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Abstract

We review the evolutionary importance of developmental mechanisms in constraining evolutionary changes in animals—in other words, developmental constraints. We focus on hard constraints that can act on macroevolutionary timescales. In particular, we discuss the causes and evolutionary consequences of the ancient metazoan constraint that differentiated cells cannot divide and constraints against changes of phylotypic stages in vertebrates and other higher taxa. We conclude that in all cases these constraints are caused by complex and highly controlled global interactivity of development, the disturbance of which has grave consequences. Mutations that affect such global interactivity almost unavoidably have many deleterious pleiotropic effects, which will be strongly selected against and will lead to long-term evolutionary stasis. The discussed developmental constraints have pervasive consequences for evolution and critically restrict regeneration capacity and body plan evolution.

1. INTRODUCTION

Evolution has produced an astonishing array of organisms, yet the branches of the evolutionary tree do not reach all profitable regions of phenotype space (Maynard Smith et al. 1985, Vermeij 2015). Evolution, thus, appears to be subject to constraints. The term constraint means restriction relative to a set of possible options. We focus on developmental constraints, that is, on the role of developmental mechanisms in restricting the range of possibly adaptive phenotypes (Oster & Alberch 1982, Müller & Wagner 1991, Amundson 1994, Schoch 2013). In practice, our view on constraints on change depends on the timescale. In evolutionary biology, it pays to distinguish at least between microevolution (changes in gene frequencies on a population dynamical timescale), mesoevolution (the evolution of quantitative traits through repeated mutant substitutions and the concomitant evolutionary diversification), and macroevolution (large-scale changes, such as innovations). Quantitative genetics and adaptive dynamics (e.g., Metz 2012) provide the main frameworks for dealing with trait evolution on the micro- and mesoevolutionary scales, respectively, whereas macroevolutionary discourse is dominated by arguments from functional morphology and evo-devo. Discussion on the existence of developmental constraints has in the past primarily focused on genetic constraints, in particular on the potential of genetic covariation due to linkage disequilibrium or pleiotropy to constrain evolution (e.g., Barton & Turelli 1989, Kirkpatrick & Lofsvold 1992, Futuyma et al. 1995, Walsh & Blows 2009, Conner 2012). Genetic covariation undoubtedly can have major effects on evolution; however, it primarily acts on microevolutionary and at most mesoevolutionary scales and is highly unlikely to cause long-term conservation (e.g., Lande 1980, Conner 2012, Hill & Zhang 2012), but see below for relational pleiotropy (sensu Hadorn 1961). Mesoevolution presumably is largely based on mutations with small effect. This expectation has both a mechanistic and a Darwinian reason. Most trait evolution appears to be regulatory (e.g., Gehrke & Shubin 2016, Maeso et al. 2017). Most mutational changes in the regulatory mechanisms may be expected to result in small changes in the relevant protein levels at different points and times in the body, and thus to result in small changes in development, physiology, and behavior. In addition, mutations with large effect tend to bring an otherwise harmoniously operating system into disarray and will therefore contribute little to mesoevolutionary change (cf. Metz 2008, 2011). These fitness effects are also relevant for macroevolution. However, on macroevolutionary scales, trait spaces themselves change through the breaking of hard constraints, permitting innovations (see Section 3.15) (Müller & Wagner 1991, Galis & Metz 2007, Varela-Lasheras et al. 2011). We focus on constraints on macroevolutionary scales.

Absolute developmental constraints on the evolution of specific adaptive phenotypes are rare. Vermeij (2015) even postulates that no such absolute constraints exist, given sufficient time. We argue below that one category of near-absolute developmental constraints exists: When development is highly interactive (has low effective modularity), the multitude of cascading pleiotropic effects (relational pleiotropy, sensu Hadorn 1961) results in high-dimensional covariation. Mutations with an effect during such highly interactive stages will almost unavoidably have a multitude of pleiotropic effects, which will be strongly selected against, and thus will constrain the evolution of traits even on macroevolutionary scales (Galis & Metz 2001, Galis et al. 2002b). Hence, such traits will be conserved owing to consistently strong stabilizing selection caused by pleiotropic effects (Lande 1978, Hansen & Houle 2008, Futuyma 2010).

We found very few well-documented examples of developmental constraints that act long term. In this review, we discuss what we think are the outstanding examples of such near-absolute developmental constraints in animals as well as their causes and evolutionary impacts: The metazoan constraint that differentiated cells cannot divide and the constraints against changes of phylotypic stages in vertebrates and other higher taxa.

