

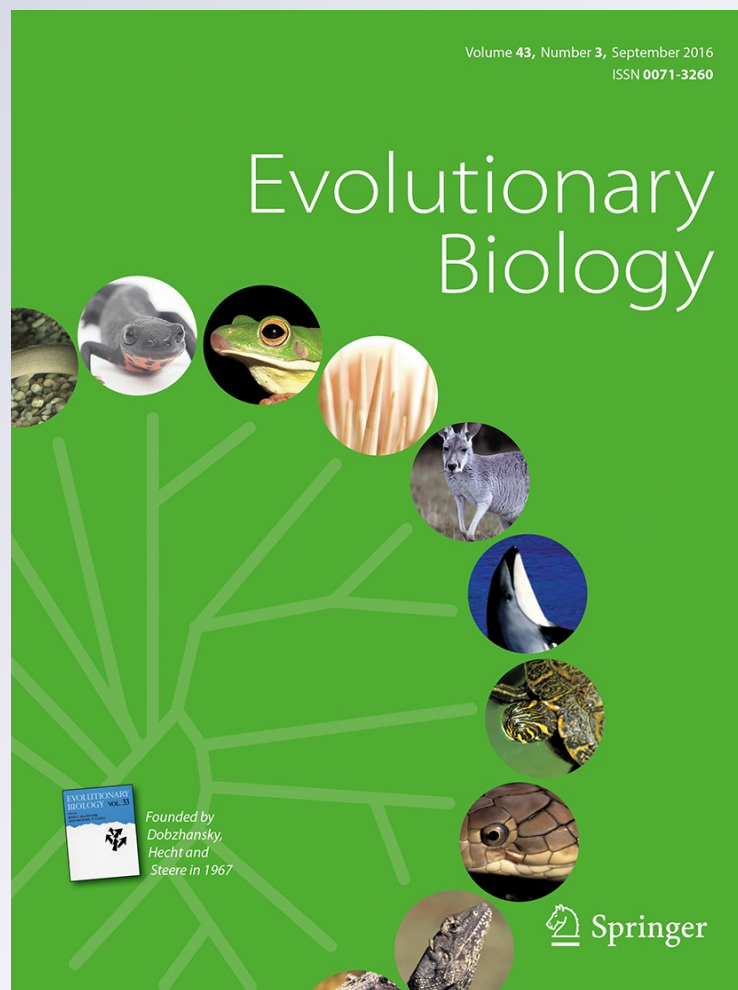
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On Novelty, Heterochrony and Developmental Constraints in a Complex Morphological Theory of Recapitulation: The Genus *Trophon* as a Case Study

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Abstract Darwin proposed *natural selection* as the main evolutionary mechanism in 1859. However, he did not think that this was the only process by which new species were generated. It was the so-called Modern Synthesis who established natural selection as the only mechanism responsible for evolution. Since then, the evolutionary process is explained by the pair mutation-adaptation: new species are generated by the appearance of new mutations, which in case of allowing new adaptations to the environment, they will be fixed and organisms will survive, therefore resulting in new species. An alternative view to the plasticity promoted by the *adaptationist program* is to think organisms as truly organized structures, having different levels of structural organization, which would mean that not every form is possible, but only those that correspond to a certain *building plan*. This would be reflected in the appearance of structural constraints, showing the limits imposed to the organism during its evolutionary development. In this work, I studied the ontogeny and development of three species of the genus *Trophon* by geometric morphometrics, in order to clarify important concepts in evolutionary developmental biology (*Evo-Devo*). Integrating theoretical and empirical investigations, I could propose a

new conceptual framework for *heterochrony* in a context of a complex theory of recapitulation. Furthermore, I could detect a developmental constraint in *Trophon*, which provided an opportunity to reconstruct the concept of constraint and propose a synthesis between heterochrony and constraint that explained evolution as a process fueled by them, that is, as directive and driving force.

Keywords Evolutionary developmental biology · Allometry · Heterochrony · Constraint · Novelty · Recapitulation

Introduction

Darwin proposed *natural selection* as the main evolutionary mechanism in 1859, with the publication of his opus magnum *On the origin of species* (Darwin 1859). However, he did not think that this was the only process by which new species were generated. In fact, he believed that another important evolutionary mechanism was the *inheritance of acquired characters* proposed by Lamarck: “I think there can be little doubt that use in our domestic animals strengthens and enlarges certain parts, and disuse diminishes them; and that such modifications are inherited” (Darwin 1859, p. 134). However, the so-called Modern Synthesis established, in a conference held in Princeton in 1947, natural selection as the only mechanism responsible for evolution (Futuyma 2005). They also recognized that it was possible the inheritance and fixation of non-adaptive mutations in small populations, a process which they called *genetic drift*.

Since then, the evolutionary process is explained by the pair mutation-adaptation: new species are generated by the appearance of new mutations, which in case of allowing

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new adaptations to the environment, they will be fixed and organisms will survive, therefore resulting in new species. This view of the evolutionary process transforms the organism in a highly malleable object to the environment. To put it all at once: the organism could have almost any structure and organization as long as it supports some interesting adaptation and/or competitive advantage in its environment (Gould and Lewontin 1979). In the words of Darwin: “It is really surprising to note the *endless* points in structure and constitution in which the varieties and sub-varieties differ slightly from each other. The whole organisation seems to have become *plastic*, and tends to depart in some small degree from that of the parental type” (Darwin 1859, p. 12).

An alternative view to the plasticity promoted by the *adaptationist program* is to think organisms as truly organized structures, having different levels of structural organization, which would mean that not every form is possible, but only those that correspond to a certain *building plan*. This would be reflected in the appearance of structural constraints, showing the limits imposed to the organism during its evolutionary development. Following a line of thought that could be traced to Richard Owen, and even Goethe, Rupert Riedl noticed that characters had a different evolutionary variation: those which were of structural kind evolved more slowly, ergo evolution seems to deviate from randomness and seems to possess a certain internal order (Riedl 1977; Wagner and Laubichler 2004). In consequence, the key question is the following: which is the cause of the different levels of variability and how do they originate during evolution? Riedl concluded that the probability of change of a character depends on the number and relevance of functions and characters depending on it. Each character evolves in accordance to its commitment in the global organism's functioning: the more assimilated a character is, the more engages with other characters in an interdependence, the more difficult will be its modification. A consequence of this is that then characters do not appear or change in isolation but, on the contrary, they are integrated in a network of functional and structural interdependences in the organism.

Meanwhile, the Spanish zoologist Pere Alberch dedicated much of his scientific activity to the study and understanding of teratologies and monstrous organisms. Alberch was aware that these anomalies were clearly not adaptive, but however they appeared recurrently in the organism's development. In this way, Alberch reached the conclusion that these anomalies were the manifestation of internal processes and general transformational rules, not exclusive in teratologies but shared with all developmental systems (Alberch 1989). Ultimately, Alberch studied developmental anomalies in order to understand the organismic rules and its building plans.

Mollusk shells represent one of the best opportunities in nature to study biological architecture and form. Naturalists, among which stands out D'Arcy Thompson (1917), have already recognized the shell as a developmental unit firmly tied by coiling rules and symmetry. Furthermore, the adult shell represents and reveals the complete organismal ontogeny conserved in its form, in such a way that the previous ontogeny participates in the posterior development of the shell, then growing in continuity with its own past (Gould 1989).

The comparison of anatomical and morphological characteristics was a central problem of Biology since its origins. The study of biological diversity was historically based on morphology description (Roth and Mercer 2000). In the last years, there has been a shift in morphological structure's analysis and quantification. A new method appeared that captures the structure's geometry and keeps morphological information along the whole analysis. This new approach is called Geometric Morphometrics (Adams et al. 2004; Klingenberg 2010; Rohlf 1998; Slice 2007).

