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A Painful Question about Genomic Coding of the Body Plan

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Abstract—One of the goals of developmental genetics is to decipher the anatomy of organisms from their genome. The study of *Drosophila* homeotic mutants has shown that individual elements of the anatomy can have clear genomic correlates. However, we are still far from a complete solution for this problem. This review analyzes the reasons why, despite a very rapid accumulation of genomic data, progress is very slow in this area. These causes are primarily determined by a large number of neutral changes (changes that do not influence the morphology) in the regulatory regions of the genome, as well as by the localization of evolutionarily important changes in noncoding regions of the genome. Therefore, it is particularly important to carry out an experimental verification of the functional role of genetic differences using crossing or methods for obtaining transgenic animals.

Keywords: body plans, homeobox genes, deep homology, developmental genes

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HISTORY OF THE STUDY OF HOMEOTIC MUTATIONS AND THE GENES THEY AFFECT

The arrangement of organs in the animal body (body plan) and the reasons why they are arranged in this way have been of interest to comparative anatomists and evolutionists since the 19th century. The mechanisms linking genes and the body plan became clearer with the study of homeotic mutations in the fly *Drosophila*. Homeotic mutations transform one part of the body into another, which should normally be located in another part of the body. For example, in Antennapedia mutants, the antennae are transformed into a duplicate of the leg. In classic works on *Drosophila* (Lewis, 1978; Bender et al., 1983; Scott and Weiner 1984; Harding et al., 1985), it was shown that mutations of this type were associated with a special group of homologous genes of the Hox family, which are arranged in the chromosome in a compact cluster, and the order of these genes in the cluster is similar to the order of segments in the body, the identity of which is determined by these genes. Each of the segments of the *Drosophila* embryo has a unique combination of active Hox genes (or levels of their activity in the case of abdominal segments) and these patterns of activity govern the differentiation of segmental structures.

It is interesting that the phenotypes of some homeotic mutations of *Drosophila* can be considered as the reproduction of the elements of the body plan of dipteran ancestors. For instance, a mutation in Bithorax leads to the transformation of the third thoracic segment into a duplicate of the second one and a mutation in halteres leads to its transformation into the second pair of wings. Halteres on the third thoracic segment are a synapomorphy of Diptera and two similar pairs of wings are characteristic of primitive insects. This naturally leads to a temptation to associate changes in the Hox gene cluster with the transformations of body plans in animal evolution.

CORRELATIONS OF CHANGES IN THE HOX CLUSTER AND BODY PLAN

Accumulation of data on the genomes of different animals involved the accumulation of examples of correlations between the features of the Hox cluster and the body plan of individual groups of animals. Most of these examples are related to reductive evolution (Fig. 1). Thus, tardigrades have no posterior Hox genes Antp, Ubx, and abd-A, which mark the thorax and abdomen in insects (Smith et al., 2016). The body of tardigrades consists of only four segments corresponding to the four anterior segments of arthropods and onychophorans (antennal, intercalary, mandibular, and maxillary segments in insects); posterior Hox genes are not involved in marking of these segments; therefore, genes that were no longer needed after body shortening were lost. A similar loss of posterior Hox genes is observed in rotifers, which, like tardigrades, underwent miniaturization and lost the posterior regions of the body (Fröbius and Funch, 2017). The even more miniaturized parasitic worms Orthonectida