2. AN ANCIENT METAZOAN CONSTRAINT: CELLS CANNOT DIVIDE WHILE DIFFERENTIATED

In 1898, Henneguy (1898) and Lenhossék (1898) were the first to hypothesize, independently, that ciliated cells of metazoans are not capable of cell division. They had both noticed that the basal bodies of cilia were transformed centrosomes, and both also remarked on never having seen a ciliated cell divide. Based on these observations, they hypothesized that in cells where the centrosome has lost its ability to form a spindle and has become involved in cilium formation, cell division is not anymore possible, at least not by mitosis. Both processes need the centrosome and are, therefore, mutually exclusive. A newly formed cell has one centrosome, comprised of two centrioles (an older mother and a younger daughter centriole) surrounded by a protein-rich matrix termed pericentriolar material. The centrosome is duplicated during the S phase of the cell cycle, so that during mitosis two centrosomes form the two poles of the spindle. In addition, one of the centrioles also serves as basal body of a cilium or flagella (Brinkley 1985, Wu & Akhmanova 2017). Indeed, several studies have found a negative correlation between the presence of cilia and mitotic activity, supporting this hypothesis (Dingemans 1969, Fonte et al. 1971). Buss (1987) extended the constraint and proposed that in metazoans a universal constraint exists on the division by mitosis of not only ciliated but all differentiated cells, as he assumed that the cilium is usually involved in the differentiation process of a cell (see also Bell 1989). His reasoning is similar, although he ascribed the idea to Margulis (1981) instead of to Henneguy (1898) and Lenhossék (1898), presuming that metazoan cells contain only one microtubule-organizing center (MTOC), the centrosome, and that any commitment of a single MTOC, whether to a cilium or to another microtubule-based structure, would preclude commitment to the poles of the mitotic spindle, thus preventing mitosis. Thus, stem cells and progenitor cells can divide and ciliated cells cannot, unless they dedifferentiate and resorb the cilium, as happens in regeneration (Tanaka & Reddien 2011) and cancer (Schwitalla et al. 2013). Buss (1987) further proposed that the constraint is a phylogenetic one, given that "evolution can only yield variants of that which it has already produced" (p. 34). He argued that metazoans inherited the limitation of having no more than one MTOC from their unicellular protist ancestors and that, in contrast, other unicellular groups such as euglenophytes, cryptophytes, and chlorophytes with multiple MTOCs do not have this constraint. These groups are capable of accomplishing simultaneous cell movement and mitotic cell division by using some centers exclusively as organizers for undulipodia (cilia, flagella) and others for cell division.

2.1. Challenges to Buss's Hypothesis

Bell (1989) stressed the importance of the proposals of Buss but disagreed with the interpretation of a phylogenetic constraint. Bell argued that it is not likely that metazoans are limited by their inability to generate more than one centrosome, given that they are capable of duplicating centrosomes in every cell cycle: The two centrioles of the centrosome are each duplicated during S phase, and both form a new centrosome together with the duplicated copy of themselves (a mother and daughter centriole). Thirty years later, the rapidly expanded knowledge of cellular dynamics has revealed several further challenges to Buss's hypothesis. Experimental removal of centrosomes has shown that they are not essential for the formation of radial spindles and mitotic cell division (e.g., Bonaccorsi et al. 2000, Basto et al. 2006). Some cells normally divide without a centrosome in early development (Courtois et al. 2012, Zenker et al. 2017). Centrosomes have been evolutionarily lost in planarian flatworms (Azimzadeh et al. 2012), and several noncentrosomal MTOCs can be found in one cell, in particular when cells are differentiated (Rios 2014, Wu & Akhmatova 2017). Furthermore, as Bell (1989) expected, an extra centrosome is exceptionally

formed in cells (Tsou & Stearns 2006, Gönczy 2015), and some cells have many centrioles and cilia.

As we describe below, novel information on the key roles of the centrosome and cilium in cell division, proliferation, and differentiation explains why there can normally be only one centrosome in a cell and not more, why the multiple noncentrosomal MTOCs cannot organize radial mitotic spindle poles in differentiated cells, and why the functions of the centrosome in mitotic spindle formation and ciliogenesis are mutually exclusive.

