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ORIGINAL PAPER

The phylotypic stage as a boundary of modular memory: non mechanistic perspective

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Abstract The concept of the phylotypic stage has been strongly integrated into developmental biology, thanks mostly to drawings presented by Haeckel (Anthropogenie oder Entwicklungsgeschichte des Menschen, 1874). They are printed in every textbook as proof of the existence of the phylotypic stage and the fact of its conservation, albeit many times criticized as misleading and simplifying (Richardson in Develop Biol 172:412–421, 1995, Richardson et al. in Anat Embryo 196:91–106, 1997; Bininda-Emons et al. in Proc R Soc Lond 270:341–346, 2003). Although generally accepted by modern biology, doubt still exists concerning the very existence or the usefulness of the concept. What kind of evolutionary and developmental horizons does it open indeed? This article begins with the history of the concept, discusses its validity and draws this into connotation with the idea of a memory activated throughout the development. Barbieri (The organic codes. An introduction to semantic biology, 2003) considers the phylotypic stage to be a crucial boundary when the genetic program ceases to suffice for further development of the embryo, and supracellular memory of the body plan is activated. This moment clearly coincides with the commencing of the modular development of the embryo. In this article the nature of such putative memory will be discussed.

Keywords Phylotypic stage · Zootype · Supracellular memory of the body plan · Modularity · Developmental pathways · Walter M. Elsasser · Semiosis

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Introduction

Inspired by Darwinian teaching, Haeckel (1874) furthered the idea into the so-called basic biogenetic law (*phylogenetisches Grundgesetz*), asserting that ontogeny recapitulates phylogeny of a given lineage in an abbreviated and rapid way, i.e., embryonic development of an individual organism passes (in an abridged form) along the same path as did its species in history. Thus, a human being starts as a single cell and then proceeds through the stages of coelenterate, planarian, fish, saurian, primitive mammal, and ape, with higher, i.e. phylogenetically later stages, becoming more and more prominent (Haeckel 1874). All species-specific differences appear at later stages of developmental sequence. The biogenetic law was later disproved, and contemporary models are safely rooted in the insight of Baer (1828) who supposed the early stages of the development to be more similar than the later stages because of their homogeneity, not because of the fact of recapitulation. Baer (1828) was the first to recognize in vertebrate development a stage common to all classes. This led him to the formulation of his *ontogenetic law*: in embryonic development, general features precede special ones; *development proceeds from undifferentiated homogeneity to differentiated heterogeneity* (Gould 1977).

For a contemporary biologist, the phylotype idea is connected with *the hourglass model* designed independently by Raff (1996) and Duboule (1994) as shown in Fig. 1; the name "phylotypic stage" comes from Sander (1983), and such a stage has so far been described for annelids, arthropods and chordates (Bininda-Emons et al. 2003), and it has been known by several names, like *pharyngula* (after the pharyngeal pouches, Ballard 1981) or *tailbud stage* (Slack et al. 1993) in vertebrates, and the *germband stage* (Sander 1983) in the development of

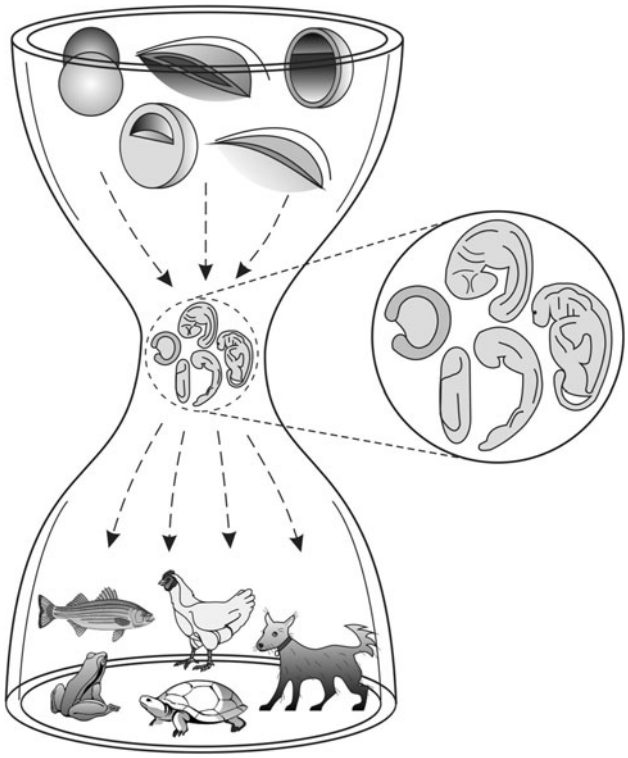

Fig. 1 The hourglass model. The developmental pathways leading to and from the phylotypic stage are quite different (even among closely related taxa), and the morphological similarity is the highest at the period of the phylotypic stage. After Jody F. Sjogren (2000)

insects. It was also already emphasized by Sander (1983) in case of arthropods that early developmental pathways leading to the phylotypic stage are highly variable even across closely related taxa. For simplicity, I focus in this article only on the vertebrates.

Characteristics of the phylotypic stage

The main differences in the early developmental periods of vertebrates generally depend on differences of cleavage, and they may exist between the taxons (meroblastic cleavage in birds, reptiles, and fishes; holoblastic cleavage in amphibians; mammals constructing a blastocyst, chorion, and amnion) or even within the same taxon (holoblastic and meroblastic cleavage in different groups of fishes). They may depend on the amount of yolk, and also on types and timing of the body axe and germ layers setup (Slack et al. 1993; Gilbert 2003; Steinberg 2003). Such differences are most probably caused by adaptation of distinct life forms to various environments—or simply they resulted from historical contingencies. Only later, after gastrulation, all the members of the same *phylum* enter the conservative period in their development during which they most resemble each other: their phenotypic divergence

