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Review

Hox Gene Collinearity with Pulling Physical Forces Creates a Hox Gene Clustering in Embryos of Vertebrates and Invertebrates: Complete or Split Clusters

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Abstract: Hox gene clusters are crucial in embryogenesis. It was observed that some Hox genes are located in order along the telomeric to centromeric direction of the DNA sequence: Hox1, Hox2, Hox3. . .. These genes are expressed in the same order in the ontogenetic units of the Drosophila embryo along the anterior–posterior axis. The two entities (genome and embryo) differ significantly in linear size and in-between distance. This strange phenomenon was named spatial collinearity (SP). Later, it was observed that, particularly in the vertebrates, a temporal collinearity (TC) coexists: first Hox1 is expressed, later Hox2, and later on Hox3. . .. According to a biophysical model (BM), pulling forces act at the anterior end of the cluster while a cluster fastening applies at the posterior end. Hox clusters are irreversibly elongated along the force direction. During evolution, the elongated Hox clusters are broken at variable lengths, thus split clusters may be created. An empirical rule was formulated, distinguishing development due to a complete Hox cluster from development due to split Hox clusters. BM can explain this empirical rule. In a spontaneous mutation, where the cluster fastening is dismantled, a weak pulling force automatically shifts the cluster inside the Hox activation domain. This cluster translocation can probably explain the absence of temporal collinearity in *Drosophila.*

Keywords: Hox gene collinearity; temporal collinearity; Noether theory; self-similarity; double strand break; split Hox clusters; chicken limb growth

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1. Introduction

In 1978 E.B. Lewis discovered a fundamental property of developmental biology: Hox gene collinearity (HGC). HGC is the long-range interaction of entities where the archetypical example is the Coulomb force relating material objects in different geometric domains. In particular, HGC relates genes (entities inside the cell nucleus) with embryonic entities. The early embryo and the cell nucleus sizes differ by about four orders of magnitude.

Hox genes play an important role in the development of most animals (plants do not have the animal Hox genes). Some Hox genes form clusters which are crucial for the embryogenesis of Metazoa. The importance of this clustering was noticed by Lewis, who studied the genetics of Drosophila [1]. He observed that some genes of the genome (later coined Hox genes) were located in order along the telomeric to centromeric direction and were denoted (Hox1, Hox2, Hox3. . .). Lewis noticed that the genes of these clusters were expressed in the same order along the anterior–posterior axis of the Drosophila embryo (Figure 1) [1]. This is an astonishing event since this correlation occurs between extremely distant domains—on the one hand the gene sequence in the cell nucleus and on the other the Drosophila embryo. Biomolecular interactions alone cannot create such correlations [2]. This surprising phenomenon was named spatial collinearity (SC). Some years later, another collinearity was observed particularly in the vertebrates: temporal collinearity (TC) [3]. According to TC, the first Hox gene (Hox1) of the Hox cluster starts being expressed. Later, Hox2 is expressed, and later on Hox3 followed until all Hox genes are expressed following the sequence Hox1, Hox2, Hox3. . . [3].