2.2. Centrosomes and Mitotic Fidelity

Centrosomes are duplicated precisely once per cell cycle. During mitosis, the duplicated centrosomes form bipolar spindles that accurately segregate the duplicated chromosomes, producing an equal distribution of chromosomes between daughter cells (e.g., Sir et al. 2013, Meraldi 2016). Centrosomes are not absolutely required for mitotic spindle formation and division in many cells (e.g., Bonaccorsi et al. 2000, Basto et al. 2006, Meraldi 2016), but mitosis in the absence of centrosomes is an error-prone process and centrosomes appear to be necessary for rapid, robust, and error-free separation of the chromosomes during mitosis (Basto et al. 2006, Sir et al. 2013, Meraldi 2016, Lattao et al. 2017).

2.3. Additional Functions of the Centrosome

Centrosomes have been found to have many different roles during cell proliferation and differentiation. For instance, they play a major role in cell cycle control, establishing cell polarity and the positioning of cell organelles; transmission of polarity to daughter cells; and adhesion, de-epithelialization, and migration of cells (e.g., Bornens 2012, Burute et al. 2017, Wu & Akhmanova 2017). They also function as signaling hubs involved in DNA damage repair (Conduit et al. 2015, Farina et al. 2016). Furthermore, centrosomes play a major role in the specification of cell fate during asymmetric divisions (e.g., Haubensak et al. 2004, Yamashita et al. 2007, Dudka & Meraldi 2017). The causal role of the centrosome presumably hinges on the asymmetry of the centrioles in the centrosome. The two centrioles of a centrosome are of different age (mother and daughter), with the older centriole having accumulated more pericentriolar proteins. After duplication, one centrosome has the duplicated mother centriole, and the other has the duplicated daughter centriole. Asymmetric cell divisions involve the differential partitioning of the two unequal centrosomes over the daughter cells, with one daughter cell renewing the stem cell (usually the one with the mother centriole) and the other initiating a differentiation path (Rebollo et al. 2007, Roubinet & Cabernard 2014). Thus, in addition to the central role in separation of the chromosomes, the centrosome plays a crucial role in cell cycle control and cell fate decisions.

2.4. Loss of Centrosomes in Planarians

The freshwater planarian *Schmidtea mediterranea* does assemble centrioles that function as basal bodies of cilia in multiciliated cells, but centrioles are not involved in cell division (Azimzadeh et al. 2012). No centrosomes are formed, and centrosomal proteins are absent, challenging the importance of the centrosome for mitosis in these metazoans. The loss of centrosomes in this species is thought to be related to the loss of spiral cleavage and oriented cell divisions. This hypothesis is supported by the presence of centrosomes in the basal, round-shaped, nonplanarian flatworm *Macrostomum lignano*, which has retained the ancient spiral cleavage and the associated oriented cell divisions (Azimzadeh et al. 2012, Bornens 2012, Wu & Akhmanova 2017; but see

Martin Durán & Egger 2012). A factor that may have played a role in the loss of centrosomes in planarians is that the reliable separation of a few chromosomes ($2n = 8$) is technically less difficult than in organisms with a large number.

2.5. Other Centrosomes

Exceptionally, de novo generation of extra centrosomes occurs. Not surprisingly, given the above-mentioned functions of the centrosome, extra centrosomes pose a grave risk and may lead to the formation of multiple spindle poles, aneuploidy, cell cycle arrest, apoptosis, genomic instability, cell migration (e.g., in metastasis of cancer cells) (Tsou & Stearns 2006, Basto et al. 2008, Godinho & Pelman 2014, Gönczy 2015), and perhaps cancer, as first proposed by Theodor Boveri (1902) and experimentally shown in *Drosophila* and mice (Basto et al. 2008; but see Godinho & Pelman 2014, Gönczy 2015, Raff & Basto 2017). The importance of having only one centrosome per cell is also supported by the elimination of the centrioles from animal egg cells before fertilization, such that the zygote receives centrioles only from the sperm cell and does not end up with two centrosomes (Boveri 1901, Bell 1989, Manandhar et al. 2005). We conclude that supernumerary centrosomes are usually seriously disadvantageous for the individual and will be strongly selected against. It is therefore unlikely that the inability of ciliated cells to form proper mitotic spindles could be compensated by extra centrosomes.