The concept of morphogenetic spaces, or morphospaces, can be interpreted as a logical extension of Waddington's epigenetic landscapes (Waddington 1970), Goodwin's epigenetic space (Goodwin 1963) and Alberch's parametric space (Alberch 1989). While these spaces are appropriate for studying a phenotype-genotype map, morphospaces are useful for inferring focal phenotypic phenomena, such as heterochrony, heterotopy and structural constraints. Besides, they enable to cover variation patterns in the fossil record (Eble 2003).

In this work, I studied the ontogeny and development of three species of the genus *Trophon* by geometric morphometrics, in order to clarify important concepts in evolutionary developmental biology. Integrating theoretical and empirical investigations, I could propose a new conceptual framework for heterochrony in a context of a complex theory of recapitulation. Furthermore, I could detect a developmental constraint in *Trophon*, which provided an opportunity to reconstruct the concept of constraint and propose a synthesis between heterochrony and constraint that explained evolution as a process fueled by them, that is, as directive and driving force.

Materials and Methods

Sample: Definition, Delimitation and Characteristics

For the present study, I focused on three species of the genus *Trophon*: *Trophon geversianus*, *Trophon patagonicus* and *Trophon plicatus*. The decision was based on the circumstance that these three species were those which could guarantee to cover the broadest spectrum of

ontogeny and development. They were the most abundant species collected in museum collections worldwide, whereas the great majority of the available specimens were housed at the Museo Argentino de Ciencias Naturales.

In this manner, the dataset consisted of 277 specimens documenting the different stages of postembryonic ontogeny of the shell of *T. geversianus* (N = 157), *T. patagonicus* (N = 92) and *T. plicatus* (N = 28). The age of specimens ranged from juvenile to adults in the three species. All the specimens used are housed at the Invertebrate Collection from the Museo Argentino de Ciencias Naturales (MACN-CONICET).

The genus *Trophon* was subjected to recent revisions in the last years (Pastorino 2002, 2005). *T. geversianus* has fusiform shells with axial (lamellae) and spiral ornamentation (cords) (Fig. 1, left). *T. patagonicus*' shells have only axial ornamentation (lamellae), which is variable among the specimens (Fig. 1, center). *T. plicatus*' shell ornamentation is similar to *T. patagonicus*, but the lamellae are low and spiny, and its shell outline is thinner than *T. patagonicus* (Fig. 1, right). *T. plicatus* also possesses a slight spiral ornamentation in the first whorls, which progressively faints on the successive whorls. Moreover, the aperture in *T. plicatus* is smaller and almost circular.

Image Acquisition

Specimens were digitally imaged using a camera Canon EOS Rebel T1i equipped with a macro lens EF-S 60 mm. Camera settings were chosen as follows: 4 s. (exposure time); F32 (aperture); 200 (ISO). Camera was fixed at approximately 50 cm from a black base (where specimens were settled), connected to a computer and distantly operated using the EOS Utility software. Illumination was regulated using a High Intensity Illuminator NI-150 (Nikon Instruments Inc.).

Shells were oriented consistently in apertural view, which captures most of the shape information and variation. Additionally, shells were aligned with the base, in order to bring the shell axis and apertural area in a plane parallel to the base plane.

Morphology Abstraction

Shell shape was captured as a set of two-dimensional coordinates, consisting of 15 landmarks (Fig. 2). The chosen set of landmarks were placed as depicted in Table 1. Landmark selection was maximized in order to be able to measure the most usual linear shell's parameters (Dépraz et al. 2009). Besides, landmarks 7–8 and 9–10 were chosen in order to lie on a perpendicular line to the shell axis.

Shape Analysis

Shell morphology, abstracted as a set of 15 landmarks, was analyzed using geometric morphometrics (Adams et al. 2004; Mitteroecker and Gunz 2009; Rohlf 1998; Slice 2007; Zelditch et al. 2012). Geometric morphometrics is a suitable methodology for morphological studies of ontogeny and development, due to its capacity to discriminate between size and shape.

Briefly, landmarks were recorded from digital images using the software *tpsDig2* (Rohlf 2010). Posteriorly, specimens were submitted to a Generalized Procrustes Analysis (GPA) (Rohlf and Slice 1990), by which location, scale and rotational effects are removed from an object. All subsequent analyses, including GPA, were performed using the *geomorph* package (Adams and Otárola-Castillo 2013) for the R programming language (Ihaka and Gentleman 1996; R Development Core Team 2012).



Fig. 1 Shells of the species from the genus *Trophon* used in this study: *Trophon geversianus* (left), *Trophon patagonicus* (middle) and *Trophon plicatus* (right). Scale bars 1 cm

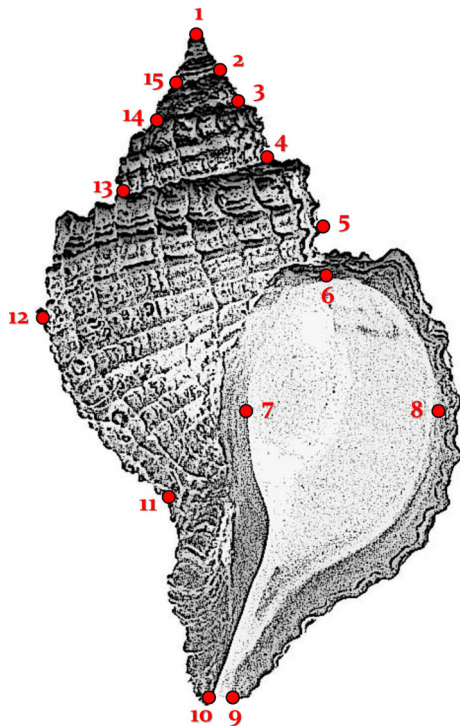


Fig. 2 Set of landmarks selected for the geometric morphometrics analysis. See Table 1 for details and definition of landmarks

Table 1 Definition of landmarks used in the geometric morphometrics analysis of gastropod shells

No.	Landmarks
1	Apex
2	Ante-antepenultimate whorl's suture (right side)
3	Antepenultimate whorl's suture (right side)
4	Penultimate whorl's suture (right side)
5	Most external point of penultimate whorl (right side)
6	Beginning of aperture
7	Most internal point of inner lip
8	Most external point of outer lip
9	End of outer lip
10	End of siphonal canal
11	End of first whorl (left side)
12	Most external point of first whorl (left side)
13	Penultimate whorl's suture (left side)
14	Antepenultimate whorl's suture (left side)
15	Ante-antepenultimate whorl's suture (left side)

From the Procrustes-aligned coordinates, I performed a Principal Component Analysis (PCA), which first components were used as shape variables for ontogenetic trajectory visualization and analysis (**plotTangentSpace**, *geomorph*, verbose mode). As a measure of size, I used the centroid size, which is defined as the square root of the sum of squared differences between landmark coordinates and

centroid coordinates. For allometric analysis, I performed a regression of shape on size, using the method CAC which calculates the common allometric component of the shape data (**plotAllometry**, *geomorph*, verbose mode). This is an estimate of the average allometric trends within groups (Mitteroecker et al. 2004). Statistical significance was assessed by Procrustes ANOVA with permutation procedures (**procD.lm**, *geomorph*, 999 iterations).