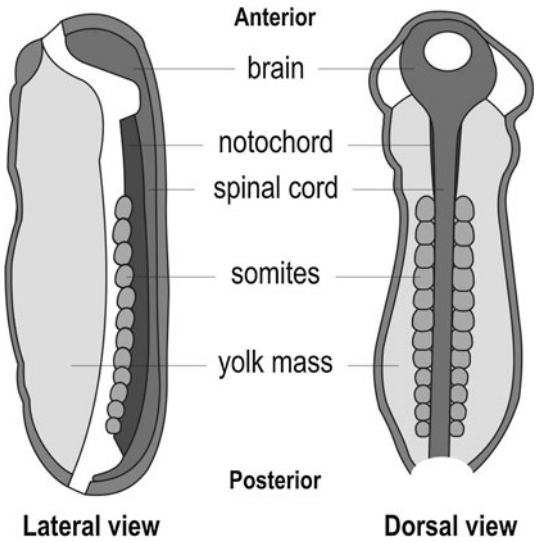

Fig. 2 The vertebrate phylotypic stage. Here, the main morphological characteristics of the vertebrate phylotypic stage are depicted (pharyngeal pouches, heart, and optic anlagen are missing)

is highly reduced, just to the point when they drastically start diverging again in subsequent development. Owing to the low phenotypic divergences in the phylum, this period is supposed to be highly evolutionary constrained (Slack et al. 1993). The hourglass model is nowadays very common, although some scientists consider the metaphor misleading or even invalid[1] (Mitteroecker and Huttegger 2009).

The morphological structure of the vertebrate phylotypic stage (i.e. the *pharyngula* or tailbud stage) is characterized by the presence of the neural tube, notochord and somites, the head with pharyngeal pouches, heart and optic anlagen and, of course, the tailbud (Richardson 1995, 1997) (Fig. 2). The phylotypic stage starts with the process of neurulation and ends when the somites are developed (Galis and Metz 2001). Wolpert (1991) considers as the most conserved period of development the early somite stage just after neurulation. For Duboule it is the period between the head fold and tailbud stage (Duboule 1994).

Slack et al. (1993) went even further: they first recognized a coupling of the phylotypic stage with the anteroposterior expression pattern of a set of specific orthologous genes. The most characteristic genes of this group are represented by the batteries of *Hox* or homeotic genes, very

[1] The authors argue from the perspective of geometric morphometrics with the impossibility to find any quantitative measure how to compare the similarities among organisms passing through the blastula stage and the phylotypic period. The differences among organisms before and after the phylotypic period are supposed to be higher- but the same measures cannot be defined for all compared species: some of the variables are not defined for all species involved; some traits at later stages are too complex to compare between each other, like the human lips or the bird's beak etc.

conservative across the phyla, and present in a broad variety of organisms like insects, nematode, amphioxus, or sea urchins. *Hox* genes probably existed in the common ancestor of *Cnidaria* and *Bilateria* (Ferrier and Holland 2001). On such a basis, they were able to unite almost the whole animal kingdom under the common concept of *zootype*. Therefore, the zootype as a genetic pattern is formally superior and evolutionarily older than the morphological structure of phylotype. (Fig. 3)

Hox genes activate or repress batteries of downstream genes by binding to DNA sequences in *Hox*-response enhancers (Pearson et al. 2005), but they can also control other executive genes. The *Hox* genes are organized in clusters, and their supposed evolution proceeded via duplication of these clusters (vertebrates have four such clusters). Their main function is the determination of the embryonic regions along the anterior–posterior axis and the specifying of the particular *identity* and *relative position* of a given structure (Slack et al. 1993). Later in development, the expression, and function of *Hox* genes they also act as region-specific selector genes in diverse structures and tissues (Caroll et al. 2006). In addition, they play a role in cell division, cell death, and cell movement (Pearson et al. 2005). Mutation in homeotic genes may lead to morphological defects or homeotic transformations (Davidson 2006). Note that *Hox* genes are best known, but by no means they are singular example of selector genes playing a crucial role in development. Slack et al. (1993) describe the phylotypic stage not only as a defining platform for an individual body plan but also as a link of this body plan to the whole animal phylogeny.

Does the phylotypic stage exist?

Richardson (1995) denies the existence of the phylotypic stage, arguing that such a "constant" must have become blurred by the plentitude of evolutionary shifts in developmental timing (heterochrony) and because of extensive variation in somite number among the members of the phylum. Heterochrony mostly concerns the development of nasal and lens placodes, heart tube, and limb buds. Richardson recognizes conservation in the pattern of gene expression at this period, but not conservation on the morphological level; he therefore prefers the term *extended phylotypic period* than phylotypic stage (Richardson 1995). Furthermore, he also describes obvious differences in body size and allometry (changes in the pattern of growth of different fields of embryo; Richardson et al. 1997).

Richardson and his group later provided support for their view by analyzing a great variety of quantitative data (Bininda-Emons et al. 2003) concerning developmental-