2.6. Noncentrosomal Microtubule-Organizing Centers

During differentiation, the microtubule-organizing capacity is partially or fully transferred from the centrosome to other cellular structures that organize the microtubules in cell type–specific arrays, suitable for specialized cell functions other than mitosis (e.g., Sanchez & Feldman 2017, Wu & Akhmanova 2017), so-called noncentrosomal MTOCs. Examples of noncentrosomal MTOCs are the Golgi apparatus, the nuclear envelope, chromatin and kinetochores, the cell cortex, and preexisting microtubules (Chabin-Brion et al. 2001, Stiess et al. 2010, Maia et al. 2013, Rios 2014, Sanchez & Feldman 2017, Wu & Akhmanova 2017). These structures organize microtubules in nonradial geometric patterns, associated with their different functions—for example, the Golgi apparatus is involved in the reorganization of the cellular architecture during the differentiation of muscle and nerve cells (Rios 2014). The division of tasks between the centrosomal and noncentrosomal MTOCs appears to be tightly regulated during cell cycle progression and differentiation, possibly in a competitive way (Wu & Akhmanova 2017). Cells with noncentrosomal MTOCs capable of both microtubule nucleation and anchoring appear to inactivate these functions at the centrosome and to transfer them to the noncentrosomal site (Wu & Akhmanova 2017), possibly because similar multiprotein complexes are involved in both processes.

2.7. Centrosome and Formation of Primary Cilium

Centrosomes change dynamically during the cell cycle. They are duplicated once per cell cycle with, as mentioned above, precisely one new centriole assembled adjacent to the mother centriole and one adjacent to the daughter centriole. The two centrosomes then form bipolar spindles that distribute chromosomes equally between two dividing cells (e.g., Sir et al. 2013, Meraldi 2016). At the exit of mitosis, cells typically form a primary cilium, unless they continue proliferating when ciliogenesis appears to be actively suppressed (Goto et al. 2017). In all other cases, also in quiescent stem cells, the mother centriole converts to a basal body and migrates to the cell surface, where it docks to the cell membrane and assembles the primary cilium that protrudes from the

cell membrane. The centrosome remains intact, as the mother and daughter centrioles migrate together and remain associated throughout ciliogenesis (Sorokin 1968, Alieva & Vorobjev 2004). Upon cell cycle reentry, ciliary resorption begins, the basal body is detached from the cell surface, and the centrosome migrates to near the nucleus.

2.8. Importance of Primary Cilia

Primary cilia were long thought to be vestigial organelles, which would complicate the reasoning of Buss that the presence of cilia forms a hard constraint for the use of centrioles for mitotic spindles. Furthermore, it was not until the 1990s, owing to technical improvements of visualization techniques, that it became known that primary cilia are present as antennae on almost all metazoan cells (Wheatley 1995). Since then, it has become apparent that they do not only function as sensory organelles but also have a key function in intercellular signaling (e.g., Dawe et al. 2007, Ludeman et al. 2014, Walz 2017). Signaling in the cilium is involved in the organization of most, if not all, developmental processes, including left-right patterning, cell migration, reentry of cells into the cell cycle (proliferation), cell size, cell shape, specification of the plane of cell division, apoptosis, and cell fate decisions. For example, in vertebrates, an important role of cilia is the coordination of many pathways, including the *sonic hedgehog* (*Shh*), *Wnt*, *transforming growth factor beta* (*TGFβ*), *liver kinase B1* (*LKB1*), and *Hippo* pathways (Breunig et al. 2008, Ohazama et al. 2009, Lancaster et al. 2011, Basten & Giles 2013, Ke & Yang 2014, Walz 2017). Underlining the crucial importance of cilia in development and tissue homeostasis is the wide range and common occurrence of diseases that are caused by dysfunctional cilia, so-called ciliopathies, which include left-right patterning defects, polycystic kidney disease, retinal degeneration, diabetes, Meckel-Gruber syndrome, microcephaly, and cancer (e.g., Wheatley 1995, Basten & Giles 2013). Furthermore, in cells with supernumerary centrosomes, extra cilia are often formed and compromise the functioning of primary cilium signaling, which may lead to cancer and other diseases (Mahjoub & Stearns 2012). Thus, far from being vestigial organelles, primary cilia are of vital importance and cannot be missed. This is, as we see below, an important cause of the constraint.

2.9. Multiciliated Cells

Some cells in animals can have hundreds of cilia, each requiring its own basal body to be assembled de novo (Dawe et al. 2007, Basten & Giles 2013). However, the pathways used to produce these centrioles are different from those used during duplication of the centrioles during the cell division cycle and are linked to differentiation rather than to proliferation. Multiciliated cells are terminally differentiated (Dawe et al. 2007, Basten & Giles 2013); for cell renewal, unciliated progenitor cells are employed (Evans et al. 1986, Bird et al. 2014). Hence, despite the large number of centrioles, these multiciliated cells do not form an exception to the rule that differentiated cells cannot divide.