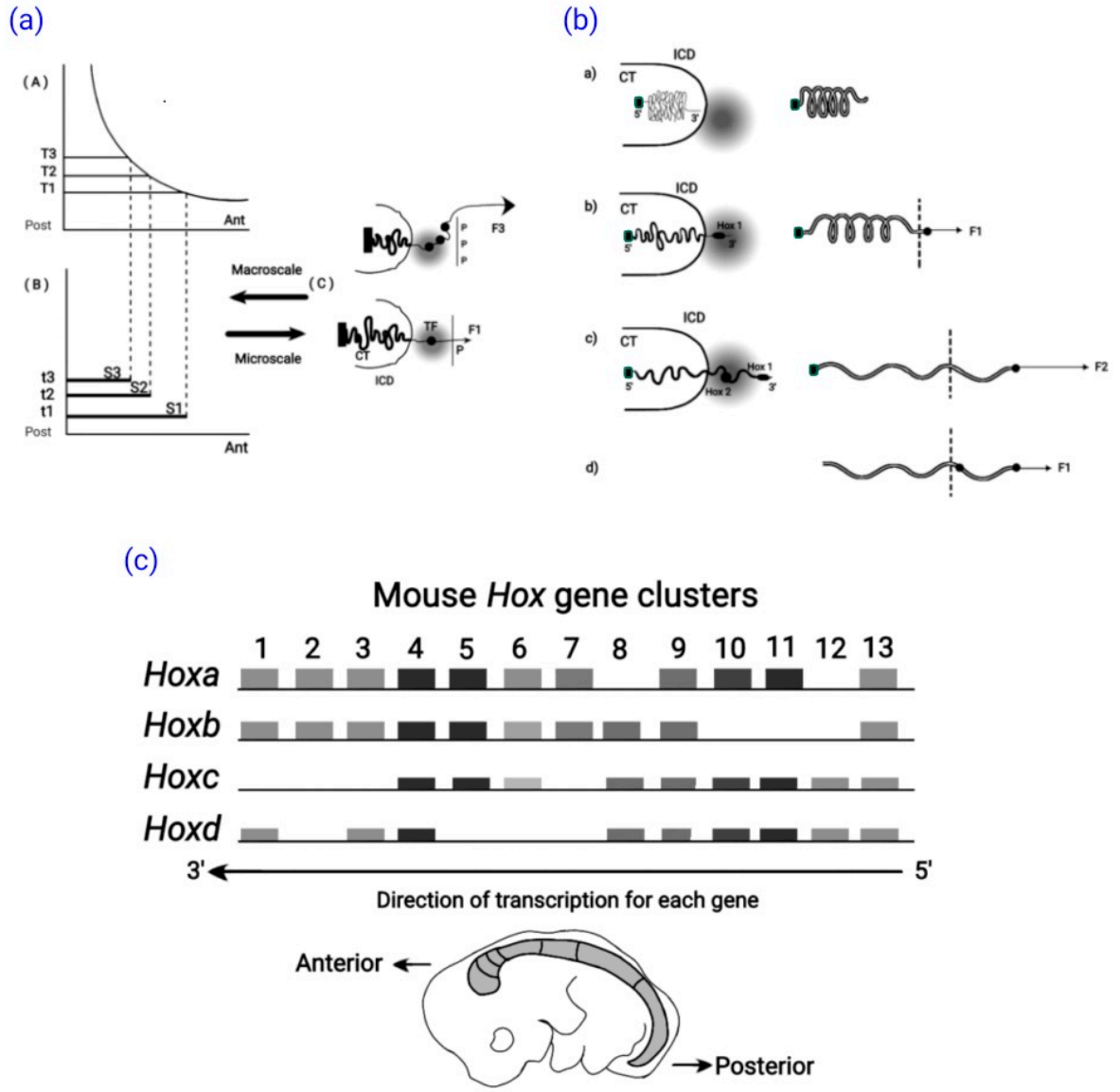

Figure 1. (**a**) **Macro-scale and micro-scale Hox gene clustering** (adapted from S. Papageorgiou, Biology 2017: 6, 32). (A) Morphogen gradient concentration (T1, T2. T3). The gradient peak is located at the posterior end. (B) Time sequence (t1, t2, t3) combine with (T1, T2, T3) for expression domains S1, S2, S3 of Hox1, Hox2, Hox3. (C) (bottom) A small force F1 pulls Hox1 out of the chromatin territory (CT) toward the interchromosome domain (ICD) and the transcription factory (TF) regime (grey domain). Polar molecule P is located opposite the telomeric end of the cluster. At a later stage (top), a stronger force F3(3P) pulls Hox1, Hox2, Hox3 out of CT toward TF. (**b**) **Mechanical analogue of Hox cluster decondensation** (adapted from S. Papageorgiou, Current Genomics 2012, 13:3). (a) (left) Before gene activation, Hox cluster is compacted inside (CT). (right) Mechanical analogue: uncharged elastic spring fixed at its left end. (b) (left) BM pulling force decondenses the cluster and Hox1 is extruded in (ICD) in the transcription factory (TF) domain (shadow disc). The cluster is fastened posteriorly. (right) A small force F1 slightly expands the spring and black spot moves beyond the dashed line. Spring fixed posteriorly. (c) (left) Hox cluster is further decondensed and Hox1, Hox2, Hox3 move in (ICD). (right) F2 > F1 and the spring is further expanded. d) (left) The fastening of the cluster is removed and, with a slight force F1, the cluster can slide beyond the dashed line. (right) The loose spring slides freely beyond the dashed line. (**c**) **Mouse Hox gene clusters** (adapted from Z. Afzal and R. Krumlauf, J DevBiol 2022) HoxA, HoxB, HoxC, and HoxD are depicted in the direction of transcription for each gene. The mouse embryo is shown in the anterior–posterior direction of Figure 1a, where the gradient peak is at the posterior end (Figure 1a).

In order to explain these phenomena, a biophysical model (BM) according to which, pulling physical forces could explain the experimental findings [2,4] (Figure 1a,b). Several predictions of BM were compared to many collected data and the comparison confirmed BM [5,6].

A simple heuristic expression for these pulling forces F was proposed [7,8].

$$F = N * P \quad (1)$$

In Equation (1), F is a simplification of the pulling Coulomb force since the distance between the electric charges (N and P) is missing. This 'quasi-Coulomb force' F is applied at the telomeric end of the Hox cluster (Figure 1a). It turns out that this arbitrary omission of the Coulomb range reflects a deep connection of these 'quasi-Coulomb' forces to the fundamental phenomenon of symmetry (see Sections 2.1 and 2.2). N and P stand for the negative and positive electric charges acting on a Hox cluster [7,8]. In the above heuristic formulation, the Hox cluster consists of a deployed finite sequence of Hox genes along the telomeric to centromeric ends of the cluster (Hox1, Hox2, Hox3. . .). The numbers assign the gene order in the cluster. These numbers determine the order membership to the paralogy group (PG) (here, Duboule's definition is followed [9]).

As mentioned above, (N) represents the microscopic contribution to F and it is a real entity—the negative electric charge of the DNA sequence in the cell nucleus. (P) represents a positively charged molecular structure located opposite the telomeric end of the Hox cluster (Figure 1). Contrary to N, P is a fictitious entity as yet, standing for the embryonic-macroscopic contribution to F. Note that the known morphogens of the present time, like sonic hedgehog, fibroblast growth factor, retinoic acid, and the plethora of other morphogenetic factors, were fictitious fifty years ago. The existence of P does not contradict any first principle, so it is legitimate to anticipate its existence as advocated in [4]. F pulls the Hox genes sequentially out of the cluster (Figure 1). As mentioned already, Equation (1) is a heuristic expression that was successfully tested in several experiments [5–8].

Hox genes control the normal development of animals (*wild type)*. Spontaneous mutation of these genes causes severe malformations (*Homeosis*), consisting of parts of the animal growing in the wrong location of the body. In *Homeosis,* PG ordering is violated.