Analysis of Ontogenetic Trajectories

After qualitative inspection, ontogenetic trajectories (Alberch et al. 1979) were thoroughly analyzed. In general terms, the approaches suggested in Zelditch et al. (2012) and Piras et al. (2011) were followed. Firstly, a multivariate analysis of covariance (MANCOVA) was conducted. This was carried out using the function **adonis()** from the *vegan* package (Dixon 2003). Depending on the result of the MANCOVA two different strategies were adopted (Zelditch et al. 2012). If the interaction term was significant, the next step was to determine whether trajectories differ in direction or in length. To determine differences in direction, the functions **common.slope.test()** and **ont.conv.test()** were used. If differences in direction were not significant, then the second strategy was undertaken. This strategy was also pursued if the interaction term of the MANCOVA was not significant. To determine if ontogenetic trajectories were parallel or overlapping, the function **int.test()** was utilized. Finally, to determine if trajectories exhibited ontogenetic scaling or paedo-peramorphosis, the function **peram.test()** was employed. All these functions were developed by Piras et al. (2011) and provided in Zelditch et al. (2012).

Spire Angle Determination

The spire angle was determined using the same landmarks chosen for the geometric morphometric analysis. Briefly, a linear regression between landmarks 2, 3 and 4, and 13, 14 and 15, was made. I considered that landmark 1 (apex), was not appropriate for spire angle determination due to the lack of protoconch in the majority of cases. Posteriorly, the angle between the slopes of both regressions was computed and considered as the spire angle. This morphometric analysis, as well as its statistics, was programmed and carried out in R.

Results

Allometry

Allometry, the study of the relationship between shape and size, has a long tradition in evolutionary studies since its

definition by Julian Huxley in 1936 (Gayon 2000). But the picture gets more interesting if we assume an intimate connection between size and age. Then, we can introduce the domain of development in evolution. This integration is not neutral: we are explicitly stating that the developmental time can interfere, modulate and route the evolutionary time.

Two of the three species under study, *T. geversianus* and *T. patagonicus*, had allometric growth, whereas *T. plicatus* grew isometrically, i.e. size increase without shape changes (Gould 1966). Table 2 summarizes these results numerically.

The fact that *T. plicatus* did not show allometric growth allows us to consider it the basal and reference behavior, that is, a situation in which development seems not to have interfered in evolution. It represents the extreme situation where development is decoupled from evolution and, therefore, it cannot exert a direct action on it. The other two species, *T. geversianus* and *T. patagonicus*, on the other hand, showed a modification of shape as a function of size. This can be interpreted as development exerting a direct action on evolution, an action responsible for the diversification of these species. In order to study this, the common allometric component (CAC), an estimate of the average allometric trend within groups (Mitteroecker et al. 2004), was plotted as a function of size (Fig. 3). *T. plicatus* is depicted as a horizontal trajectory with a slope near to zero, which demonstrates its isometric, non-allometric, growth. From this isometric trajectory two other allometric trajectories seem to originate at different timing: *T. geversianus* and *T. patagonicus*' ontogenetic trajectories. The first one deviates from the reference at about 1, whereas the second one at about 1.5 (log centroid size). Therefore, we are at the presence of divergent ontogenetic trajectories. *T. geversianus* and *T. patagonicus* seem to have evolved from a stem group, represented today by *T. plicatus*, by allometric modifications. Moreover, *T. geversianus*' allometric trajectory seems to have a steeper slope than *T. patagonicus*, which is consistent with its lower *P* value. In conclusion, different allometric change onset, the cause of different ontogenetic trajectories, led to the production of different species. The species diverging earlier in their ontogeny from the reference trajectory differ more profoundly from the other species, comparing their adult morphology.

Table 2 Results of the multivariate regression of shape on size, i.e. allometry

Species	Rsq	F	<i>P</i> val.
<i>Trophon geversianus</i>	0.086131	14.609	0.001
<i>Trophon patagonicus</i>	0.031332	2.9434	0.012
<i>Trophon plicatus</i>	0.071133	1.9145	0.064

Significant *P* values are given in bold

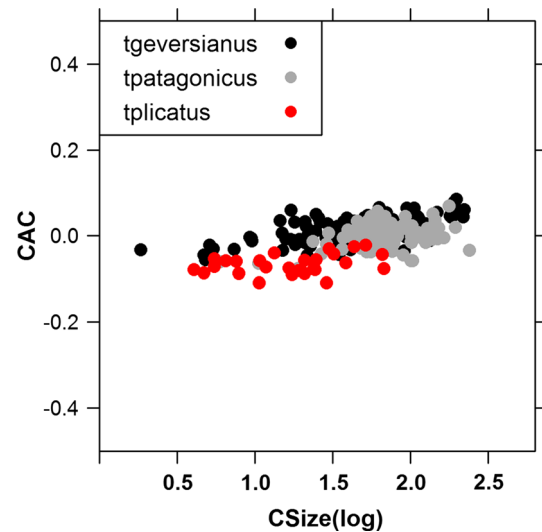


Fig. 3 Allometric plot viewed as the common allometric component (CAC) versus size (log centroid size)

Heterochrony

In recent discussions and debates about heterochrony, there has been a growing consensus considering that heterochrony is explanatory and meaningful if and only if ontogenetic trajectories do not diverge in shape space: “The definition of heterochrony implies that the species compared share the same ontogenetic trajectory of shape change (i.e., overlapping trajectories in shape space)” (Gerber and Hopkins 2011). Therefore, “Only if the trajectories of two related species lie along exactly the same path in shape space can the classic terminology of heterochrony apply and pure dissociation of size change against shape change be detected” (Mitteroecker et al. 2005). However, this statement arises from a particular conception of the term, from a certain amount of premises, and derived from a special conceptual framework. Actually, the concept of heterochrony has a long history in evolutionary biology and has undergone a series of theoretical modifications during its path (Gould 1977), which must be revised in order to fully comprehend the concept.

Heterochrony was a term coined by Ernst Haeckel in 1875, in order to describe a series of exceptions to his theory of recapitulation. According to Klingenberg (1998), heterochrony referred to “a temporal shift of the appearance of an organ relative to other organs of the same organism”. However, we must dig deeper into Haeckel’s theory of recapitulation in order to acquire a clear comprehension of the concept of heterochrony, and its proper place in the context of the theory of recapitulation.

Haeckel thought that the evolutionary process was caused by the integrated acceleration of the adult ancestor into earlier ontogenetic stages of descendants. In this context, that is, in this harmonic and integrated acceleration

of development, he coined the term heterochrony for cases in which this integration was not maintained and, therefore, there was a modification in the phase of development of the different organs in the organism. This definition led to the comparison between animal parts rather than whole animals.

Here, I must state that I cannot agree with Gould when he declared that Haeckel “originally coined “heterochrony” to describe exceptions to global recapitulation based upon changes in developmental timing of one organ with respect to others in the same body”, whereas De Beer “redefined heterochrony as a change in developmental timing of an organ or feature relative to the same structure in an ancestor” (Gould 2000). It has no sense to compare changes in developmental timing of one organ with respect to others in the same body. The comparison, even in the same example given by Gould at that point, is always relative organ appearance during history, i.e. among different species, even though it is not made explicit.

Thus, it is important to consider and evaluate the whole conceptual architecture of the theory of recapitulation. Haeckel defined his first principle of Biogeny as follows: “the evolution of the germ (Ontogeny) is a compressed and shortened reproduction of the evolution of the tribe (Phylogeny)” (Haeckel 1879, p. 12). In this context, Haeckel distinguished between *palingenetic processes* and *kenogenetic processes*: “The term *Palingenetic process* (or reproduction of the history of the germ) is applied to all such phenomena in the history of evolution as are exactly reproduced, in consequence of conservative heredity, in each succeeding generation, and which, therefore, enables us directly to infer the corresponding processes in the tribal history of the developed ancestors. The term *Kenogenetic process* (or vitiation of the history of the germ) is applied to all such processes in the germ-history as are not to be explained by heredity from primæval parent-forms, but which have been acquired at a later time in consequence of the adaptation of the germ, or embryo form, to special conditions of evolution. These kenogenetic processes are recent additions, which do not allow of direct inference as to the corresponding processes in the tribal history of the ancestral line, but which rather falsify and conceal the latter” (Haeckel 1879, p. 10).