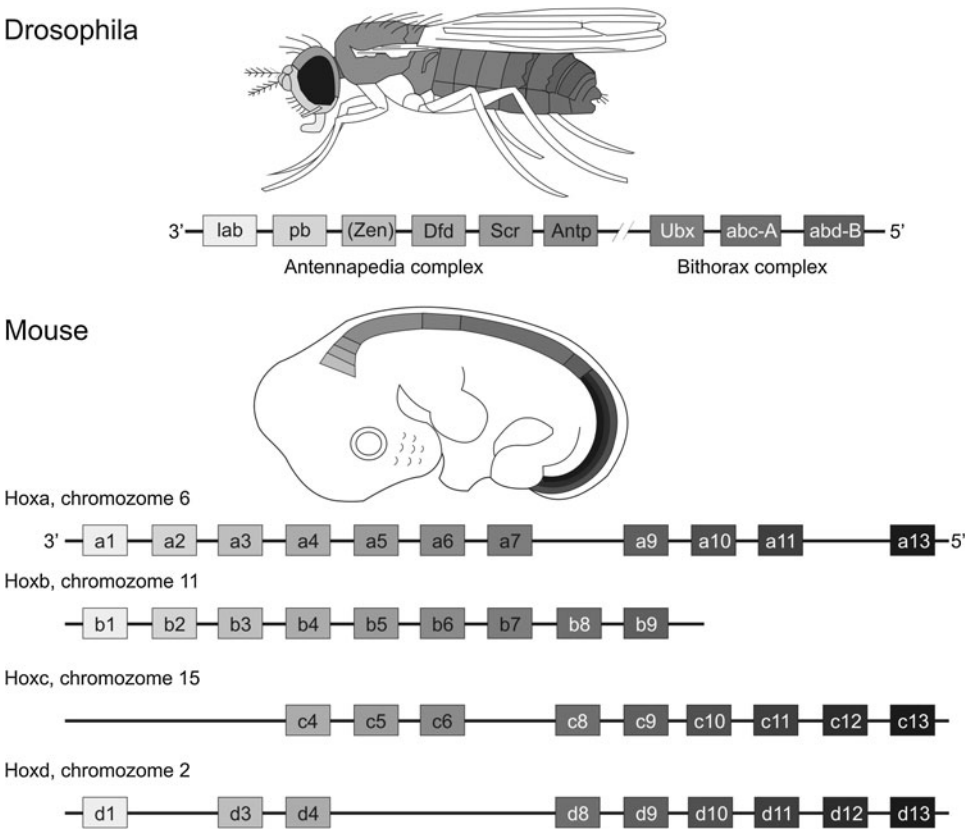

Fig. 3 The zootype transcription pattern in *Drosophila melanogaster* (one Hox complex) and *Mus musculus* (mouse and other vertebrates) have 4 Hox complexes. *Source* www.bio.miami.edu

timing across different vertebrate taxonomic groups, and within the group of mammals.[2] As remarked above, the hourglass model presupposes that phenotypic divergence between lineages should be minimal at the phylotypic period, when compared with earlier or later stages. The authors show, however, that phenotypic variation, i.e. variation in the timing and size of structures appearing at the time of the putative phylotypic stage, was surprisingly high.[3] Mitteroecker and Huttegger (2009) criticize this approach as too simplifying—in their study, the timing of the homologous events was compared, but the morpho-spatial variation of the studied structures were not taken into account.

In contrast, Irie and Sehara-Fujisawa (2007) argue that the expression pattern of the orthologs of vertebrate developmental genes are very similarly right when the supposed phylotypic stage appears (e.g., in mouse development at day 8–8.5). Furthermore, Hazkani-Covo et al. (2005) confirmed the evolutionary conservation at the level of gene expression products, and located the phylotypic stage between the first somite stage and the formation of the posterior neuropore.

Galis and Metz (2001) discovered a web of intense interactions among organs of primordia due to which, any small, laboratory-induced mutation (exposure to teratogens) during the period of the supposed phylotypic stage causes pleiotropic (even lethal) effects in the whole embryo; later on, the effects of such mutations are not as fatal. The phylotypic stage is obviously a very conservative period of development, naturally resistant to any mutational change (Galis and Metz 2001), and maximally interconnected (Raff 1996).

High level of interaction among the traits of the developing embryo was also confirmed during the development of the zebrafish *Danio rerio* (Schmidt and Starck 2004): stages between 15 and 19 h post fertilization are resistant to selection because changing any trait would affect all others that are functionally linked.

Most recently,[4] Domazet-Lošo and Tautz (2010) studied the gene expression in zebrafish genome from the point of

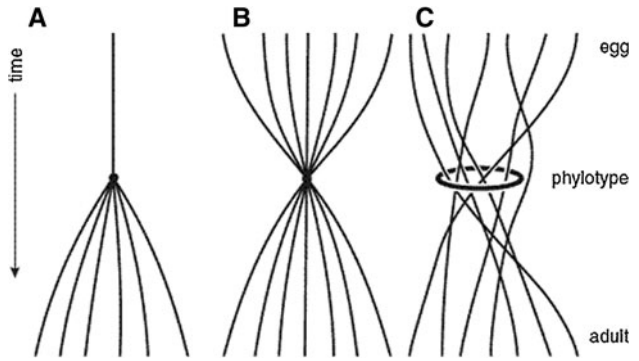

Fig. 4 The sheaf model. Three different models of the development: **a** the broom model represents Haeckel's biogenetic law, **b** the already mentioned hourglass model, and **c** the sheaf model recognizing the conservation of the phylotype but also allowing a loosening of individual straws and circumventing the straw binder (Markoš et al. 2009)

times of origin of various gene sets (i.e., the gene set typical for all living forms, for animal phylum, or for vertebrata). The supposed phylotypic stage is characterized by the expression of the evolutionary oldest sets, whereas during earlier and later stages, evolutionary younger genes are activated. Such results seem to confirm the hourglass model, and the expression of the oldest gene set during this period is explained either by adaptive constraints or by internal constraints which do not allow new gene sets to be involved.

However, shifts in the timing of gene expression and particular protein appearance, of cellular communication patterns and heterochronies in the appearance of homological structures, all obviously take place, although the principal structures are always present at the phylotypic stage. The developmental constraints leading to the phylotypic stage may differ in diverging taxa (Schmidt and Starck 2004); therefore, definition on the morphological level remains more general and broader. Moreover Markoš et al. (2009) came with the "sheaf model" (Fig. 4) reflecting the fact that the phylotypic stage is much looser than that defined by Raff, although the phylotypic constriction is recognized.

Should we set aside the heterochrony and the somite number differences emphasized by Richardson (1995, 1997), we can accept the idea of the phylotypic stage at the mentioned more general level. This phylotypic stage is then defined by the basic morphological structures (notochord, somites, neural tube, optic anlagen, and pharyngeal pouches), by highly conservative zootype transcription pattern, i.e., by the transcription of the orthologous genes

[2] The developmental events being taken into account were transformations (i.e., first appearance of a defined morphology or morphogenetic movement), as they take place during the whole of the mid-embryonic period. Most of these developmental events shared features present in all the species studied: the first dataset consisting of 14 vertebrate species and 41 developmental events and the second 14 mammal, plus two amniote outgroups with 116 developmental events (Bininda-Emons et al. 2003).

[3] For a criticism on the conservation of phylotype, based on interspecific variation in amphibians observed during the development of neural crest, see Collazo (2000).