2.10. The Centrosome Cycle Regulates the Cell Cycle

Recent research has shown that the cell cycle is not so much regulating centrosome and cilium dynamics—instead, the dynamics of the centrosome and primary cilium actively regulate cell cycle progression and arrest or exit followed by differentiation (Masuda & Sato 1984, Basten & Giles 2013, Ke & Yang 2014, Goto et al. 2017, Walz 2017). For instance, the presence of the primary cilium appears to block cell division, whereas primary ciliary resorption is thought to unblock cell division, and the length of the cilium influences cell cycle duration, with cell cycle length influencing cell fate decisions.

2.11. Pleiotropic Constraint on Changes of Centrosome Number

Cellular dynamics in multicellular metazoans are, thus, extremely complex, and the centrosome and primary cilium are key players regulating almost all major cellular processes including mitosis and differentiation. A further contribution to the complexity and tight interactive control comes from the competitive interactions between the centrosome and noncentrosomal sites that organize microtubules. As a result, mutations affecting centrosomal and ciliary functions have many deleterious pleiotropic effects that can have an impact on cell cycle progression and may cause apoptosis, genomic instability, aneuploidy, and cancer (e.g., Boveri 1902, Godinho & Pelman 2014, Gönczy 2015, Dudka & Meraldi 2017). As a consequence of the pleiotropic constraint, only one centrosome is in a cell and can function either in cell division or in ciliation and differentiation. The pleiotropic constraint causes a developmental constraint, as ciliated and differentiated cells cannot divide by mitosis. In metazoans a pleiotropic constraint is, thus, most likely the cause of the strong conservation of the centrosome and primary cilium dynamics that regulate the cell cycle, including the number of centrosomes, with the sole exception thus far being planarians that have lost centrosomes (Azimzadeh et al. 2012). As Buss (1987) has lucidly pointed out, the uncontrolled proliferation of individual cell lineages is the major challenge to be overcome by multicellular individuals in the early evolution of multicellularity. Once the control is at the level of the individual, strong anticancer selection will be exerted against mutations that lead to unchecked proliferation of cell lineages (Galis & Metz 2003).

The pleiotropic constraint is associated with a cost of lower fidelity of meiotic divisions in the ovum, as the centrosome in all animal ova is degenerated, presumably to avoid the problematic presence of a second centrosome in the zygote. It is thought that the centrioles of the oocyte rather than of the spermatocyte are degenerated because of the necessity for the motile sperm cell to have a centriole organizing its tail (Manandhar et al. 2005). Thus, the acentriolar spindle formation during oogenesis can be expected to lead to increased rates of aneuploidy.

2.12. Developmental Constraint

A direct consequence of the strong pleiotropic constraint in metazoans is a developmental constraint: Ciliated and differentiated cells cannot divide by mitosis unless they first dedifferentiate, as occurs for instance in regeneration and in cancer.

The hypothesis of Henneguy (1898) and Lenhossék (1898) that ciliated cells cannot divide by mitosis remains thus far uncontested for metazoans. As virtually all differentiated cells appear to have a primary cilium, this essentially equates the hypothesis to that of Buss—that a constraint exists on mitotic divisions of differentiated cells. Even lymphocytes, which were long thought to be exceptional as not having primary cilia, are now thought to have a modified primary cilium (Finetti et al. 2009, Dustin 2014). Furthermore, in the differentiation of some cells the cilium or centrosome is discarded, which also prevents further cell division (Bornens 2012, Das & Storey 2014). The few claims that differentiated cells can divide during cell renewal and regeneration are controversial (Dor et al. 2004, Brennand et al. 2007, Teta et al. 2007, Smukler et al. 2011, Razavi et al. 2015, Beamish et al. 2016, Afelik & Rovira 2017).

2.13. Evolutionary Consequences of the Developmental Constraint

The constraint that cells can either divide or differentiate has a pervasive influence on the evolution of metazoans. It is a major force in shaping development, regeneration, and the evolution of body plans.