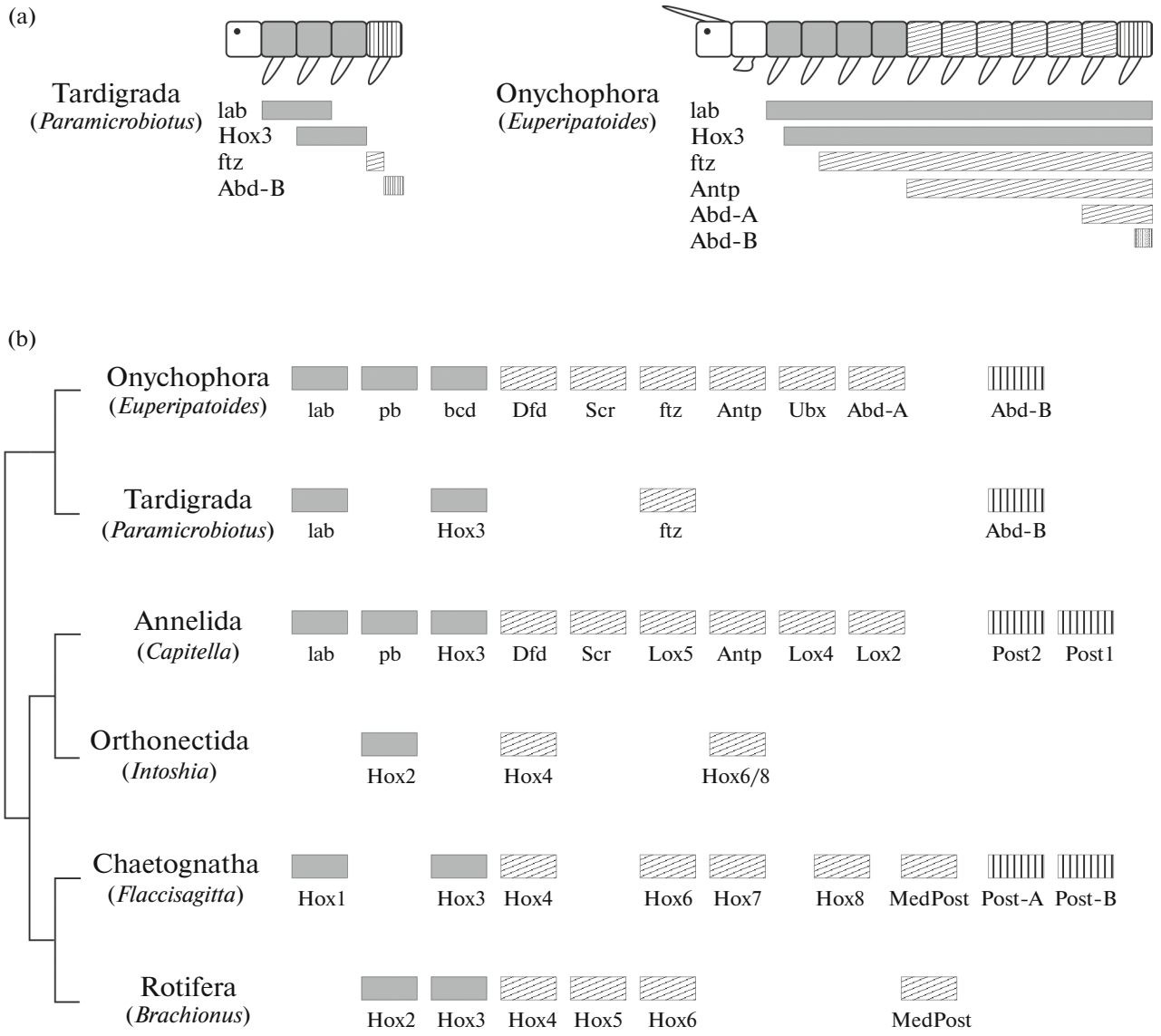

Fig. 1. Structure of the Hox cluster and expression zone of Hox genes of protostomes: (a) expression zones of Hox genes in two representatives of Panarthropoda: tardigrades and onychophorans: tardigrades differ from other Panarthropoda in the oligomerization of the body and the disappearance of homeobox genes such as Antp, Ubx, and Abd-A, which regulate the marking of the thorax and abdomen of insects; as a result, the expression zones of the anterior Hox3 gene (homologous to Bcd in arthropods) and posterior Abd-B gene contact each other in tardigrades; (b) structure of the Hox cluster in individual representatives of Protostomia: in several evolutionary branches (Tardigrada, Orthonectida, and Rotifera), the body was oligomerized and most of the Hox genes marking the posterior part of the body disappeared; in Orthonectida, the Hox cluster disintegrated and the three remaining Hox genes are in different regions of the genome.

and Dicyemida also lost many Hox genes. The genome of *Intoshia linei* (Orthonectida) has preserved only three Hox genes (anterior Hox2 and middle Hox4 and Hox6–8); the posterior Hox genes are completely lost (Mikhailov et al., 2016). On the contrary, *Dicyema* has lost the anterior Hox genes and preserved the posterior genes (Zverkov et al., 2019).