About twenty years ago, an important advancement was achieved concerning the transfer of specific molecules from outside the cell into the inner domain of the cell nucleus [10–13]. For example, it was noticed that significant amounts of activin are gathered outside the cell nucleus. Controlled amounts of this activin were transduced inside the nucleus, causing specific modifications on the genome. It is assumed that BM combined with the action of transduction technology can affect Hox gene expression. This possibility is incorporated in the present review.

BM is based on the hypothesis that pulling forces are applied at the telomeric end of the Hox cluster. This hypothesis was elaborated in detail, and it was concluded that the cluster is elongated along the direction of the force [14]. This BM prediction was later confirmed [15–17]. In some cases, the measured elongation of the activated Hox cluster was five times longer than the length of the inactive Hox cluster [16]. When Hox cluster activation is initiated, a weak force (F1) pulls the first gene of the cluster (Hox1) out of its niche toward the interchromosome domain (ICD) (Figure 1a,b).

Particularly Hox1 is directed towards the transcription factory domain (TFD), where Hox gene activation (expression) is possible [18,19]. The pulling forces increase irreversibly: (i) increasing along the gene location from Hox1 towards Hox13 (Hox1 → Hox13), and (ii) following the time course t1 → t3. Under force F2 (F2 > F1), Hox1 and Hox2 are extruded from their niche. This process continues until all Hox genes of a complete Hox cluster are transferred to the TFD. In the mechanical analogue, for the efficient function of an elongated elastic spring, besides the pulling force at one of its ends, a proper fastening should be applied at its other end. Accordingly, the Hox cluster should be fastened at the centromeric end of the cluster (Figure 1b).

The vertebrate Hox clusters comprise four homologue clusters (HoxA, HoxB, HoxC, and HoxD), as shown in Figure 1c [11]. Each homologue cluster is included in a separate chromosome. In these homologue clusters, the PG identity is conserved. However, in the course of evolution, modifications of the mouse genes are possible up to the point of gene deletion. These ordered mouse Hox clusters are reminiscent of a 'ratchet', allowing motion in one irreversible direction only [8]. Note that some 'teeth' of the ratchet may be missing (corresponding to Hox genes deleted in the course of evolution).

Besides vertebrates, the contemporary cephalochordate Amphioxus is a descendant of the ancestor Amphioxus. This ancestor Amphioxus was the ancestor of both Drosophila and vertebrates [9]. Amphioxus lived after the Cambrian period of evolutionary explosion 500 million years ago (Mya). Vertebrates and Drosophila appeared a few Mya later. Amphioxus has 14 Hox genes, whereas vertebrates and Drosophila have 13 (Hox14 is missing).

2. Symmetries

2.1. Symmetry Is the Cornerstone of Science and Several Other Human Intellectual Activities

Many distinguished scientists have proposed their definition of the term. When an action is applied on any material object (or physical system), it causes its change. If this change leaves the system invariant, the system is symmetric. This means that any point of the system moves to another point contained in the system. I consider the compact definition of Frank Wilczek (in the form of an aphorism) appropriate: symmetry is a change without change [20]. The human intellect incorporates a wider realm than pure scientific thinking. Therefore, Wilczek's definition of symmetry could be complemented with unusual thoughts e.g., 'symmetry is complicated', 'symmetry is beautiful', or even 'lack of symmetry is ugly'.

2.2. Noether's Theory in Developmental Biology

In 1918, Emmy Noether formulated and proved in classical mechanics a fundamental theorem on symmetry. In simple terms, Noether proved that any physical system obeying a symmetry law is escorted by a conserved physical quantity [21]. For example, if the physical system is invariant under time translations (that means it is independent of when the origin of time measurement is set), the energy of the system is conserved [21]. The significance of Noether's theory is evident. Its applications extend from the symmetries in classical mechanics to the complicated symmetries of the elementary particles—the numerous constituents of the universe [20–22].

Besides the obvious external symmetries in space and time, there appeared in the last century the need to introduce several internal symmetries and particularly in the field of elementary particles where these material constituents are given exotic names like bosons, quarks, charmed particles, mesons, etc. [22,23]. Historically, in 1932, W. Heisenberg was the first who introduced such esoteric terms (e.g., the isotopic spin or isospin to describe the symmetry of interchanging protons and neutrons under the strong nuclear interactions [23]).

Another important application of Noether's theory is the following: assume that the equations of a dynamic system are invariant under space translations. According to Noether's theory, such a symmetric system is necessarily followed by a conserved quantity—in this case, it is the momentum [22,23].

The heuristic equation introduced in the Introduction has the form of Equation (1), which is a 'quasi-Coulomb' force. The proper Coulomb force (CF) is

$$CF = (Q1 * Q2)/R^2 \quad (2)$$

R is the distance between charges Q1 and Q2. The quasi-Coulomb force depends only on the electric charges N and P. The electric charges (Q1 and Q2) (or N and P) may be attractive (if one charge is positive and the other negative) or repulsive (if both charges are either positive or negative).

In the quasi-Coulomb force F, the dependence on R is missing. This arbitrary absence of geometry is motivated by sheer simplicity as mentioned in the Introduction. However, it turns out that this simplicity is unexpectedly related to the internal symmetries [23].

Among its numerous applications, Noether's theory was used in the study of important biological issues. Self-similarity is the particular symmetry of objects which, although different, look the same if depicted under suitable scale units. Such objects are fractals, where the part looks like the whole [24]. A typical example is the Barnsley fern that can be easily drawn using a computer program. B. Mandelbrot invented this branch of applied mathematics and introduced the term fractals [24].