Buss (1987) postulated that metazoans inherited the constraint from their unicellular protist ancestors and that their inability both to divide and to move with a cilium provided a selective advantage for multicellularity (see also Trestman 2013). Multicellularity allows a division of labor, with some cells taking care of mobility and feeding and some undergoing mitosis. Buss further proposed that the evolution of gastrulation in early free-living metazoans is a consequence of the constraint, because ciliated dispersing blastulas can continue to develop only when ciliated cells from the outside migrate inward and prepare for mitosis by retracting their cilia, leaving as many ciliated cells as possible on the surface. Bell (1989) argued instead that gastrula formation with flagellated cells on the outside and unflagellated cells on the inside may be a selectively advantageous hydrodynamic configuration. Be this as it may, the incompatibility of simultaneous cell division and ciliation has without a doubt crucially shaped developmental processes, with zones that produce pluripotent stem cell colonies that subsequently migrate to other places in the embryo to initiate their paths of differentiation. Additionally, signaling pathways are of major importance for all aspects of development and, as we now know, cilia are required for the coordination of many signaling pathways; hence, this coordination cannot happen when cells are dividing. Furthermore, niches of tissue-specific progenitor cells must be developed and maintained in adult tissues and organs to function in cell renewal, wound healing, and regeneration (Tanaka & Reddien 2011).

Wound healing and regeneration would presumably be much more effective if all differentiated cells could divide to replace damaged cells of the same type. Regeneration now typically proceeds from a blastema of undifferentiated cells that are either dedifferentiated or already locally present progenitor cells (Brockes 1997, Tanaka & Reddien 2011). Complex interactions then are required, often similar to those that occurred during development of the to-be-regenerated part (Galis et al. 2003). This requirement of developmental interactions after dedifferentiation seriously limits the possibility of regeneration in many metazoans, at least in more complex ones (see Section 3.11). It is perhaps not accidental that *S. mediterranea*, which has lost centrosomes and does not employ them for spindle formation in mitosis, has virtually unrivalled powers of regeneration, whereas the platyhelminth *M. lignano*, which has not lost centrosomes, has a more limited capacity for regeneration (Azimzadeh et al. 2012, Bornens 2012). *S. mediterranea* and other planarians have broadly distributed adult stem cells (neoblasts), some of which are totipotent, and as a result small pieces of tissue can reconstitute the entire organism (Baguñà & Auladell 1989, Gentile et al. 2011, van Wolfswinkel et al. 2014). Adult stem cells in most other metazoans have lost pluripotency and are fate-restricted progenitor cells, which limits the regenerative capacity.

In addition, the conflict between ciliation and mitosis powerfully constrains the evolution of body plans. Unlike plants, in which new organs like branches and leaves can develop during adult life, we cannot grow an extra arm or eye. In almost all metazoans, the evolution of the number of organs is constrained. The specification of complex body parts must happen early on in the embryo, usually during early organogenesis. The most flexibility is provided by changes in the number of segments, which allow the multiplication of legs and other organs of the segment (see Section 3.14). Another solution to this problem is the vegetative production of modules that are morphological repeats of the body plan—for instance, those found in cnidarians, bryozoans, and colonial ascidians (Bell 1982). With increasing complexity, the constraint against simultaneous differentiation and division will have a stronger influence on the conservation of development (cf. Section 3.12).

Finally, the constraint against having more than one centrosome in a cell not only leads to a low fidelity of meiotic divisions as discussed above but also provides a powerful developmental constraint against parthenogenesis. A centrosome is generally necessary to initiate mitotic divisions (e.g., Riparbelli et al. 1998, Eisman & Kaufman 2007, Bornens 2012), hence the absent paternal contribution of centrioles to the oocyte lowers the chance for successful mitosis of the

unfertilized zygote. The problem of the missing centrosome in unfertilized ova in obligatory or regularly parthenogenetic animals is usually solved by the de novo assembly of a centrosome (e.g., Riparbelli et al. 1998, Tram & Sullivan 2000, Riparbelli & Callaini 2003, Ferree et al. 2006, Hiruta & Tochinai 2012). Special adaptations have evolved for this—for example, special cytoplasmic organelles (accessory nuclei) in haplodiploid insects (Tram & Sullivan 2000, Ferree et al. 2006). In contrast, in sporadic parthenogenesis, it leads to malfunctioning centrosomes that interfere with proper mitosis and ploidy restoration (Eisman & Kaufman 2007). In *Drosophila mercatorum*, problems with the de novo assembly and maturation of centrosomes are an important contributor to the high rates of early embryonic death, contrasting with the high hatchability and viability of the obligately parthenogenetic *Drosophila mangabeirai* (Riparbelli & Callaini 2003, Eisman & Kaufman 2007). Another solution for the absent centrosome is found in species that utilize sperm-dependent parthenogenesis, gynogenesis (pseudogamy), or hybridogenesis. In gynogenesis, a sperm cell of a related sexual species or conspecific is necessary to initiate mitosis of the ovum, but the sperm does not contribute genes (Beukeboom & Vrijenhoek 1998, Lampert & Schartl 2010). It is generally assumed that the centriole is the paternal contribution that is necessary to initiate mitosis after centrosome formation (Tournier et al. 1989, Riparbelli et al. 1998).