There are interesting data on the Hox genes of echinoderms. The echinoderm body plan has undergone a radical transformation. Their initial bilateral symmetry is superimposed by a secondary radial, usually five-rayed symmetry. A catastrophic metamorphosis occurs in the development of many echinoderms; the axes of the body of adult individuals and larvae do not coincide at all in this metamorphosis. The work (David and Mooi, 2014) showed that the three anterior genes of the Hox cluster (Hox1–Hox3) of sea urchins underwent inversion and were translocated to the end of the cluster, behind the posterior genes, so that Hox1 became the last gene of the cluster (Fig. 2). Moreover, it was shown that the translocated anterior genes were expressed in those organs which

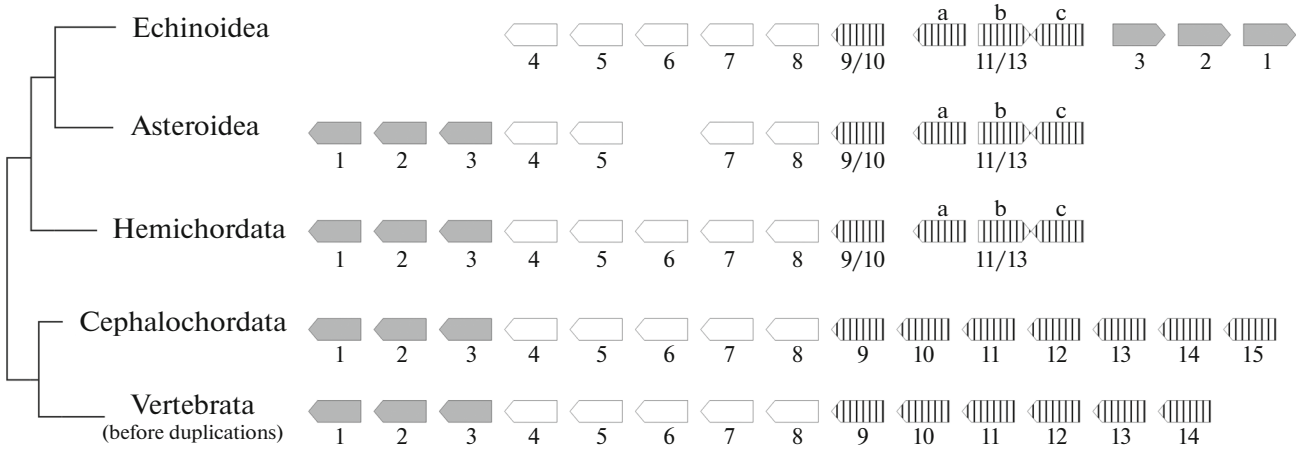

Fig. 2. Structure of Hox clusters in Deuterostomia: there were attempts to associate the inversion of the three anterior Hox genes and their translocation to the end of the cluster in sea urchins and holothurians (Echinozoa) with the radical transformation of symmetry in echinoderms; however, this translocation inversion is absent in Asterozoa and Crinoidea; therefore, it cannot be considered a correlate of the five-rayed symmetry of echinoderms; in addition, deuterostomes generally differ from protostomes in duplications of the posterior Hox genes; additional posterior Hox genes are involved in marking the postanal tail in chordates and also in limb development in vertebrates.

were echinoderm innovations (David and Mooi, 2014). This suggested that this Hox1–Hox3 translocation inversion is a genomic correlate of the radical transformation of the body plan in echinoderms (David and Mooi, 2014).

Further studies refuted this bold assumption. After reading the genome of starfish, it was found that its Hox cluster did not have this translocation inversion and completely coincided with the Hox cluster of hemichordates (*Saccoglossus*) with respect to the gene order, which have a common bilateral symmetry (Byrne et al., 2016). The Hox1–Hox3 translocation inversion is typical only of sea urchins and sea cucumbers (Echinozoa clade).