[4] In the same issue of *Nature*, Kalinka et al. (2010) confirmed the phylotypic status of the germband stage in insects, comparing the expression levels of selected genes and their specific temporal

Footnote 4 continued
relationships among six *Drosophila* species, revealing that the temporal gene expression pattern is the most conservative during the mid-embryonic period.

shared among vertebrates, and by high level of interactiveness within the phylotypic body.

What is the cause of phylotypic conservation?

Duboule (1994) suggested that the conservation of the phylotypic stage is caused by the colinearity of the homeotic genes.[5] Sander (1983) suggested the phylotypic conservation to be caused by pleiotropic nature of interaction among developmental modules. Moreover, Galis and Metz (2001) suppose that interactiveness, rather than the colinear organization of the homeotic genes, causes the fatal effect of any mutation during the phylotypic stage (Galis and Metz 2001). Furthermore, they suggest (2001; see also Raff 1996) that the robust interactiveness observed at the phylotypic stage will later be apparent again between semi-dependent, loosely coupled, developmental modules with different functions and outputs. This is the reason why experimentally induced mutations will exert much lesser impact, by affecting only the selected parts of the developing organism. Hence, the absence of modularity at the phylotypic stage (or phylotypic stage functioning as one interactive module) is one of the key aspects causing its conservative character. Schmidt and Starck (2004) prefer to emphasize high morphologic integration, i.e., high degree of interconnectivity at the period of the phylotypic stage, rather than a lack of modularity, because the different degrees of modularity is hard to test and reconcile. Intuitively, there is a correspondence between both interpretations.

Modularity in the development

Module: general definition

As mentioned above, the phylotypic stage is defined by a high degree of interactiveness within the embryo, while later development operates on the level of discrete semi-autonomous modules. Pioneers emphasizing the role of modularity in development were mainly Riedl, Lewontin, Bonner, and Raff (see Nelson 2004; Wimsatt and Schank 2004). The module is a special integrated and relatively autonomous unit (Schlosser 2004) with high degree of internal and a low degree of external interactions (i.e., with other modules of the given structure). The integration of the module means that the input–output relationship of the module depends on the particular connectedness of its components, not only on the additive superposition of these components. An autonomous module is insensitive to perturbation of the context in which they are embedded (Schlosser 2004). Insensitiveness means that the module is able to maintain the same function in abnormal tissue environments (e.g., ectopically, by transferring the bud, or anlagen, to different location of the embryo).

The functions of each module had to be unified early in evolution, i.e., it has relatively similar genetic and developmental background in different lineages. Every change in the genetic network of a single module leads to the pleiotropic effects *only* within such a module.

Developmental and evolutionary function may reside in canalization and environmental perturbation (modularity leads to higher phenotypic stability during development), or in selective buffering against pleiotropic effects on the whole organism, which facilitates adaptation (or escape from adaptive constraints) (Wagner et al. 2005).

Different types of modules and examples of redeployment

In general, we can observe modularity on many levels of structures emerging during development. Schlosser (2004) makes a distinction between *gene regulation*, *signaling*, *positional*, *cell type*, and *organ modules*.

A *transcriptional activation module* requires a cooperative assembly of many upstream transcription factors (*trans*-factors) on the promoter and/or *cis*-regulatory sequences (Davidson 2006) (Fig. 5a, b). The specific set of *trans*-factor inputs present at a given time will define which downstream genes (outputs) will become regulated at that time, as well as where and how this happens. The modules can gain new functions by new combinations of inputs (new combinatorics of transcriptional factors), by mutations affecting the *cis*-sequences, and by new relations between outputs (generating new regulatory networks influencing their downstream genes). It is clear that not only the DNA binding specificity of the trans-factor but also the interaction among different transcription factors during the gene regulation is crucial. Grenier and Carroll (2000) compared two trans-factors, O-Ubx in *Acanthocara kaputensis* a D-Ubx1a in *Drosophila melanogaster*. Both have a similar homeodomain, but the rest of the protein body differs to a great extent between both species. O-Ubx can repress *surf wings* gene and drive the expression of *decapentaplegic* in the visceral mesoderm as does the D-Ubx1a. On the other hand, O-Ubx cannot repress *distalless* gene as D-Ubx1a can (Wagner 2007). The authors believe that both proteins are engaged in different teams of transcription factors mainly because of differences in their

[5] Colinearity means that the order of the gene cluster on the chromosome corresponds to the order of their expression along the antero-posterior axis of the organism. In vertebrates, colinearity is not only spatial, but also temporal, as genes corresponding to the anterior part of the body are expressed earlier than the genes corresponding to the posterior parts.