3. PHYLOTYPIC STAGES

Embryologists have long noticed that within many higher order taxa (e.g., phyla or classes) early organogenesis is less variable morphologically than both the earlier stages of cleavage and gastrulation and the later stages (e.g., Seidel 1960, Sander 1983, Hall 1997). Recently, many studies have shown that strong conservation of the expression of genes also occurs during early organogenesis. This was first observed for *Hox* genes (Slack et al. 1993, Duboule 1994) and subsequently for many other genes, in particular regulatory ones (e.g., Davis et al. 2005, Domazet-Lošo & Tautz 2010, Kalinka et al. 2010, Irie & Kuratani 2011, Levin et al. 2012, Ninova et al. 2014, Drost et al. 2015, Hu et al. 2017; see also Cheng et al. 2015). The conservation includes not only gene expression but also DNA sequences, and it was found to correspond with high evolutionary gene ages (Domazet-Lošo & Tautz 2010, Hu et al. 2017). Furthermore, epigenetic mechanisms, such as demethylation of specific embryonic enhancers, were found to be conserved in vertebrates at this stage (Bogdanović et al. 2016). Some support has also been found for conservation of earlier stages (e.g., Piasecka et al. 2013). Sander (1983) came up with the term phylotypic stage and the attendant concept, although nowadays the concept is extended to other higher taxa than phyla (e.g., classes in insects)—an extension that in the past has generated some controversy (Scholtz 2000). Note that the conservation is not to be taken overly strictly. What matters is that in many higher taxa development is remarkably conserved during phylotypic stages, more strongly than during earlier or later embryogenesis (Galis et al. 2001, Sander & Schmidt-Ott 2004).

3.1. Global Interactivity, Pleiotropy, and Stabilizing Selection

As in mitosis, it has been hypothesized that the complex and highly controlled global interactivity of early organogenesis in the embryo causes strong pleiotropic constraints against evolutionary change (Sander 1983, Raff 1996). Strong global interactivity contrasts with effective modularity of development. It causes even slight disturbances to cascade into deleterious pleiotropic effects in other parts of the embryo, and these become amplified as development proceeds. The result is strong stabilizing selection against changes (Galis et al. 2001, 2002b; Kalinka et al. 2010). Thus, in this scenario, deleterious pleiotropic effects and stabilizing selection against these effects are key to the conservation. We have termed this the pleiotropy hypothesis (Galis et al. 2002b). In the

following sections, we discuss the rapidly growing body of empirical support for the pleiotropy hypothesis and also discuss why evolutionary constraints for change at earlier and later stages are weaker.

3.2. Vulnerability and Interactivity of Mammalian Phylotypic Stages

Teratological data on rodents strongly support the pleiotropy hypothesis: Early organogenesis was found to be more vulnerable to disturbances than earlier or later stages, because disturbances led to more lethality and abnormalities (Galis & Metz 2001). The pattern of multiple induced abnormalities (pleiotropic effects) indicates that high interactivity and effective modularity is the root cause of the vulnerability of the stage. Hence, the vulnerability is not due to one vulnerable process (e.g., neural tube closure), as is the case with cell division in cleavage (see below), but is due to the global interactivity. This finding implies that a particular, potentially useful change of this stage (e.g., the induction of an extra digit) almost always will induce other abnormalities and lethality even before the organism is exposed to ecological selection. For example, in humans, ~90% of individuals with a supernumerary digit are dead at birth (Opitz et al. 1987, Galis et al. 2010), and a multitude of deleterious pleiotropic effects are associated with this abnormality (Galis et al. 2001, Biesecker 2011). Similarly, induction of a change of the highly conserved number of seven cervical vertebrae (usually manifested as a cervical rib, i.e., a partial rib on the seventh vertebra) virtually always leads to early lethality: Approximately 90% of the individuals with a cervical rib are dead at birth, and a strong association is seen with cardiovascular, nervous, urogenital, and other abnormalities (Galis et al. 2006, ten Broek et al. 2012). Additional selection against cervical ribs, after birth, comes from a significant association with childhood tumors (Galis 1999, Galis & Metz 2003). Cervical ribs are extremely common, found in ~50% of deceased fetuses and infants (versus ~1% in the general population). This finding implies that an astonishing ~8% of human conceptions appears to die after disturbances of early organogenesis, which emphasizes the vulnerability of the stage (Galis et al. 2006; see also Reumer et al. 2014, van der Geer & Galis 2017). As organ primordia typically originate during the phylotypic stage, this implies that the conservation of the stage leads to conservation of the number and earliest development of most organs (e.g., lungs, kidneys, limbs, eyes, ears; see Sections 3.12 and 3.13).