Haeckel considered kenogenetic processes as “a gradually occurring displacement of the phenomena” caused by “adaption to the changed conditions of embryonic existence” (Haeckel 1879, p. 12). If this displacement was in place, the process was to be called *heteropy*; if it was in time, it was to be called *heterochrony*. Heterochrony is evidenced “in the fact that in the germ-history (Ontogeny) the sequence in which organs appear differs from that which, judging from the tribal history (Phylogeny), would be expected” (Haeckel 1879, p. 13). Additionally,

heterochrony could be manifested as acceleration or retardation.

Therefore, Haeckel saw in palingenesis the true process of evolution, caused by constant heredity and reflected in complete reproduction (recapitulation). On the other hand, Haeckel saw in kenogenesis a vitiation, a corruption or decay, in the historical process of evolution. However, I think Haeckel was wrong at this point. I think that he could not see here the true process of novelty in evolution.

From a different point of view, heterochrony, and kenogenesis in general, could be regarded not as an exception, vitiation or corruption in the theory of recapitulation, but rather as a complication and enrichment of it: the key towards a complex theory of recapitulation. This change of perspective could allow us to realize that evolution by changes in developmental timing would not be a homogeneous, but, in fact, a heterogeneous process, in which the ultimate cause remains the same.

The above discussion established the following implicit statement: heterochrony was not a concept directly applied to shape analysis in its conception. Its domain of applicability was confined to developmental stages and organ appearance. Having said that, is it possible to apply the concept of heterochrony to shape analysis studies? I think not without a redefinition, a whole reconstruction of the concept. What would be the importance of such a concept in a morphological framework? For that purpose and with this intention, we must reevaluate the Haeckel’s theory of recapitulation, since only in this context the concept of heterochrony would remain valuable and coherent.

In the first place, developmental stages and organ appearance must be translated into morphological parameters and terminology. By doing this, all the changes considered by Haeckel’s theory would be informed as changes in shape. Since heterochrony was applied strictly to organs, i.e. parts of an organism, then the focus of shape analysis should be restricted to them. The following question appears: what happens to overall shape when heterochrony takes place? In the materiality of discourse and domain of applicability of Haeckel’s theory, the overall structure would remain the same, since it is in fact the cause and *raison d’être* of the concept of heterochrony: displacement of relative timing between the organs, without a complete acceleration of developmental stages. But taking into account the new interpretation of heterochrony as a complication in recapitulation, would not be such displacement (i.e. heterochrony) a new developmental stage? Would not be such displacement the source of evolutionary novelty in a theory of recapitulation?

The answer seems to be affirmative. If so, one should consider overall structure as a novelty and, therefore, in a morphological framework, as a new shape. Should the theory of recapitulation, in view of this conceptual

development, be discarded as obsolete or dispensable? Certainly not. The spirit of the theory of recapitulation, the assimilation of ancestors into the developmental stages of descendants, would keep alive. In a morphological framework, this would be reflected in shape recurrence at earlier age/size. Under these conceptual determinations, *simple recapitulation*, i.e. appearance of integrated morphology of ancestors in developmental stages of descendants, would be manifested as recurrence of shape at earlier age/size. Meanwhile, *complex recapitulation*, i.e. appearance of isolated organ morphology of ancestors in developmental stages of descendants, would be manifested as novelty in shape at the same age/size.

Let us study the above statements in more detail. The ontogenetic trajectory of an ancestor determines a recapitulatory morphospace, which is, consequently, a space containing all the shapes adopted by the ancestor during its ontogenetic development. In a model of two dimensions (shape vs. age/size), this space can be represented by a continuous area extending from the starting point of the ancestor's ontogenetic trajectory to its end. In this model, simple recapitulation, that is, recurrence of shape at earlier age/size, would imply that the descendant's ontogenetic trajectory falls within the recapitulatory morphospace (Fig. 4a). Conversely, complex recapitulation, that is, novelty in shape at the same age/size, would imply that the descendant's ontogenetic trajectory falls outside the recapitulatory morphospace. The only way for the descendant's ontogenetic trajectory to develop without repeating (recapitulating) the shapes of the ancestor is adopting a complete change in trajectory's direction (Fig. 4b). Meanwhile, in a model of three dimensions (shape 1 vs. shape 2 vs. age/size), the situation is different. In this case, the recapitulatory morphospace is transformed into a plane which is perpendicular to the plane shape 1–shape 2. In a situation of simple recapitulation, again, the descendant's ontogenetic trajectory must fall within the recapitulatory morphospace (Fig. 5a). This would determine that, in a view

perpendicular to the plane shape 1–shape 2, the two trajectories are superimposed (Fig. 5b). In a situation of complex recapitulation, the requirement to fulfill this condition is just that the descendant's ontogenetic trajectory leaves the plane imposed by the ancestor (Fig. 6a). In this way, in a view perpendicular to the plane shape 1–shape 2, the two trajectories must not be superimposed (Fig. 6b).

From another point of view, the relation between size and age (time) would be paved by means of this new approach. In fact, age and time would become superfluous parameters, since the only important parameter necessary for detecting heterochrony would be shape, changes of shape, specifically, divergence in ontogenetic trajectories. As a matter of fact, although it is a central concept in Haeckel's theory, it seems to me that time is not (should not be) the central point in recapitulation, especially in a morphological framework. The focus should be at shape recurrence and novelty during development, regardless of developmental timing. The focus should be at shape (developmental stage) ordination rather than their relative timing. I think that time by itself, by means of mere accelerations or retardations, cannot explain the creative process called evolution.

In this manner, I decided to test for heterochrony in *Trophon* by means of this new approach, studying the behavior of shape as a function of size. For that purpose, I plotted in three dimensions the ontogenetic trajectories of the different species. Two dimensions represented shape, and consisted of the first (PC1) and the third (PC3) components of a PCA (PC2 was omitted from the analysis as it did not show any relevant or additional information, data not shown). Meanwhile, the third dimension represented size, and consisted of the logarithm of the centroid sizes of the specimens. As can be seen in Fig. 7a, the ontogenetic trajectories of the three species have a common origin, depart from each other at an early developmental stage, i.e. at small sizes, and follow very different directions on their

Fig. 4 **a** Simple recapitulation in two dimensions (shape vs. age/size). **b** Complex recapitulation in two dimensions (shape vs. age/size). Ancestor's ontogenetic trajectory (*black*). Descendant's ontogenetic trajectory (*grey*). Colored area denotes ancestor's morphospace