Self-similarity is a continuous symmetry applying to all spatial lengths. The finite sequence of ordered Hox genes associated with the ordered embryonic units constitute a very limited 'primitive' self-similarity, since it applies to only two discrete spatial dimensions. Consequently, it is expected for only a remnant symmetry to emerge, as shown in Figure 1c.

In this figure, in the mouse homologue Hox gene clusters, only PG is preserved. The set of Hox genes is reminiscent of an irreversibly advancing 'ratchet', where some Hox genes are missing [8]. In this remnant self-similarity, only PG ordering is conserved and it is here assumed that it constitutes the conserved quantity of Noether's theory. Some Hox genes of the cluster may fade out up to extinction during the whole genome duplication of the evolutionary process, as mentioned above [8,11].

In another biological application, Noether's theory was recently used in a comparison of DNA sequences of different animal phyla [25].

In any measurement, symmetry in a variable quantity requires that this variable is absent in its constituent equations. In order to clarify this point, the reasoning of Iliopoulos is followed [23]. Consider a completely symmetric body (e.g., the sphere in 3D space) as described in a Cartesian system of axes (x, y, z) or a Polar coordinates system (z, φ, θ). Any measurement in the sphere contains the variable angles θ and φ. It turns out that the constituent equation of the sphere is:

$$x^2 + y^2 + z^2 = R^2 \quad (3)$$

where R is the radius of the sphere. In this equation, the variables (θ, φ) are indeed missing in agreement with the above symmetry requirement: no angular dependence is involved [23].

In Equation (1) for the quasi-Coulomb force F, the term R^{-2} is missing, so F is an even simpler equation than the proper Coulomb force. The meaning of this omission is that the heuristic pulling force of the BM is a quantity independent of the 3D geometric space.

Note that in this space, the symmetry of F becomes internal, reminiscent of Heisenberg's internal variable—the isotopic spin [23].

3. Insertion of a DNA Fragment in a Hosting DNA Sequence

Besides the ordering of Hox genes on a finite straight line, in many early larva embryos (e.g., the echinoderms) a circular ordering is superimposed on this finite line, as shown in Figure 2a(A).

In Figure 2a(B), the two ends of the circular cluster are attached to the 3′ and 5′ ends of the flanking chromosome. If the 3′ end of the flanking chromosome is attached to Hox1 (and 5′ to Hox13), no novelty is created and the *A.planci* normal Hox gene order gene is reproduced (Figure 2a(B)). In contrast, if the 5′ end of the flanking chromosome is attached to Hox1, Hox2, Hox3 (shown in Figure 2a(C)), a novelty is created. A second breaking follows, leading to a new Hox gene order, which corresponds to the Hox gene order of the *sea urchin* [8].