In the chordate type, the Hox cluster is enlarged based on duplications of the posterior Hox genes. In the lancelet, the number of genes in the Hox cluster reached 15, which is one of the highest values among animals (Fig. 2). The common ancestor of vertebrates had 14 Hox genes in the cluster and the common ancestor of chordates had at least 12 Hox genes (Pascual-Anaya et al., 2013). Additional posterior Hox genes are involved in marking of the postanal tail characteristic of chordates and can probably be considered correlates of this feature of the chordate body plan.

Posterior Hox genes are also involved in the development of vertebrate limbs (Sordino et al., 1995). In fish, gene expression patterns are similar both in the rudiments of paired limbs (dorsal and ventral fins) and in the rudiments of unpaired fins (caudal, dorsal, and anal fins) (Cotoras and Allende, 2015). Presumably, the additional posterior Hox genes of vertebrates became a preadaptation that enabled the development of complex limbs.

Examples of correlations between changes in the set of Hox genes and the body plan can also be found in the further evolution of vertebrates. For instance, repeated cases of loss of limbs and rearrangement of axial marking (particularly in snakes) are recorded in the order of scaly reptiles (Squamata). Hox clusters of squamates are evolutionarily variable; they lost regulatory regions (which are conserved in other vertebrates) and insertions of mobile elements many times. However, specific changes associated with the rearrangement of the body plan of snakes and legless lizards have yet to be clarified (Di-Poï et al., 2010).

NEUTRAL EVOLUTION OF THE HOX CLUSTER

Differences in the structures of Hox clusters of animals belonging to different types and classes will not necessarily be genomic correlates of differences in the body plan. There are examples of how the rearrangements of the Hox cluster are not accompanied by any significant changes in the anatomy. Thus, comparison of the Hox-clusters of 13 fly species of the genus *Drosophila* (Negre and Ruiz, 2007) revealed five inversions and six transpositions that occurred during the evolution of this genus. Nevertheless, all the *Drosophila* species have an identical body plan and many of them cannot even be distinguished from each other by a non-specialist.

EXPERIMENTAL VERIFICATION OF GENETIC CORRELATES OF ANATOMICAL VARIATIONS

Examples of the neutral evolution of the Hox cluster show that the putative genomic correlates of ana-

tomical differences are not necessarily so. Ideally, they should be verified and necessary genetic changes should be made in the genome of a species that does not normally have these correlates. This is technically possible only when comparing sufficiently close taxa: species, genera, families, and, at most, orders. In the case of intraspecific differences, crossing can be used. Examples of such studies are few; however, they exist and are of great value; therefore, let us consider them in more detail.

One of such studies revealed the genomic underpinnings of the features of the female reproductive system of moles (Talpidae), which is rather unusual for mammals. Mole females have a high level of androgens (the same as in males); instead of typical ovaries, they have ovotesticles (chimeric gonads with both ovarian and testicular tissue). The seminiferous tubules in the mole ovotestis are histologically distinguishable but nonfunctional, and the Leydig cells function normally and secrete male hormones (Barrionuevo et al., 2004). Analysis of the expression of genes involved in the development of the gonads showed an increased expression of the transcription factor of differentiation between FGF9 testes and the enzyme of synthesis of CYP17A1 androgens in moles compared to other mammals (Real et al., 2020). Amino acid substitutions detected in these mole genes and transcription factors influencing them were also found in mouse and human mutation databases; however, none of them led to the development of an ovotestis. Further studies showed that moles had a tandem triplication of the CYP17A1 gene and an inversion of a chromosome region with a length of about 26 million base pairs at a distance of 1.3 million base pairs from the FGF9 gene. The fragment subjected to inversion has no genes involved in the development of gonads; however, it contains insulator loci (noncoding DNA regions that govern chromatin packing). Inversion leads to a change in chromatin packing and the convergence of FGF9 with an additional enhancer. Experiments involving transgenic mice with the constitutive expression of FGF9 showed that the inversion led to the formation of an ovotestis or the complete transformation of ovaries into testes in mice with a female chromosomal sex (Real et al., 2020). Therefore, the main genomic correlate of phenotypic changes for the mole ovotestis is the inversion of a chromosome region that does not disrupt any coding sequences and is located at a considerable distance from the gene with an expression directly altered by the development of the ovotestis. To detect such chromosomal rearrangements, it is necessary to assemble the genome sequence to whole chromosomes and study chromatin packing using cross-linking technology (Hi–C), which is much more complicated and expensive than the simple reading of the entire genome sequence using next-generation sequencing.