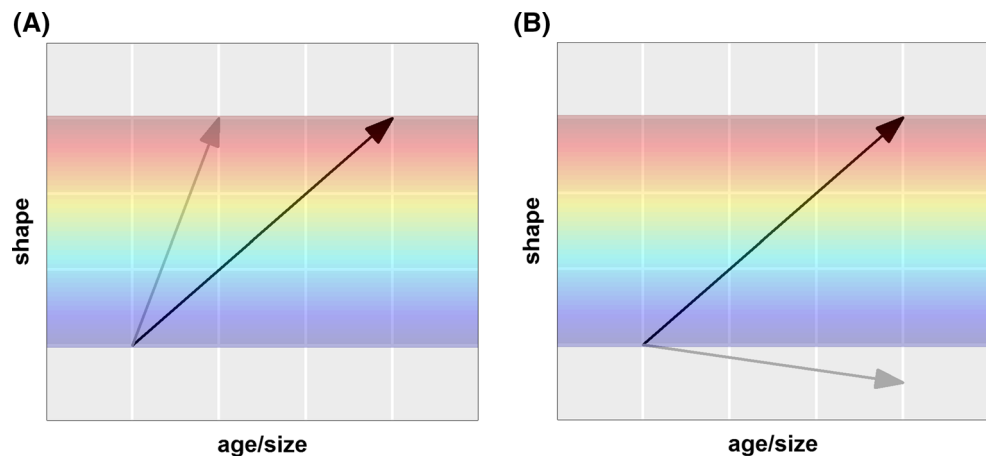


Fig. 5 Simple recapitulation in three dimensions (shape 1 vs. shape 2 vs. age/size). Ancestor's ontogenetic trajectory (black). Descendant's ontogenetic trajectory (grey). Colored area denotes ancestor's morphospace. **a** 3D view, **b** transversal view

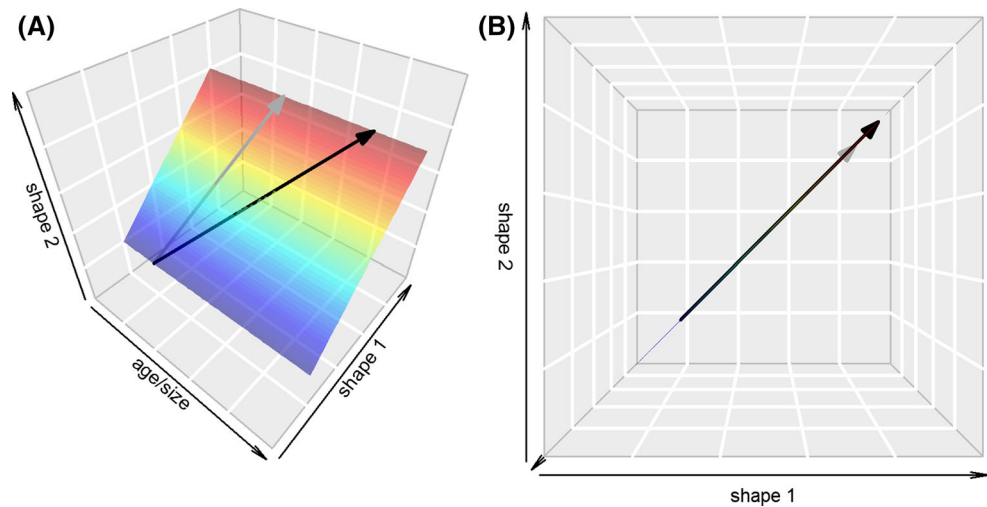
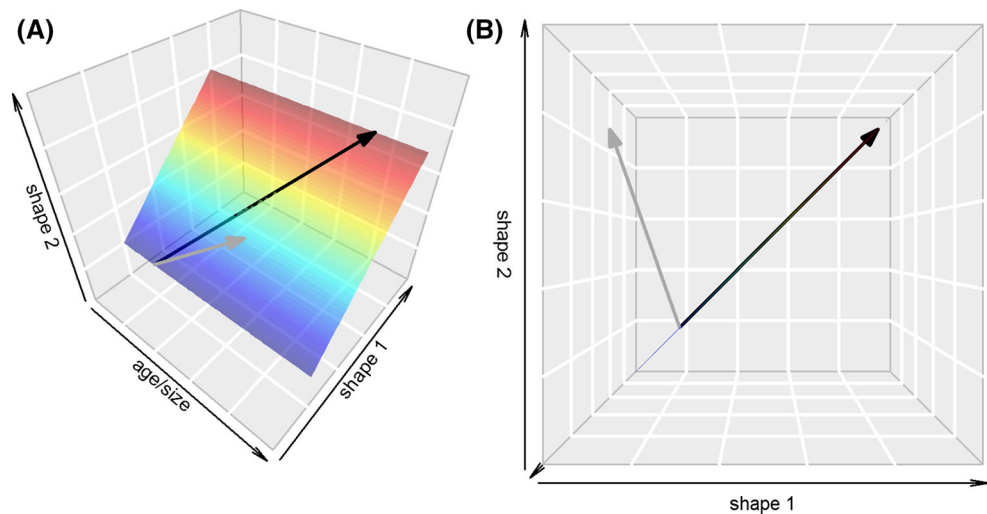


Fig. 6 Complex recapitulation in three dimensions (shape 1 vs. shape 2 vs. age/size). Ancestor's ontogenetic trajectory (black). Descendant's ontogenetic trajectory (grey). Colored area denotes ancestor's morphospace. **a** 3D view, **b** transversal view



subsequent development. This could be detected and visualized better by rotating the view in a way that ontogenetic trajectories could be discriminated by its projection in two dimensions (Fig. 7b). From this new perspective, we can see that young specimens are much more similar than adult specimens. Moreover, if we remember the models of recapitulation in three dimensions discussed previously, and its corresponding properties, we could be able to realize that we are in a situation of complex recapitulation: the *T. geversianus* and *T. patagonicus*' ontogenetic trajectories completely deviate and diverge from the recapitulatory morphospace (plane) determined by the *T. plicatus*' ontogenetic trajectory (Fig. 8).

In conclusion, ontogenetic trajectory divergence is a symptom and evidence of the occurrence of heterochrony, in the way it was defined specifically in this work. This was clearly detected in the species under study.

Analysis of Ontogenetic Trajectories

Posteriorly, I decided to excel the previous qualitative study and undertake a thoroughly analysis of ontogenetic trajectories. From the above analysis, we concluded that ontogenetic trajectories had a common origin, depart from each other at different developmental stages and followed different directions. Thus, I aimed to test this statistically.

Firstly, I performed a multivariate analysis of covariance (MANCOVA) in order to test for differences in ontogenetic trajectories. As shown in Table 3, MANCOVA was highly significant ($P < 0.05$), including the interaction factor, which led to the conclusion that ontogenetic trajectories differ in direction or rate of allometric growth.

As interaction was significant, I then performed the common slope test and the ontogenetic convergence test in order to check if trajectories differ in direction. Both tests