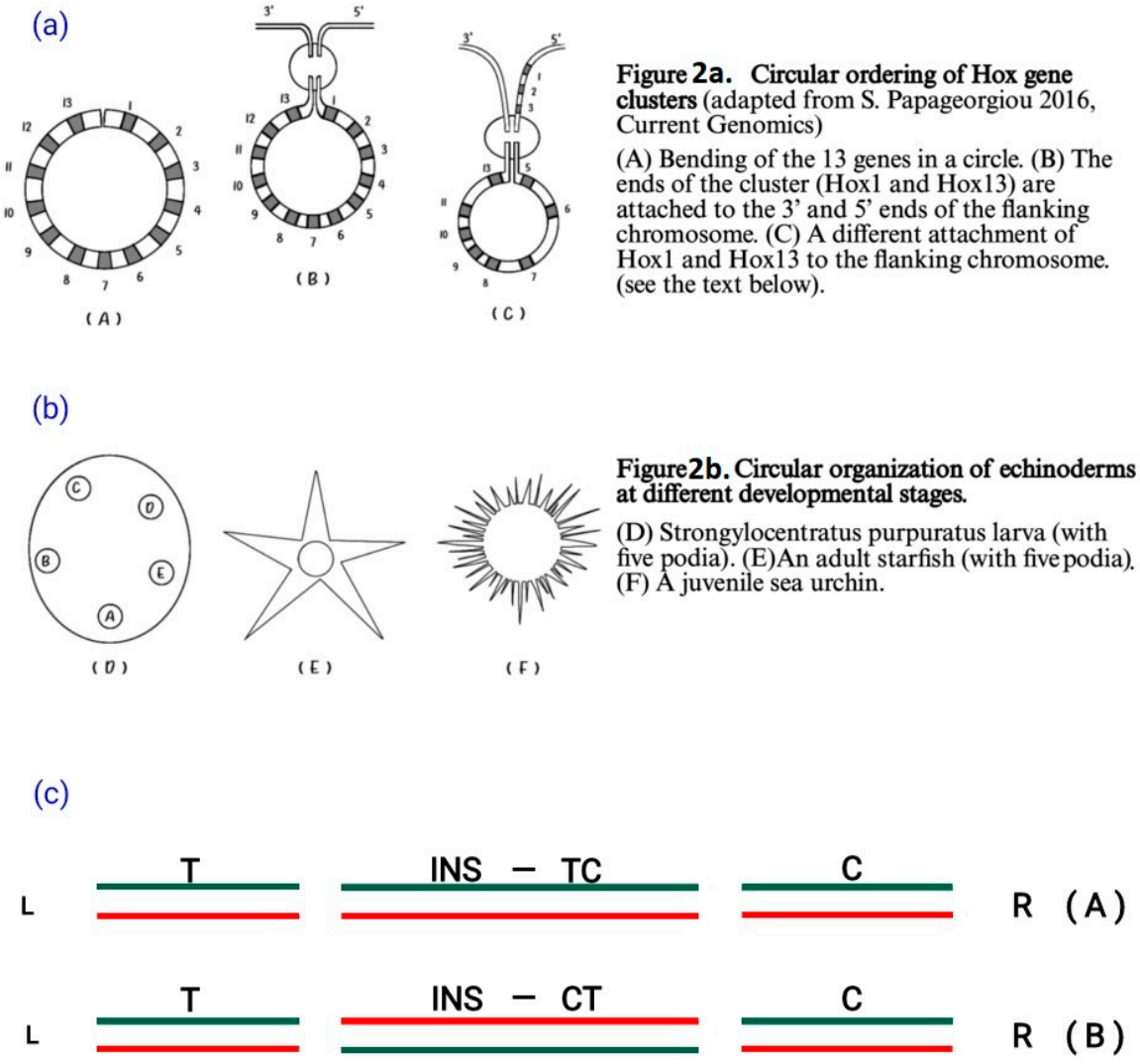

Figure 2. (**a**) Circular ordering of Hex gene clusters. (**b**) Circular organization of echinoderma at different development stages. (**c**) (A) L: Double strand break left (T) and right (C). In the middle, (INS-TC) follows orientation (T → C) R(B) L: Double strand break left (T) and right (C). In the middle, (INS) follows orientation (T ← C) R.

The circular Hox gene clusters can be incorporated in the flanking DNA sequence of the genome. A recent review by T. Hanscom refers to the well-known technique of double strand break (DSB) [26,27]. In the above review, besides the usual medical applications of the DSB methodology, the novel trends of research are extensively emphasized to explore how DSB can leverage genome evolution. In Figure 2, the incorporation of a Hox cluster (INS---) in the flanking genome is schematically depicted.

In Figure 2c(A), the inserted graft in the middle (INS) follows the orientation T → C. In Figure 2c(B), the inserted graft in the middle (INS-CT) follows the reverse orientation (T ← C)

4. Complete vs. Split Hox Clusters

Hox Gene collinearity (both spatial and temporal) has been unequivocally confirmed in the vertebrates. However, in recent years it was found that this is not true in many other animal species, particularly in invertebrates. For instance, it was observed that Hox collinearity is violated in the lophotrochozoa, and this violation was associated with the brachiopods whose Hox cluster is broken [26,27]. In brachiopods, both spatial and temporal collinearities are violated, while lophotrochozoan morphological novelties result from Hox collinearity violation [26]. It was argued that for the insertion of a circularly organized Hox

cluster in the flanking genome, a break (split) of the cluster is necessary. It is clear that Hox cluster splitting is a necessary step for evolutionary novelties.

It has been emphasized that tight Hox clustering is lost during evolution [28–31]. More specifically, D. Ferrier and P. Holland observed that Hox clusters are constrained by TC in their gene order [28,29]. Moreover, complete Hox clusters are associated with the spectrum of Hox gene expressions along the whole anterior–posterior embryonic axis. Otherwise, if the gene expression does not extend along the whole A–P axis, the Hox gene cluster is split. In this cluster splitting tendency, the Hox clusters may fall apart when TC is no longer needed [28,29]. Similar arguments were put forward by several other authors before and after the above observation [29,30].

Drosophila has a typically split Hox cluster. The Drosophila Hox cluster has 13 genes, consisting of two subclusters, ANT-C and BX-C, depicted in Figure 3 [29].

