gene ordering. (c) If Hox5 is connected to the 3' end and Hox13 to Hox3 on the flanking chromosome, the linear ordering is the sea urchin Hox cluster arrangement as shown in Fig.3.

In this figure are depicted the 13 Hox genes in a circular ordering like a clock face where the clock 'hours' and the sequencing is self-similar to the rotationally symmetric larva body elements [34]. How this symmetry change is imposed remains to be clarified. After the establishment of the new symmetry the circular contour has to be incorporated in the DNA sequence. The DNA fiber undergoes a double strand break shown in Fig.4 (middle). Such events are spontaneous and they are the powerful force of Evolution. This break enables two switching combinations to the ending Hox1 and Hox13 genes of the Hox cluster.

If Hox1 and Hox13 are attached to the 3' and 5' chromosome ends respectively, no novel DNA sequence is created. This case could explain the Asteroid planci Hox cluster ordering. Although this Asteroid is rotationally symmetric, its Hox gene cluster ordering is comparable to the normal vertebrate ordering [32]. However, a novel Hox gene sequence will be created if Hox1 and Hox13 are attached to the 5' and 3' ends of the flanking chromosome respectively (Fig.4, right). If a subsequent break occurs at the level of Hox4, the reshuffling indicated in Fig.4 (right) leads to the Hox gene linear ordering of Fig.3b. At the location of the break, the Hox4 is

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deleted. This resulting gene sequence is the observed ordering of Hox genes in the sea urchin Hox cluster [34].

4 Hox Gene Collinearity and Noether's theory

a) Since HGC is a 'primitive' symmetry of self similarity, it is reasonable to examine whether a modified NT could be applied to this case. The main exploration is to search for an associated constant quantity in every directly developing animal species. From an inspection of embryos with an Anterior/Posterior structural organization and their Hox gene orderings, the ever increasing 'ratchet' type gene ordering could serve as a partly conserved quantity as depicted in Fig.1b. In this ordering no gene reversal is allowed. It seems that the lost Hox genes play an important role in the directly developing animals (see Fig.1b). Therefore, HGC with its primitve self similarity and the linked ratchet-type ordering of the Hox genes constitutes an approximate NT as applied to Complex Systems like Biology.

b) The above approximate NT for embryos developing on a linear A/P axis can be extended to rotationally symmetric animals. In this case there are no missing Hox genes in the circular ordering as shown in Fig. 4(left). As in the directly developing animals, the symmetry is a primitive self similarity of a circular organization applied to two scales only (the Hox cluster and the embryo). In the case of the sea urchin, the circular Hox cluster is incorporated in the flanking chromosome (Fig.4). Then a double strand break occurs in the domain of Hox4 and the 'open' Hox cluster is incorporated in the flanking DNA. The resulting gene ordering is shown in Fig.3b. The deleted Hox4 is the only missing gene (Fig. 3b). In vertebrates the missing genes were related to a partially conserved quantity- the ratchet type ordering of the Hox genes. It should be interesting to clarify if the Hox4 loss is the cause or the effect of a partially conserved gene ordering in the sea urchin.

5 Conclusions

During Hox gene activation, Hox clusters are gradually elongated. Such elongations were observed by super resolution imaging (STORM) in the mouse HoxD cluster [35]. This finding supports the hypothesis of pulling forces of BM with the associated ratchet -type ordering of the Hox genes. HGC is the outcome of these forces [17, 25]. The vertebrate Hox gene clusters obey a primitive symmetry of self similarity and the ratchet- type ordering of the Hox genes could be the partly conserved quantity in the Noether theory spirit. Therefore, HGC is the remnant of a Noether theory as applied to animals developing along an Anterior-Posterior axis.

The various phyla of rotationally symmetric animals should be compared to the Vertebrates which retain the common ancestor's Hox gene cluster ordering. This comprehensive comparison could probably reveal, following NT, a relation between the animal morphological symmetries and the different gene orders in their Hox clusters.

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Several spatial and temporal features of the Hox gene expressions in echinoderms are already reported [32]. A similar analysis for other rotationally symmetric animals with differing morphologies and Hox gene cluster orderings could be illuminating.

A hypothesis was proposed according to which, during Hox gene activation, preceding physical forces organize the geometry of the Hox clusters followed by the biomolecular interactions [35]. This prediction is in agreement with the conclusion that 'the data suggest the structural organization of the HoxD gene cluster may predate transcriptional activation' [19].

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