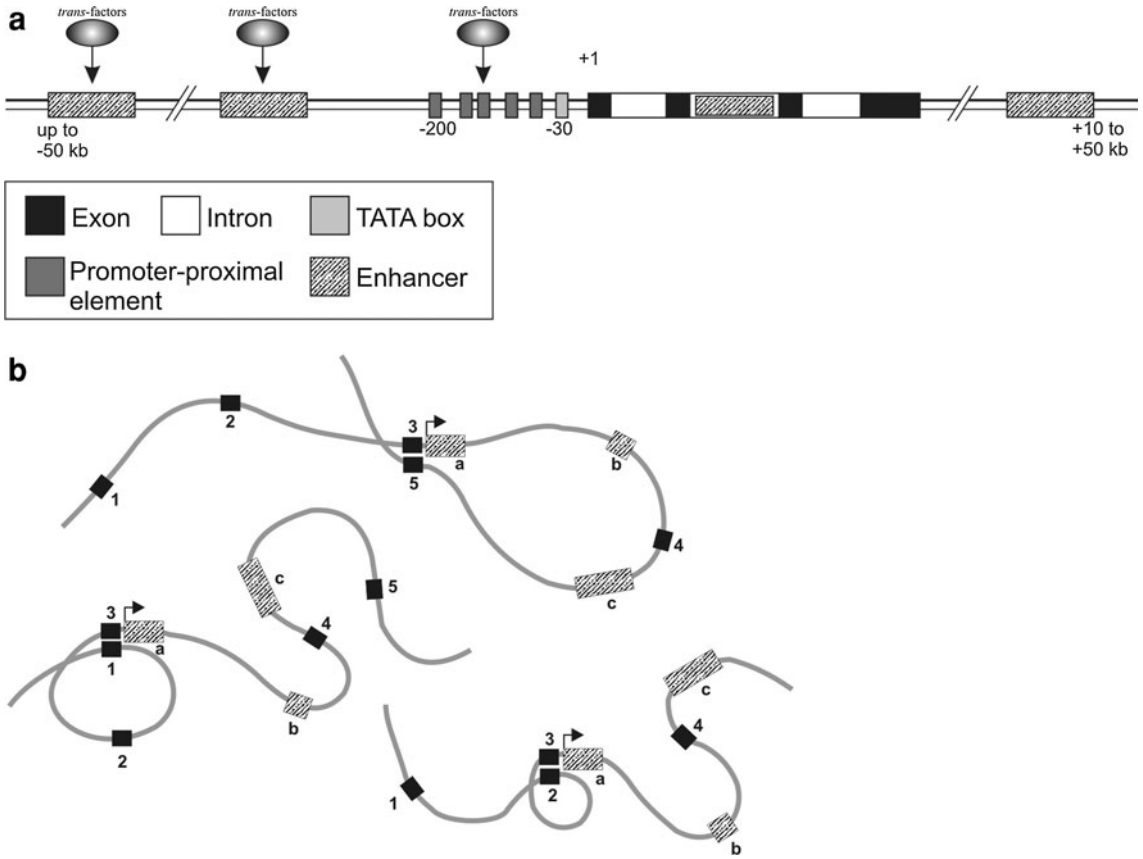

Fig. 5 **a** The *cis–trans* module; **b** DNA looping. **a** The specific assembly of combinations of different transcription factors on the promoter or enhancer/silencer of a given gene is crucial in gene regulation. Promoter (*promoter-proximal element*) with TATA box (starting point of transcription by binding of RNA polymerase) and the enhancer/silencer are the so called *cis*-sequences (draw according to Lodish et al. 2003). The enhancer can lie even within the intron region and the gene can have more than one enhancer. The transcription of the gene is regulated by the binding of transcription factors on *cis*-sequences and their interactions among each other. **b** The dynamic of interactions can be achieved by alternative loopings of DNA (drawing according to Davidson 2006). The orchestration of transcription activation is a lot more complicated than herein described: also the interactions with chromatin structures or other regulating sequences can be included (e.g., *insulators*, which prevent the enhancer to control the neighboring gene, or *global control regions*, which regulate gene transcription over large chromosomal domains, Alonso 2008)

protein domains—which results in diverse regulatory capacities.[6]

S*ignaling modules* between communicating cells represent a plethora of extracellular or intracellular signaling pathways (e.g., the Sonic hedgehog, receptor tyrosine kinase, Wnt, TGFβ, or Notch pathway; e.g., see, Schlosser (2004). These modules are active in different developmental contexts in different tissues. Such pathways are generally initiated by the binding of the ligand on the cell receptor, which activates a transduction pathway resulting in the release of an activator or repressor of some target gene. Most components of such pathways are, again, conserved from insect to vertebrates, acting as independent modules in several tissue environments. For example the Sonic hedgehog pathway is active in wing disk, leg disk or eye disc formation in *Drosophila*, or in vertebrate in dorso-ventral patterning of somites and neural tube and in antero-posterior, and proximo-distal patterning of limbs. In vertebrates, the Sonic hedgehog pathway also participates in gut, pancreas, lung, or tooth formation (Borycki 2004). The universality of signal transduction module is often hacked by cross talk among pathways.

Positional modules are based on the function of positional-specific selector genes. The selector gene is

[6] Alternative splicing of the gene transcript provides yet another source of *trans*-factor heterogeneity: the differences in products of a single *Ubx* gene, i.e. different proteins are spliced from the same gene. In *D. melanogaster* six such different isomorphs were observed (Alonso 2008). *Ubx* determines the segment specificity for many cell types, in epidermis, central and peripheral nervous system and mesoderm. The transcript isomorphs of Ubx gene differ in the presence of short additional regions (microexons): isomorphs containing microexons are expressed especially in epidermis, mesoderm and peripheral nervous systems. Isomorphs lacking the microexons are expressed only in central nervous systems. Functional specificity of the selector genes is therefore generated also on the level of RNA splicing.

expressed in relative space and time, which determines its relationship to other selector genes. This means that the selector genes (García-Bellido 1975; Caroll et al. 2006), such as the *Hox* genes, which control the development of a given module are very conservative across the whole kingdom of organisms, and do not mutate frequently. However the main differences emerge not on the level of genes, but on the level of their regulation by duplication or rearrangements of the *cis*-regulatory sequences, or by changes in the time and place of the expression of a given gene (see Fig. 5). An illustrative model based on Caroll (2005) shows the example of Hox6 expression in vertebrate development (Fig. 6).

Hence, a mutation in a given selector gene similar to a *Hox* gene can have a pleiotropic effect on the functioning of the whole module (i.e., of all genes responsive to it). On the contrary, the rearrangement of inputs (different combinations of TF) and outputs (target *cis*-regulatory sequences) of gene regulatory networks enables the module to gain a completely new development function (Caroll et al. 2006). Complexity in development increases by adding new regulatory states (Davidson 2006), and specificity and diversity in the usage of the same modules arise from the combinatorial control of inputs and outputs of the given module. These modules are therefore quite autonomous: they work in the context of completely unrelated tissues and are able to act ectopically, i.e. in abnormal cellular environments (Schlosser 2004) (Fig. 7).

We shall not discuss the *cell type* and *organ modules* in detail. The first represent the control of determination and differentiation of given cell type, which is usually ruled by a small group of genes that act as cell-specific selector genes. *Organs* (Fig. 8) are modules because their development function relatively independently from other organs (Schlosser 2004), developing in the fashion of morphogenetic field. The best studied example of organ module is vertebrate limb development.