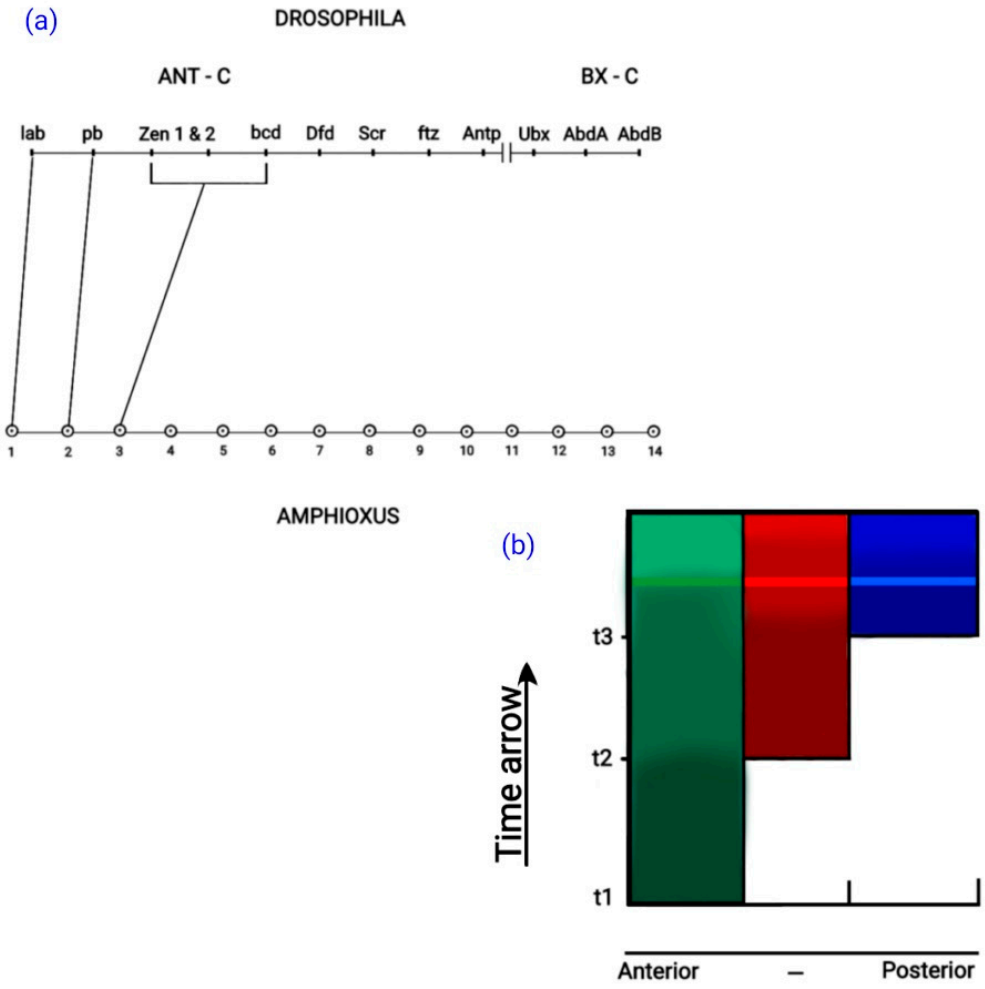

Figure 3. (**a**) **The ANT-C and BX-C subclusters of Drosophila and the complete Amphioxus cluster** (adapted from D.E.K. Ferrier in Hox Gene Expression editor S. Papageorgiou. Editions Springer and Landes Bioscience 2007). Only a part of the cluster correspondence between Drosophila and Amphioxus. The Drosophila [Zen1 and 2, bcd] genes have evolved from the ancestral Amphioxus 3 gene. (**b**) **Quantitative collinearity** The split clusters of Section 3 and quatitative collinearity of Section 5.3 are represented by the same figure. The horizontal narrow strip (above t3) is in agreement with PP—the intensity of the posterior part of the strip is stronger than the anterior part (see Section 5.4).

Drosophila, together with the vertebrate Hox cluster, originates from a large ancestral Hox cluster. Cloning had later identified Amphioxus as the common ancestor of insects and vertebrates [28], and a one-to-one correspondence between the Amphioxus Hox genes and

the Drosophila Hox genes was confirmed [28,29]. However, in this correspondence, some Drosophila Hox genes of the ANT-C subcluster developed novel evolutionary non-Hox functions. For instance, the Drosophila complex of Hox genes (zen1, zen2, bcd) corresponds to the ancestral Amphioxus Hox3 gene. Some central genes have evolved from tandem evolutionary duplications [29] (see in [29] Figure 1). The BX-C subcluster consists of the three last genes (Ubx, Abd-A, Abd-B) of the Drosophila Hox cluster. The summarizing conclusion from the above analysis is that TC is responsible for a complete Hox cluster. If this is not possible (or not needed), the Hox cluster is split [28]. In any case, SC is a necessity for a Hox cluster.

As stressed above, if TC extends to only a fraction of the Hox cluster range, the cluster is expected to split. In a way, Hox expression in a range between anterior and posterior ends ('space') is translated into 'time', where TC coordinates a 'Hox clock'—more exactly a 'Hox timer' since the time course is irreversible [30].

5. Discussion

5.1. Empirical Rule on Complete and Split Hox Clusters

With the above established knowledge, D. Duboule recently formulated a useful empirical rule (ER) for the Hox gene clusters [30]: A complete Hox cluster controlling development in time along the anterior–posterior axis is non-split, whereas animals developing according to a time-independent mechanism to produce their main body axis are licensed to split their clusters.

In a significant experiment of extended posterior upstream excisions, T. Kondo and D. Duboule noticed that several Hox gene expressions (and particularly Hoxd4 and Hoxd10) were unexpectedly absent as if temporal collinearity (TC) had disappeared [30]. In this case, TC disappearance is not real—it is only fictitious [31]. However, in a different interpretation of the above Kondo and Duboule experiment, a 'prediction in retrospect' of BM was formulated according to which TC disappears actually (and not fictitiously) [32–34] (notice analogies in the following Sections 5.2 and 5.3).

5.2. Development in the Secondary Developmental Axis

In chick limbs, it was observed that the apical ectodermal ridge (AER) controls development responding to morphogen fibroblast growth factor (FGF) [32]. The last Hox13 gene of the cluster switches off when the AER is cut-off. [32]. The consequences from this experiment are illuminating [32]: Hox13 expression can be initiated again if beads soaked in FGF are implanted distally. This can occur after a fixed time interval. If the FGF dose is increased, the Hox13 rescue occurs earlier. Furthermore, the rate of Hox13 spreading is faster initially and slower at later stages—a sign that passive diffusion is the main mechanism of signal propagation [32]. In the above chick limb bud experiment in the secondary developmental axis, Hox13 expression is most sensitive to AER excision [32]. However, Hox10 and Hox11 are less sensitive to this excision, indicating that TC is not uniform along the developmental axis.

5.3. Development in the Mouse Primary Anterior–Posterior Axis

It is interesting to compare the above limb findings to a similar experiment of upstream DNA excision in mouse embryos, as described in [33,34]. In this excision experiment, TC disappearance was in agreement with the BM pulling forces model [33]. According to BM, it is eventually expected for TC to reappear. This expectation remains to be tested [33]. To this end, the reverse experimental path (the insertion of TGF-beta signals) was proposed (a detailed description is included in [34]). The proposed disappearance experiment and the eventual reappearance of TC was not completed. The direct course of disappearance was confirmed but the palindromic course of TC reappearance remains to be tested and its eventual experimental confirmation will be decisive [34].

5.4. Quantitative Collinearity

Relying on Lewis' observation [1], A. Durston proposed a Hox cluster property (posterior prevalence (PP)) to guarantee the dominance of posterior Hox gene expressions over simultaneously expressed anterior Hox expressions [35,36].