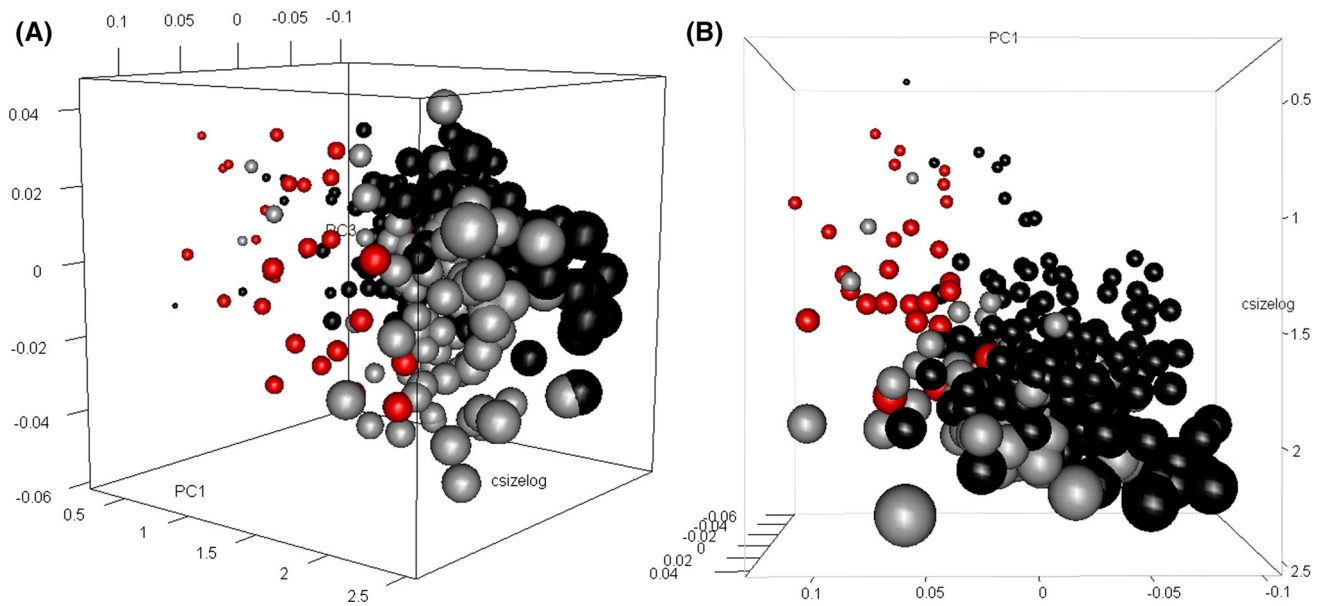
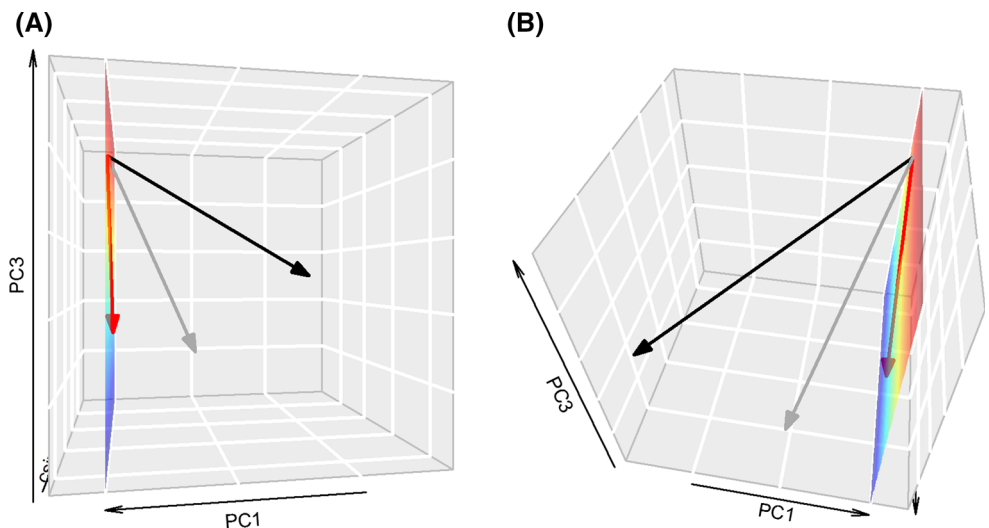


Fig. 7 Ontogenetic trajectories depicted in shape-size space, with PC1 and PC3 as shape dimensions and log centroid size as size dimension. *Dot* size is proportional to the centroid size of specimens. *T. geversianus* (black), *T. patagonicus* (grey), *T. plicatus* (red). **a** 3D view, **b** upper view (Color figure online)

Fig. 8 Scheme of ontogenetic trajectories depicted in shape-size space, with PC1 and PC3 as shape dimensions and log centroid size as size dimension. *T. geversianus* (black), *T. patagonicus* (grey), *T. plicatus* (red). Colored area denotes *T. plicatus*' morphospace. **a** transversal view, **b** opposite view (Color figure online)



were negative ($P > 0.05$) and do not show any divergence or convergence between predicted shapes at common small and large sizes (Table 4). Therefore, both tests suggest that trajectories are parallel.

The next step was to test elevation or overlapping in ontogenetic trajectories. For that purpose, I carried out the multivariate intercept test. The results indicated that there was elevation between *T. geversianus* and *T. plicatus* ($P < 0.05$), whereas the other pairwise comparisons were not significant, thus suggesting, taking into account the previous test, that these last trajectories were overlapping (Table 4).

Finally, overlapping trajectories, although they were apparently at the same line, may be subjected to processes of acceleration and retardation, implying the need of

Table 3 Results of the multivariate analysis of covariance (MANCOVA)

	F. model	R ²	Pr(>F)
LCS	18.394	0.056	0.001
Species	18.172	0.110	0.001
LCS:species	1.657	0.010	0.035

LCS log centroid size

Significant P values are given in bold

testing that hypothesis. The peramorphosis test was highly significant in all comparisons ($P < 0.05$), indicating a wide occurrence of paedo-peramorphosis (Table 4). This was

Table 4 Results of pairwise comparison of ontogenetic trajectories

Pairwise comparison	Common slope test	Ontogenetic convergence test	Multivariate intercept test	Peramorphosis test
<i>T. geversianus</i> <i>T. patagonicus</i>	0.613	1.000	0.433	0.001
<i>T. geversianus</i> <i>T. plicatus</i>	0.130	0.500	0.009	0.001
<i>T. patagonicus</i> <i>T. plicatus</i>	0.595	0.431	0.101	0.002

Significant *P* values are given in bold

especially important in the comparison between *T. patagonicus* and *T. plicatus*, which from the previous test we knew they were overlapping. Thus, it seemed that *T. patagonicus* was a species derived from *T. plicatus* by peramorphosis (hypermorphosis).

In conclusion, the statistical analysis of ontogenetic trajectories suggested a situation in which *T. patagonicus* arised from *T. plicatus* by hypermorphosis, and *T. geversianus* arised from *T. plicatus* by preformation or pre-displacement. In consequence, both species arised from its ancestor by a process of peramorphosis, that is, by overdevelopment of the descendant (Klingenberg 1998; Lieberman et al. 2007).

However, we cannot disregard the statistically significant allometry detected in *T. geversianus* and *T. patagonicus* (allometric growth), which suggested a dissociation between size/age and shape with respect to *T. plicatus* (isometric growth). Therefore, I think that the situation is better explained by the occurrence of a process of acceleration at different developmental stages: *T. geversianus* departed from the *T. plicatus*' ontogenetic trajectory at an early age, whereas *T. patagonicus* departed from the *T. plicatus*' ontogenetic trajectory at a late age, which could be confounded or interpreted as a simple process of hypermorphosis (overdevelopment without dissociation between size and shape).

The most important point that I would like to stress from these results is the fact that the dissociation between size and shape, i.e. allometry, seemed to be the process responsible for the divergence of ontogenetic trajectories, which, under our underlying assumptions, is the manifestation of heterochrony.

A Developmental Constraint in *Trophon*

From the very beginning of this work it was quite evident that, besides the morphological analysis by geometric morphometrics, there was a kind of constant feature in the different species from the genus *Trophon*, that it could not be detected or reflected clearly by this approach. Specifically, it was almost certain that a different and constant spire angle was distinctive in each species. I, therefore, decided to test this hypothesis empirically.

For that purpose, the spire angle of all specimens was computed as described in [Materials and Methods](#), and plotted

for the different species. As shown in Fig. 9, a clearly different and distinctive spire angle was detected in the three species considered from the genus *Trophon* ($P < 0.001$ by ANOVA): *T. geversianus* (48°), *T. patagonicus* (42°) and *T. plicatus* (36°). More interestingly, the difference among these spire angles was also constant, differing by approximately 6°. This situation may be pointing out the existence of a developmental constraint in *Trophon*.