The versatility of different developmental modules of development can be observed on various levels of description: the same gene modules have as outcomes not only different morphologic structures in a variety of species, but also in the different contexts within the same developing body. Developmental processes leading to very similar morphological structures can differ even among closely related species[7] (Swalla and Jeffery 1996).

The evolution of animal form is shaped thanks mainly to the spatiotemporal shifts in gene activation, different partners in protein interaction (different combinations of transcriptional factors), and differences in the activation of downstream targets. Other processes like variable alternative splicing or RNAi are also included. These new regulatory states are responsible for new interpretations and new usage of the same modules in different tissue environment.

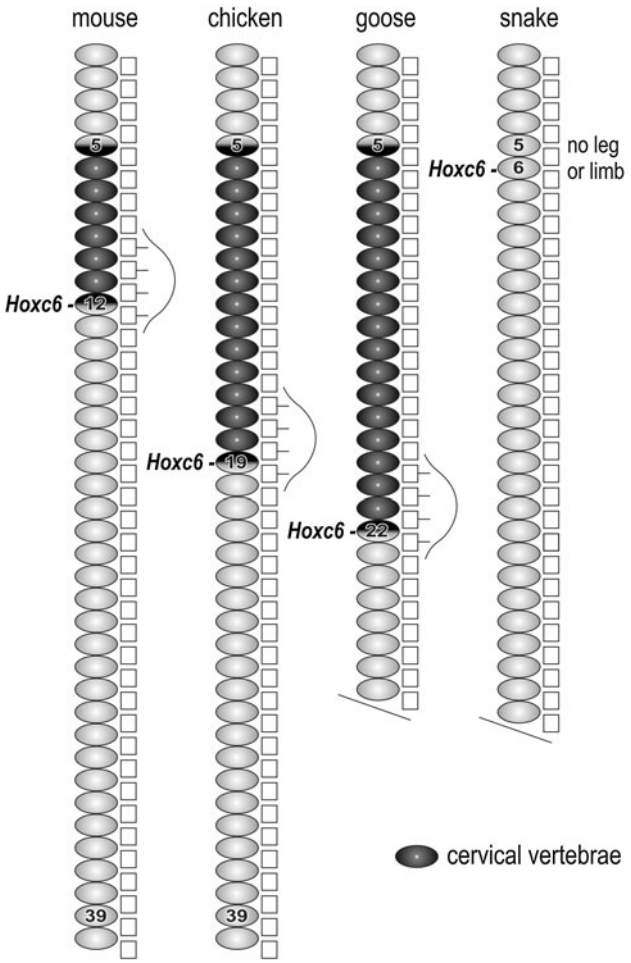

Fig. 6 Cervical and thoracic vertebrae. Various vertebrates have different numbers of cervical and thoracic vertebrae and therefore mice have a short neck, geese long one, and snakes none (only one long torso). The boundary between the cervical and thoracic vertebrae corresponds with the expression of gene Hox6, which forms the interface between the neck and chest. Hox6 gene is activated in every vertebrate species, but its position with regard to the whole body is different. For all four-legged vertebrate, forelimb arises at this boundary. In the case of snakes, there is no obvious boundary between the cervical and thoracic vertebrae, and the expression of Hox6 is spreading forward to the head (and no limbs are formed)—drawing according to Caroll 2005

Why phylotypic stage?

How, then, is the organism to reconstruct its specific three-dimensional morphologic layout, when the genetic background is very similar across disparate taxonomic groups of fish, bird or mammal (not to mention insect and other evolutionary distanced groups of organisms)? How is the

[7] Two related ascidian species undergo different developments: either a conventional tadpole larva, or a tailless larva (Swalla and Jeffery 1996). In addition, changes in sea urchin cytoplasmic determinants can generate sea urchins that develop without larvae, yet accomplishing a normal adult (Gilbert 2003).

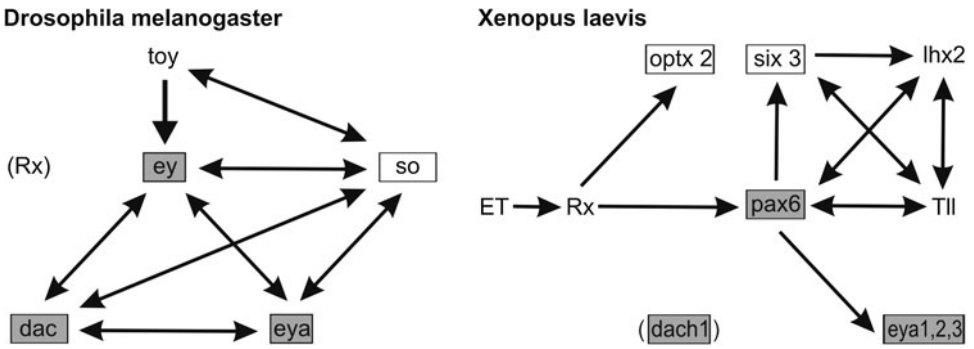

Fig. 7 Pax6 module. Very famous examples of using the same modules in the development of different organs within the same and within different taxa include Pax,-Six,-Eya-Dach gene families which form the regulatory network functioning as a multiply deployed module in vertebrate and also insect development. In *D. melanogaster*, the gene *eyeless* (*ey;* with homology to the Pax gene family in vertebrates) is necessary for eye development. *ey* is part of a small network that includes another transcription factor, *sine oculis* (*so*; with homology to the six gene family in vertebrates), and two transcriptional cofactors, *eyes absent* (*eya*) and *dachshund* (*dac*), and is activated by a paralogue of *ey* called *twin of eyeless* (*toy*) (drawing and text according to Wagner 2007). *Pax6* induce the ectopic eye in *D. melanogaster* as well as in *Xenopus laevis* (Halder et al. 1995; Chow et al. 1999). *so* and its orthologue, *Six1*, play role in *Drosophila* eye development, and in vertebrate myogenesis and ear development (Kardon et al. 2004). The figure depicts two scenarios of *eye* genetic regulatory network *D. melanogaster* and *Xenopus laevis.* In *D. melanogaster*, *toy*, *ey*, *so*, *eya*, and *dac* are necessary for eye development. *Toy*, *ey*, *eya a dac* are sufficient for the induction of eye development and can also mutually induce their own expression. In insects, *ey* is regulated by *toy*, whereas in vertebrates, the transcription factor gene *retinal homeobox* (*Rx* or *Rax*) is upstream of *Pax6*. *Rx* gene is also present in *Drosophila*, but not active during the eye development. *Optx2* a *Six3* are paralogous genes to *so*. Similarly, in vertebrates, *Eya1*, *2*, *3* (homologues of *eya*) do not regulate *Dach1*, the homologue of *D. melanogaster dac*. Orthologous genes are in *gray* boxes, paralogous in *white* boxes