Besides spatial and temporal collinearities, a third collinearity has also been traced: quantitative collinearity (QC). This was a puzzling issue, and for a long time it was examined following a parallel path with the PP hypothesis. QC is determined following two directions in the two-dimensional plane. First is the direction along the down–up time irreversible direction (Figure 3b) and second is the expression intensity along the anterior–posterior axis. For the HoxD expression in a sequence of cells along the horizontal dashed line, the intensity is stronger at the posterior side (Figure 3b) [14]. The intensity at any point depends on its distance from the morphogen source. It turns out that in the limb, passive diffusion is the main signal propagating mechanism whose size of spreading depends on the vicinity to the morphogen source [4,14]. It was measured that this size is higher near the morphogen source compared to the size of spreading at a distant location [32]. A similar mechanism applies for the expression of split clusters as mentioned in Section 3.

5.5. A Spontaneous Mutation in the Drosophila Case

In the Introduction, it was stressed that spontaneous genetic mutations can lead to evolutionary novelties, as in the case of *Homeosis*. If, in a spontaneous Drosophila mutation, the Hox cluster fastening is dismantled, as shown in Figure 1b, the slightest pulling force will automatically shift the cluster in the transcription factory domain. The repercussion on both genetic structure and function of the cluster will be dramatic: TC will automatically (and not gradually) disappear (Figure 1b).

According to the generally accepted argument mentioned before, TC is constrained to inexistence if TC is no longer needed [28–31]. This occurs when the complete Hox cluster slides inside the transcription factory domain. Therefore, the loss of TC in Drosophila could be ascribed to a spontaneous genetic mutation that suppresses Hox cluster fastening.

6. Complex Expression Patterns

According to BM, it is expected that complex patterns can be created by splitting the early (simply connected) Hox gene expression into expression domains separated by a 'ditch' zone (cf. Figure 4a) [37,38]. This splitting was already confirmed in 2013 [37]. Following this line of thought [37,38], BM predicts that a small DNA strip containing Hox10 and Hox11 has a strange expression behavior in time (Figure 4). These genes can be pushed in and out of the Hox cluster activation domain, which is depicted in the dark blue circle (Figure 4b). In the left graph, Hox10, Hox11, and Hox12 are activated. In the middle graph, Hox10 and Hox11 are pushed out of the activation domain. In the right graph, Hox10 and Hox11 reenter later in the activation domain, following the increased force of BM in the time course [37,38].

Recently, a local expression disappearance in the interdigital area was unexpectedly observed in limb digit condensations, as shown in Figures {2 (m, o) and 2 (n, p)}in [39]. These figures are reminiscent of the (Hox10 and Hox11 disappearance in Figure 4b of the present review. The above reminiscence is a further confirmation of the pulling forces of BM.

It is interesting that the theoretical prediction 'Biophysics precedes Biochemistry' [38] was experimentally confirmed soon after '. . .structural organization of HoxD cluster may predate transcriptional activation' [16].