This developmental constraint is interesting due to the fact that different spire angles may be reflecting different developmental processes involved in this genus. In this manner, this constraint not only would be limiting the possible forms adopted by the organismal shell, but it would also be actually channeling its development. This leads to a reinterpretation of the concept of constraint that will be analyzed in the following discussion.

Discussion

Structural Reconstruction of Recapitulation: Towards a Complex Theory of Recapitulation

Haeckel's theory of recapitulation rested on two fundamental concepts: (1) *terminal addition*: evolutionary

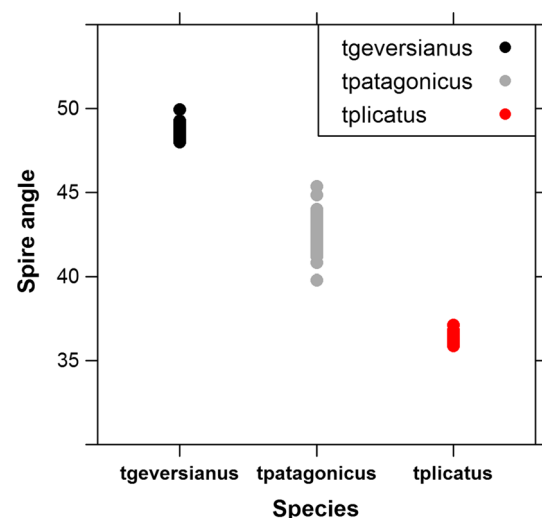


Fig. 9 Determination of spire angle in the three species considered from the genus *Trophon*. Statistical significance determined by ANOVA ($P < 0.001$)

change works by adding new developmental stages to the end of the ancestor's ontogeny; and (2) *condensation*: development accelerates as ancestral traits are relegated to earlier stages of descendant's ontogeny. This conceptual architecture works fine when dealing with simple recapitulation, but it is not a good framework when dealing with complex recapitulation, where heterochrony takes place and is no longer appropriate to speak only in terms of timing of developmental stages. What would be the proper conceptual architecture for a complex theory of recapitulation?

A complex theory of recapitulation would be founded in the following concepts:

1. *Heterochrony*: the displacement and phase difference of part of the structure affecting the morphological correlation of the parts. This would be manifested in the occurrence of allometry, i.e. dissociation between size/age and shape, and departure from the ancestor's ontogenetic trajectory.
2. *Assimilation*: ancestor's morphological structure is incorporated into earlier stages of development of descendant's ontogeny in a complex manner. This would mean that ancestor's developmental stages may not be obviously recognized at the descendant's ontogeny. The process is complex and, therefore, must be analyzed correspondingly.

The process of assimilation could be represented and compared to similar processes described by Riedl (1977), Waddington (1970) and Butler (1878), in different fields of knowledge. This process would consist of an increasing internalization in deeper levels of organization over time. In this context, development would represent the acquisition and display of these increasingly complex levels of organization.

It is true that the descendant's ontogeny does not reproduce its ancestor's adult morphologies: it is most likely that the process of diversification, i.e. descendant generation with variation, occurs during development, that is, before ancestors complete development and reach adulthood. Moreover, this allows us to stress the important fact that evolution occurs *during* development: development enables evolution. I consider that assertion so literally, that I am almost prone to say that evolution *is* development and, reciprocally, development *is* evolution. In morphological terms, this would mean that as long as there is disrupting change of shape, i.e. heterochrony, there is evolution. This reformulation of the theory of recapitulation, as it was the original version, is also compatible with the inheritance of acquired characters. Acquired characters would be acting as the source of change, morphologically manifested as heterochrony, which could enter into the process of assimilation, be passed to earlier

developmental stages, i.e. deeper levels of organization, and be, henceforward, inherited.

What about the phenomena of pure acceleration (hypermorphosis) and retardation (hypomorphosis) in development? From this new perspective, they are not *per se* sources of novelty, they only consist of difference in timing with maintenance of overall structure with respect to the ancestor. The only source of novelty is heterochrony, which under this formulation is *diverging* allometric growth, i.e. with the generation of a new potential direction.

Comments on the Definition and Interpretation of Constraint in Evolution

Variation in a Darwinian context is gradual and continuous, and appears as an unrestricted and limitless event. On the other hand, adaptation, understood as fitness to the environment, appears as the primary factor responsible for its channeling. But could the cause of the shell's morphological evolution and development be ascribed to such concepts? The shell is the external manifestation of the snail developmental growth, that is to say, the final shell morphology is the history of its development. To think that morphology is driven by a limitless event, and channeled by an external factor, seems rather inadequate to explain such a complex and structural process as the one involved in development. On the contrary, shell morphology is rather the final architecture of a historical created and progressively modified building plan, a *Gestaltung*.

A developmental constraint was found in *Trophon*. The species studied in this work grew with a specific and constant spire angle. These angles were not continuous: they were separated by a surprisingly constant angle increase among the species (approximately 6°). Both facts point out to different developmental processes involved in the genus *Trophon*.

The concept of developmental *constraint* is one of the most controversial and debated topics in evolutionary biology since the publication of the renowned paper from Stephen Gould and Richard Lewontin (Gould and Lewontin 1979). The focus of all the discussion of that paper, and of this work, is Chapter 6 from *On the origin of species* (Darwin 1859). That chapter, entitled "Difficulties on theory", deals with the irritable topic of transition. Darwin opens the chapter asking himself, almost rhetorically: "why, if species have descended from other species by insensibly fine gradations, do we not everywhere see innumerable transitional forms? Why is not all nature in confusion instead of the species being, as we see them, well defined?". What is called into question is, then, the "old canon in natural history": *Natura non facit saltum*. This is a key issue in Darwin's evolutionary theory: the whole

conceptual edifice of his theory rests on gradual (continuous) variation.

It is not an accident or fortuitous event that Darwin has left out development from his consideration in the development of his theory (redundancy is intended and appropriate in this case). Development is the occasion for change and novelty. If one were inclined to look for gradualism or constraint, development would be the proper opportunity. With this, it is not intended to say that Darwin did not talk about embryology and development in his work, or even not considered and analyzed these topics in view of his own theory of evolution by natural selection. However, this is quite different from including development in the epistemological and conceptual framework of his theory. This could be done, for instance, by including such concept as constraint in the “mechanistic” explanation of the theory. However, as it is widely known, Darwin’s theory is predominantly functional rather than structural, and, what is more, a rather atomistic functionalism and adaptationism (Gould and Lewontin 1979).

Constraint is a concept that contains ambiguity in itself, and this fact makes it a very interesting concept in biology. Constraint is derived from the Latin *stringere*, which in turn is derived from the Proto-Indo-European *streg*. From this term, the words *string* and *strength* were generated. Therefore, from its very own origin the term contains the double and apparently contradictory concepts of (1) restriction, limitation; and (2) strength, potency, impulse. In other words, a thing that has in itself strengths and weaknesses. However, both concepts can be included in the more exact translation of *compression*. A thing that is compressed is restricted but, at the same time, pressed, i.e. moved. One can imagine at this point the act of fisting. However, this is a peculiar fisting in which the thing that clenches is the one which is squeezed. In fact, one may think that the concept of constraint is loaded with a kind of negativity that generates a twisting of the thing in itself, in which a lack impulses the next movement, the next necessary step, of what is considered to be the developmental process. In consequence, constraint is intrinsic to development and is actually the motor of development itself. In this view, constraint would be not only the directive force, but also the driving force of development and, subsequently, evolution.