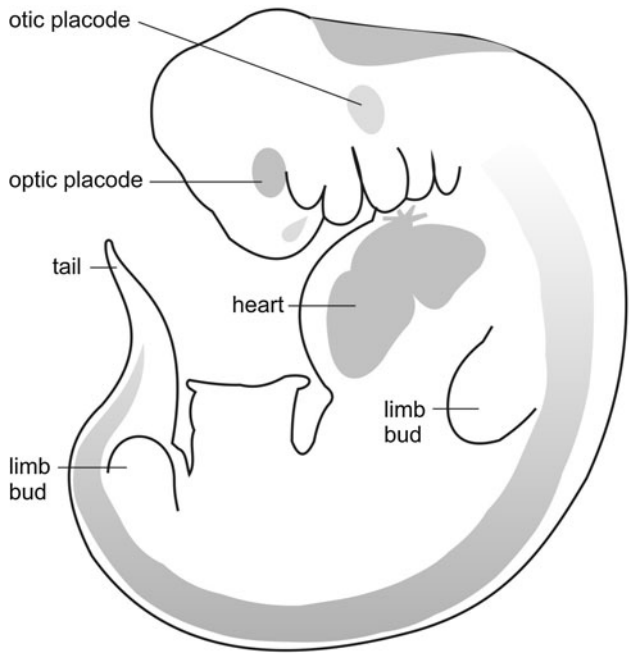

Fig. 8 The vertebrate organ modules. This picture depicts the main morphologic structures acting as modules throughout development

continuity and informational stability of the developmental processes maintained, if most phenotypic characteristics of the whole metazoan are generated not by individual genes acting alone, but by networks of interacting gene products (Salazar-Ciudad and Jernwall 2004)? Why does animal development require a conservative phylotypic stage?

On the level of zootype, all the members of the same phylum put the same genetic toolkit in use (Caroll 2005, 2006) to start their embryonic development. Barbieri[8] noticed that it is the very phylotypic stage that launches new, qualitatively different types of development. In the first period (up to the phylotype), development is very quick and directed only by the hardwired genetic program. In the second period, development is also coordinated by processes working on the basis of the bodily, i.e., supracellular memory of the body plan. The supracellular memory is empty in the beginning (Barbieri 2003); through many rounds of iterative processes, the tight coordination of function at the phylotypic stage allows a gradual "reconstructing" of the phenotype from incompletely inherited information. Today's knowledge of evolutionary developmental biology coincides with Barbieri's opinion: phylotype itself acts as a highly connected module; and immediately after this period, the embryo divides into several semi-dependent modules. This is the time period

[8] In his book *Organic codes*, Barbieri (2003) distinguished several different types of memories. The first level is genetic – in DNA. The second type of the memory works on the basis of different epigenetic codes, e.g. histone code. Such codes are created and re-written thanks to quasi-digital marks, such as histone modification or DNA methylation (Markoš and Švorcová 2009). Epigenetic memory determines the state of every cell in the body and maintains their differentiation. Barbieri also speaks about the neuronal memory and the memory of the immune system at the supracellular level. In his opinion, such memories represent deposits of epigenetic information acquired in ontogeny.

during which the spatiotemporal expression of orthologous genes is activated. This independent spatial and temporal regulations of gene expression permits individual genes to have different but specific functions in different contexts (Caroll et al. 2006). The architecture of hierarchical regulatory domains or different level modules can well represent the phenomenon, which Barbieri calls the supracellular memory of the body plan.

Barbieri (2003) considers the body plan to be simultaneously a three-dimensional structure and a deposit of information. The information about spatial organization of body plan cannot be transferred without *the three-dimensional structure* of the conservative phylotype which is typical for the whole phylum. In this case, we should actually speak about four-dimensional structure: the heterochronic events dependent on the species lineage have to be taken into account as well. The concept of supracellular memory may therefore help us further answer the question of conservation of the phylotypic stage.

In Markoš et al. (2009), the term "Barbieri's platform" was introduced. It represents the meeting point between the period of development ruled by hardwired genetic information and the period when this information is mollified "from above" by semiotic processes. This mechanistic, hardwired, one-module platform is a starting point of the species-specific modular development. Phylotype and zootype form together bodily and genetic toolkit of the body plan. It is also a meeting point for Barbieri's semantic biology and the language or hermeneutic metaphor of life (Markoš 2002; Markoš et al. 2009).

Organic memory of the body plan: is it a mechanistic storage?

The concept of the organic memory in the development is traceable back to Walter M. Elsasser (1987), a physicist, who came up with the concept of holistic memory as a general principle in the reproduction of cells and organisms. This memory is based on the process of *homogenous replication*, i.e., replication based on the molecular stability behaving according to the laws of chemistry and physics and *heterogenous reproduction*, i.e., creative selection from *immense* reservoir of possible patterns in nature. The term "creative" means: tied in with the "laws" of physics and chemistry, but not only with them. Both processes provide the stability of heritable information and cannot be fully separated from each other (Elsasser 1984). Elsasser drew his inspiration from the analogy with cerebral memory, and criticized the mechanistic approach to the processes of reproduction of living forms. In his concept, memory is not a simple mechanistic storage (here, Elsasser is inspired by Henri Bergson's book *Matter and memory* published in 1896), but a process of heterogenous reproduction. Holistic memory is a primary phenomenon of nature existence of which is postulated but cannot be deduced from any "law" (Elsasser 1987).