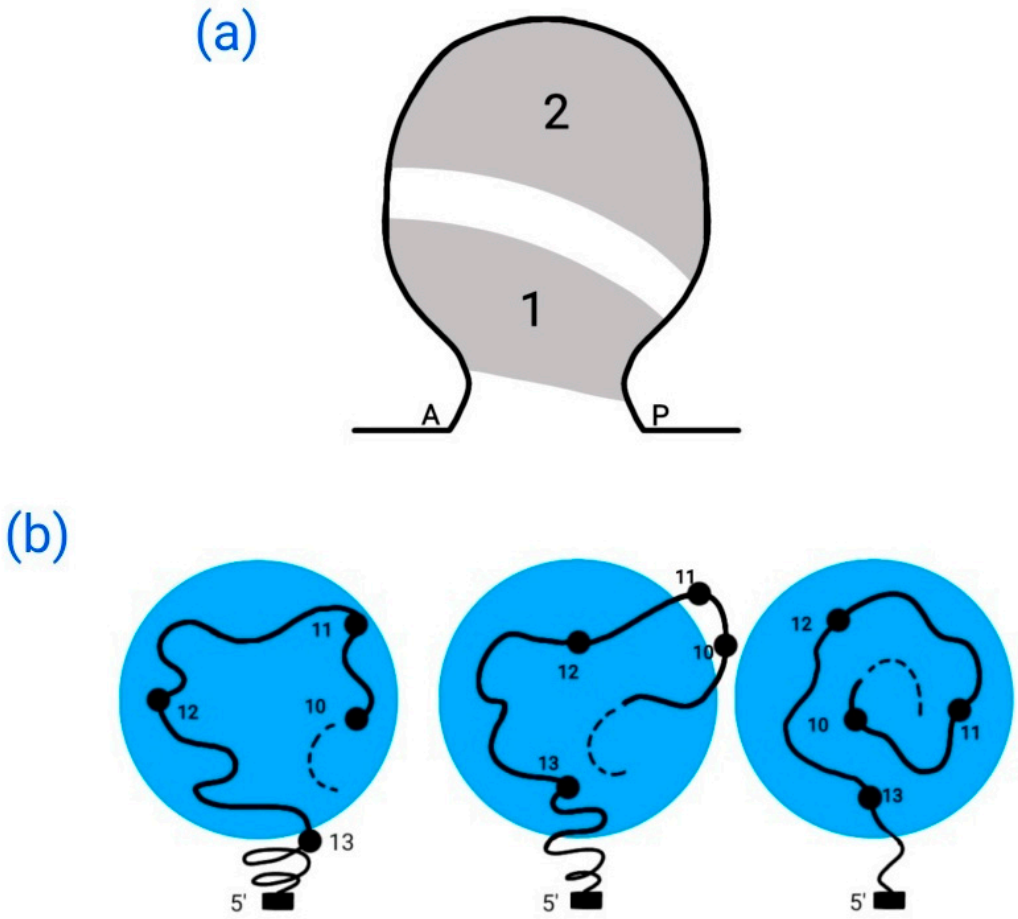

Figure 4. (**a**) Hox10 expression at stage E11.5, with a 'ditch' zone separating distal domain 2 from proximal domain 1. A and P are the anterior and posterior ends of the limb. (**b**) Big blue discs indicate the expression domain of the Hox genes. In the left domain, Hox10, Hox11, and Hox12 are activated. Hox13 is not yet activated. In the middle domain, Hox12 and Hox13 are activated but Hox11 and Hox10 are pushed out of the activation domain. In the right domain, all Hox genes are activated and Hox11 and Hox10 reenter in the activation domain.

7. Recent Findings Caused by BM Physical Forces

7.1. Physical Forces May Cause a Tension in the Hox Clusters

New technological advances (e.g., STORM—Stochastic Optical Reconstruction Microscopy) made possible the measurement of quantities and properties that were inaccessible before. Physical tension in Hox clusters is such a case, more specifically the tension of DNA topological domains which are important for Hox gene activation. Amândio et al. have recently measured mouse HoxD clusters under physical tension [40]. The origin of this tension is elusive. This team has even considered the possibility of the BM physical forces to be responsible for this phenomenon. In this case, they argue that 'the forces would be generated by the local chromatin interactions themselves, rather than through an asymmetrically localized point of attachment to the nuclear environment' [40]. Indeed, this is possible, and it is worth further examining.

7.2. Proposal for a Test of Section 7.1

The origin of tension due to BM pulling forces could be tested by manipulating the Hox cluster's gradual deletion of its fastening [40] (Figure 1b). If the tension is really caused by the BM forces and the manipulations start under full cluster tension, it is expected that this tension will be gradually deleted and the cluster be free of tension. This is worth performing an experiment.

7.3. Confirmation That FGF Causes the Necessary (but Not Sufficient) Condition for Limb Growth

As early as 2001, it was observed in C. Tickle's Laboratory that FGF signaling in the chick limb is only necessary but not sufficient for the expression of the last gene of the HoxA cluster [32]. Recently, this important finding was further confirmed by the novel genetic techniques of Sedas-Perez et al. [39]. It was found out that Fgf signaling creates a permissive environment necessary for the HoxA13 gene expression for both the mouse and the chick. Furthermore, Fgf signaling is unexpectedly dispensable once it is activated.

8. Conclusions and Predictions

8.1. Transition of External Symmetry to Internal

The starting point is the long-range Coulomb force (CF)

$$CF = (N * P)/R^2$$

where N and P are the corresponding negative and positive electrically charged complexes and R the distance between them. In formulating a heuristic expression for the pulling force of the biophysical model (BM), I proposed for the 'quasi-Coulomb Force' F the equation $F = N * P$, where the geometric term $(1/R^2)$ of CF is missing for sheer simplicity. This arbitrary heuristic force F is reminiscent of Heisenberg's isospin invariance in neutron–proton interchange under the strong nuclear forces. This was the prototype of a plethora of exotic particles obeying internal symmetries. Unexpectedly, the pulling forces of the BM are related to the internal symmetry of these particles crowned by Higgs boson. It turns out that F (the BM force) is the simplest internal force, since it depends on only two electric charges (N and P) free of any geometric property.

8.2. According to the Conventional Representation, the DNA Sequence Is Deployed along a Meandering One-Dimensional Line

However, it is more realistic if this deployment expands as a long two-dimensional strip with the DNA sequence gaining another degree of topological freedom. Besides DNA bending and stretching, a novel topological operation is possible: tape twisting that leads to a 'Moebius strip' [8]. Furthermore, the two-dimensional bending and twisting may be extended to three-dimensional surfaces, the so called 'Moebius torus', that can accommodate more realistic DNA sequential structures [8].

8.3. Besides Hox Gene Quantitative Collinearity

Figure 3 can also describe the split Hox cluster activation. It is strange that so divergent phenomena can be described by the same mechanism. This may hint at a scarcity or universal parsimony of the developmental mechanisms. Is this an evolutionary advantage or disadvantage? I believe this is an evolutionary advantage since it can accommodate several divergent developmental pathways, as for instance in the case of primary and secondary developmental axes. Note that the morphogen source in the limb is located at a quite different position—namely the AER in the distal tip of the bud [32].

8.4. Acording to Chick Limb Bud Experiments

Morphogen signaling for Hox gene expression is necessary but not sufficient [32]. Therefore, complementary cues must come into play for a proper gene expression. For instance, such cues can distinguish gene expression in the liver from the heart or even when this should occur.

The BM forces vary along the developmental axes, following the distances from their origin. Passive diffusion is the main signaling mechanism and the closer to the source origin, the stronger the force, as noticed in [32]. For a more accurate source localization, more accurate recent techniques have been exploited (CRISP technology).

In the chick limb experiments mentioned above, the excision of the morphogen source causes the disappearance of HoxA13 at the distant tip [33,34]. However, this expression

reappears by exposure of the bud to an FGF soaked bead. It would be interesting to perform the analogous experiment in the primary mouse axis and compare the results as proposed in [34]. Non-Hox gene expressions may be involved with proper Hox gene contributions preserving HGC [41,42].

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