The loss of legs in snakes was associated with a change in the ZRS enhancer of the Shh gene. The Shh (Sonic hedgehog) gene is a multifunctional regulator of morphogenesis that is involved in the formation of many organs; it has many enhancers triggering its expression in different sites. Shh expression in limb buds is driven by the ZRS enhancer. Transgenic mice in which the ZRS is replaced by a homologous cobra or python enhancer are born with significantly underdeveloped limbs, while the replacement of mouse ZRS by coelacanth or human ZRS does not influence the limb development (Kvon et al., 2016). The ZRS enhancer in snakes differs from that in other vertebrates in the deletion of the 17-nucleotide region containing the binding site for HoxD transcription factors (Leal and Cohn, 2016). The chimeric enhancer of python with recurrent deletion triggers the development of normal limbs in transgenic mice (Kvon et al., 2016).

Other studies revealed genetic bases for differences in the relative position of anatomical structures based on the example of color variations in butterflies (*Heliconius*), bumblebees and the arrangement of spines in sticklebacks (*Gasterosteus*).

Bumble bee *Bombus melanopygus* has two morphs, with a red or black spot on three–four abdominal segments (Tian et al., 2019). This trait is ecologically important, since the two morphs of *B. melanopygus* imitate other different species of bumblebees living in the same region (California). Laboratory crossings show that the color morph was inherited according to Mendel as a monogenic trait and the red spot is dominant. The peak expression of homeobox gene Abd-B is observed only in the morph with the red spot; it is recorded in the zone of this spot at the late stages of the pupa, when the expression of other homeobox genes decreases. The genetic differences between the two morphs are associated with the intergenic region between genes Abd-A and Abd-B, more precisely, with almost 50 nucleotide substitutions in the 4 kb long region. This region contains no known coding sequences or transcription factor binding sites; therefore, it is unclear how these nucleotide differences lead to the alteration of Abd-B expression. Other Californian *Bombus* species with similar color morphs have nothing similar to these alleles of the intergenic Abd-A:Abd-B region and color dimorphism is apparently determined by completely different loci (Tian et al., 2019).

Different stickleback species have intraspecific polymorphism in the number of dorsal spines. It is assumed that different numbers of spines may be adaptive in water bodies with different sets of predators, since long spines prevent predatory fish from swallowing stickleback but facilitate their grasping by invertebrate predators, such as dragonfly larvae (Morris et al., 1956; Marchenko, 2009). The work (Wucherpfennig et al., 2022) studied the genetic basis of the polymorphism in the number of spines in two stickleback species, *Gasterosteus aculeatus* and *Apeltes quadracus*. In both

cases, crossing and mapping indicated a noncoding region between homeobox genes HOXD9B and HOXD11B. Morphs differ in HOXD11B expression zones. In *Gasterosteus*, the multispined allele differs in two transposon insertions and the deletion of the AxE enhancer; in *Apeltes*, it differs in single nucleotide substitutions in the same AxE enhancer. Therefore, the number of spines in different stickleback species changes under the influence of different mutations in the same region of the genome.

As in bumblebees, color variation in butterflies (*Heliconius*) is associated with mimicry. Their genetic basis has been studied in a number of works and is mainly associated with changes in the expression zones of regulatory genes Optix, Cortex, and WntA (Reed et al., 2011; Martin and Reed, 2014; Van Belleghem et al., 2017; Mazo-Vargas et al., 2022). As in the above-described bumblebees and sticklebacks, cases of emergence of similar phenotypes as a result of different mutations were shown in *Heliconius*. In addition, cases of interspecific transmission of adaptive alleles as a result of introgression (Dasmahapatra et al., 2012) and inheritance of ancestral polymorphisms by different descendant species (Gallant et al., 2014) were found.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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