With regard to the holistic theory we should also mention Russian biologist Ivan Ivanovich Schmalhausen who is known for his holistic approach to the development of organisms. Schmalhausen, strongly influenced by his teacher Alexei N. Sewertzoff, criticized the neo-Darwinian concept of the organism as a mosaic sum of genetically determined characters (Olsson et al. 2010; Levit et al. 2006; Levit 2007). In his conception, the organism was understood as an interconnected whole defined by the relative integrity, i.e., by mutual adaptedness of all parts and functions of the organism to each other, providing the stability of the developing system. According to Schmalhausen, the organism develops as a whole at all developmental stages because of the regulative correlations (genomic, morphogenetic and functional)—in this sense, has Schmalhausen already anticipated the modular character of ontogeny (Schlosser and Wagner 2004).

Here, I do not deal with the conflict between holism and reductionism, yet must attend the theory that embodies memory without storage: memory that is not fixed-inscribed into some permanent code such as DNA.

Nowadays, genocentric neodarwinian biology still dominates, with the opinion that every phenotypic trait is represented in the form of string(s)—shorter or longer—of DNA molecule (together with some epigenetic modifications). In contrast, supracellular memory of the body plan probably operates in the way of *heterogenous reproduction*, choosing specific way in which to use the co-opted regulatory pathway. Developmental processes leading to similar morphological structures can differ even among closely related taxa (Newmann and Müller 2000); the same spatial pattern can be generated by various independent ways acting at roughly the same time (Salazar-Ciudad and Jernwall 2004). What is important is not only the inner representations of orthologous genes, but also various contexts, time, and space in which the products of these representations meet. However, such developmental structure itself (the lineage-specific usage of the *toolkit*) is not completely stored in any mechanistic sense of mere digital representations; it is stored in the *bodily form* of the supracellular memory of the species, and in the pattern of the interactive developmental network. Thus, the direct correlation between genotype and phenotype vanishes, and the communicating tissues and cells are the primary level of description, not genes as mere representations. The memory processes the inputs from the developmental program and from the environment and provides the coordination of species specific processes.

A semiotic perspective

Barbieri argues that the memory of the body plan follows specific codes, in order to reconstruct the phenotype of a given organism. Such codes are implemented in the phenotype itself. This author and coworker have already tried to deal with the ontology of codes (Markoš and Švorcová 2009) and found disagreement with Barbieri's concept.

In their conception (Markoš and Švorcová 2009), there is no semiosis without interpretation; the coding–decoding procedure (the set of character strings, and operations thereupon, Searls 2002) is just a derivative, i.e. the outcome of the semiotic negotiation in terms of natural language. Code does not provide meaning to the informational structures—interpretation does. Code is a well-established interpretation (habit of interaction—in the Peircean sense). Therefore, codes are not simply implemented (stored) in the phenotypes; they are negotiated. Living beings are able to reinterpret their developmental circuits based on the same genetic *toolkit,* and these *historically* created and integrated interactions are able to maintain through the following generations. In the particular study of this topic, it becomes obvious that living beings are primarily historical entities capable of forming habits in the form of regulatory circuits, which are homologous and co-opted in evolution. Barbieri would call this habit a code, but this author and coworker believe that the path leading to a code or habit is the interpretation, in the hermeneutic sense, where the receiving system is capable of learning, of following its own history and experience (Markoš 2002).

In this metaphor, memory represents the deposit of habits, which are unique for every species. This approach should not be considered as a vitalistic point of view, they do not postulate any type of hidden vital principle entering the body from outside, the discussed memory representing the memory of every single species embraces the whole unique recruitment and set-up of the same co-opted evolutionary-developmental modules used in different contexts. Such a memory is never empty at the beginning as Barbieri suggests; it comes with the bodies of the germ cell (Markoš et al. 2007). Owing to supracellular memory, living beings are able to deal with an enormous amount of informational processing on many levels of embryonic development. The organic memory maintains the convention, continuity, and coherency of the species.

Extrapolation from the past evolution of developmental circuits is always difficult, and the semiotic perspective describing the evolution of bodily memory remains an ontological claim that even at the cellular level, there is a semiosis. Yet to assume that the complete memory of the body plan is reconstructed based on representations, such as DNA, or recorded codes of mechanistic nature, would be a larger ontological claim (Markoš and Švorcová 2009).

Conclusion

In this article, I have tried to highlight a significant phenomenon of evolutionary and developmental biology, the concept of phylotype. I focused on the history of discussion on this phenomenon, on its role as a key period in the evolution of the phylum, and also on other characteristics associated with this concept (the zootype transcription pattern, and modularity of development). Although the phylotype is probably not such a strongly conservative period in evolution, as Haeckel and Raff had suggested, at a general level this concept can certainly be accepted. The phylotype is then defined by general morphological structures (as, in chordates, notochord, neural tube, or somites), by the conservative transcription pattern of orthologous genes, and by the high level of interactiveness within the embryo at this time.

This article supports Barbieri's idea of the phylotype as a bodily boundary between two types of development: one strictly based on internal representations in form of DNA, and another having the modular and contextual character of specific usage of the same orthologous *toolkit*, where the bodily form precedes the quasi-digital form of genes.

By attempting to describe the nature of supracellular memory in semiotic terminology, we get to the ontological character of living beings. Davidson (2006) deals with this challenge using the computational metaphor of the embryonic development as a programmed computational network with many hierarchically coordinated nodes. In this approach, genes represent the protocols directing the communication, and the regulatory nodes are the control units of informational processing. These metaphors always imply an external creator or coder (programmer) and consider the animal as a nonautonomous unit.

We therefore propose further another, semiotic, metaphor, wherein the memory of the body plan is represented as a field of semiotic habits, negotiated historically during the life of the species in the sense of "semiotic scaffolding" (Hoffmeyer 2007) or in the sense of species as a cultural entity (Markoš 2002). Such a semiotic perspective, of course, remains an ontological claim to be tested and developed further.

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