Darwin’s solution to the problem of transition is, as is well-known, to ascribe the lack of continuity to the incompleteness of the fossil record and the isolation of biogeographical niches. He left the problem of “Unity of Type” to the end of Chapter 6. There, he subordinated Saint-Hilaire’s unity of type to Cuvier’s conditions of existence, and both of them to his principle of natural selection, that is to say, the adaptation of “the varying parts of each being to its organic and inorganic conditions of

life” (Darwin 1859, p. 206). Darwin considered the unity of type included in his principle of natural selection “through the inheritance of former adaptations” (Darwin 1859, p. 206). What he precisely wanted to imply with that asseveration is not made very clear. A past adaptation cannot act as a present constraint: a constraint must be present from start in order to act as such. If there are only adaptations, and the accumulation of adaptations, there is no room for the concept of constraint in evolution.

At this point, one could argue that inheritance is a kind of constraint, and that Darwin considered inheritance, therefore, he did consider constraint. This is quite a complex and interesting question to analyze. In the first place, we could say that the consideration of inheritance as a kind of constraint depends on what it is understood by inheritance. If by inheritance is understood the appearance of isolated and independent characters of the ancestor in the descendant, which is the predominant notion of the term in a Darwinian context, then it can hardly be considered as a sort of constraint.

We could find a more thoughtful explanation of constraint in Darwin’s discussion of correlation of growth. By correlation of growth Darwin understood the concomitant and coordinated modification of parts during development: “I mean by this expression that the whole organisation is so tied together during its growth and development, that when slight variations in any one part occur, and are accumulated through natural selection, other parts become modified” (Darwin 1859, p. 143). After recognizing that “the nature of the bond of correlation is very frequently quite obscure” (Darwin 1859, p. 144), Darwin discussed various cases of correlation of growth in nature, such as “cats with blue eyes are deaf”.

Now, is the “cats with blue eyes are deaf” a legitimate case of correlation of growth? In other words, is this an example of co-development? Or, otherwise, it is an event of co-inheritance? With co-inheritance we mean the probabilistic association of appearance of related characters based, for example, on their proximity in a given chromosome (in Mendelian conceptology). It seems that the statement “cats with blue eyes are deaf” appeals to a process similar to co-inheritance, rather than to a process by which “the whole organisation is so tied together during its growth and development”, which is Darwin’s definition of correlation of growth.

I think there is an ambivalence (not ambiguity) in the concept of correlation of growth used by Darwin. I think that he was, in fact, dealing with two different concepts of correlation. The first one, exemplified by the “cats with blue eyes are deaf”, is a concept of correlation that we could call “accidental correlation” or, more moderately, “atomistic correlation”, which opened the door for the entrance of Mendelism into Darwinism, and derived in the

modern concept of *genetic linkage*. The second one is the actual concept of correlation of growth, which we could call, in order to do justice to the concept by choosing a more precise and specific term, “structural correlation”. As I think that development is a structural process, it could also be called “developmental correlation”. It would be a rather controversial and hard task to check how many of Darwin’s examples are true structural correlations. But one thing is for sure: the true master of structural correlations was undoubtedly D’Arcy Thompson (1917).

What seems to be concluded from the above discussion is that Darwin conceived the action of a process such as correlation of growth, confined to the domain of development and, therefore, not inherited. The reason for this is that Darwin subordinated such a process to natural selection: “These tendencies, I do not doubt, may be mastered more or less completely by natural selection” (Darwin 1859, p. 143). Ultimately, a character will be transmitted to the descendants if natural selection allows it. As this mechanism is essentially an atomistic process [i.e. natural selection acts at each individual part of the organism, regardless of its relations with the rest: “natural selection will always succeed in the long run in reducing and saving every *part* of the organisation, as soon as it is rendered superfluous, without by any means causing some other *part* to be largely developed in a corresponding degree” (Darwin 1859, p. 148)], then there is no room for the inheritance of structural properties.

Recapitulation, Heterochrony and Constraint: A Synthesis

Arthur provided an interesting conceptual framework for evolutionary developmental biology (Arthur 2002). He considered evolution as a process with directionality. He believed that the concept of constraint was loaded with an inevitable negative connotation and that Gould’s attempt to broaden its definition brought even more confusion to the concept (Arthur 2004). For that reason, a new concept was necessary that was responsible for the orienting role in evolution. He proposed, then, *developmental bias*. Arthur defined developmental bias as situations in which “the probability of [the ontogenetic trajectories to] being rerouted in some directions is higher than for other directions” (Arthur 2004). He, thus, defined developmental bias in terms of probability. In this way, the causal factor is lost and the explanation is, therefore, missing.

Arthur included a negative and a positive component in his concept of *developmental bias* (Arthur 2002, 2004). The negative bias was represented by *constraint*, which referred to developmental trajectories that are hard to produce. Meanwhile, the positive bias was represented by *developmental drive*, which referred to developmental

trajectories that are, on the contrary, easy to produce. Therefore, Arthur envisaged the existence of a pair of complementary factors acting in the evolutionary process, but he could not conceive a reciprocal causal relation between them.

What is also lacking in Arthur’s proposal is an agent responsible for the assumed directionality in evolution. There must be an instance of decision and choice in order to propose a direction in the evolutionary process. The same consideration applies if a selective process is postulated. I think that it is necessary to move to the realm of behavior in order to comprehend this deeply.

I consider Arthur’s approach to be very valuable. However, I think that the concept of evolution must embrace the inherent contradiction of its dialectical process. I think Gould was right in trying to broaden the concept of constraint, although he made no clear definition of the evolutionary developmental process as a dialectical process fueled by constraint, i.e. by its negativity. There is a fundamental epistemological obstacle to break here. An *epistemological obstacle* is a concept conceived by Gaston Bachelard, in order to stress the fundamental role of rationality and epistemology in the scientific enterprise, and the necessity to overcome the mental patterns (obstacles) at work in science.

What if the concepts of heterochrony and constraints were related? What if they could explain evolution and development as a process fueled by them? Heterochrony, the generation of novelty, would create an organismal imbalance which is translated as a negativity, i.e. a constraint. This constraint, its fundamental action, is to reestablish the order which conforms to the organismal norms. It is an inward action, a loop, which drives the next movement towards the generation of novelty, the next step in a creative evolution. The principal manifestation of this process is the existence and directedness of ontogenetic trajectories. These ontogenetic trajectories reflect the working of a directed and structural driven developmental process, in which each developmental stage promotes and regulates the following. This process cannot be accounted or explained by such concepts as *adaptational plasticity* or *aleatory gradual variation*. Moreover, this interpretation provides a synthesis between constraint and evolutionary drive, and an explanation for its cooperative and synergistic activity.

On the other hand, what has been called inheritance in a genetical conceptual framework, it is in this context the ordered unfolding and displaying of the historically-sedimented organismal norms. What the observer/researcher sees while witnessing and studying this process morphologically, is what has been called, by means of simplification, a process of recapitulation, that is, the reproduction of phylogeny through ontogeny. However, this must be

understood properly: ontogeny is an ever new and present-actual process of norm unfolding, which is constantly prone to deviate and departure from its expected trajectory.

Concluding Remarks

I would like to say, before ending, that I think that the future comprehension of evolution rests on the development of new concepts and new epistemological devices. I do not think that descending in the levels of biological organization will provide per se new levels of conceptual explanation. In other words, passing from morphological considerations to genotypic considerations is nothing more than transferring the involved conceptual issues and epistemological obstacles to another domain of explanation. We will have to break these obstacles in order to capture the living nature of the concept of evolution.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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