3.3. Vulnerability and Conservation

Early embryonic cleavage is vulnerable to toxicants and radiation, albeit at relatively high doses (e.g., Russell 1950, Shenefelt 1972, Galis & Metz 2001, Jacquet 2004). However, in contrast to early organogenesis, the vulnerability of cleavage has not led to strong conservation, at least not within insects or vertebrates. This is probably because the dividing cleavage cells are all highly similar and capable of self-renewal: Either too many cells are killed and the embryo dies, or the damage is reversible and development proceeds largely normally (Russell 1950, Shenefelt 1972). This is known as the "all or none" phenomenon in medicine (Jacquet 2004, Adam 2012). The crucial difference from phylotypic stages is that the vulnerability is caused by disturbances that mainly affect one sensitive process, cell division. In the phylotypic stage, the strong global interactivity restricts the potential for reversal of damage. Mutations that affect earliest development presumably have more of a chance to be successful, as it is more difficult to destabilize a simple pattern than a more complicated pattern. In this spirit, Galis & Sinervo (2002) hypothesized that the weaker conservation of type of cleavage and gastrulation is probably due to the greater simplicity of the early forms.

3.4. Developmental Fields, Modularity, and Pleiotropy

The global interactivity and low effective modularity of phylotypic stages are probably largely because this early in development there are only a few developmental fields and few signaling centers (Opitz et al. 2002). Galis et al. (2006) proposed that the low developmental robustness of early organogenesis in vertebrates results from the well-documented interactions between the patterning of the three body axes and the coupling of axial patterning with simultaneously occurring morphogenetic processes, such as the division and migration of cells, cell shape changes, segmentation (somitogenesis), and the active maintenance of the bilateral symmetry of somites (e.g., Diez del Corral et al. 2003, Cordes et al. 2004, Ninomiya et al. 2004, Vermot & Pourquié 2005, Durston et al. 2011). The opposing and antagonistic gradients of *Fgf/Wnt* and retinoic acid in interaction with the segmentation clock play a major role in the coordinated organization of these processes. In addition, many auto-regulatory and cross-regulatory interactions, including physical interactions, provide feedback to these organizers, which adds to the interactivity in this developmental field that occupies a large part of the embryo, in particular the trunk (e.g., Diez del Corral et al. 2003, Cordes et al. 2004, Ninomiya et al. 2004, Vermot & Pourquié 2005, Durston et al. 2011). Another large developmental field during this stage is the neural crest–related cardio-craniofacial developmental field (Grifone & Kelly 2007, Keyte & Hutson 2012). Hence, the large size of the developmental fields and the intense signaling both within and between them probably cause a major part of the pleiotropy in space associated with the phylotypic stage. Additionally, pleiotropy is expressed over time owing to cascading downstream effects in later stages. Furthermore, many of the conserved transcription factors active at this stage, including *Hox*, as well as common signaling pathways, such as *TGFβ*, *Hedgehog*, *Fgf*, and *Wnt*, are known to be also active at later stages (although for more restricted functions), which could cause additional pleiotropy (e.g., Sakata & Chen 2011, Akhshabi et al. 2015). Data on deceased human fetuses and infants provide strong support for the coupling of axial patterning and morphogenetic processes as a cause of the vulnerability (ten Broek et al. 2012). The pattern of abnormalities in ∼1,300 cases shows a significant association between defects of A-P patterning of the paraxial mesoderm (in particular cervical ribs) with defects of left-right patterning, midline formation, segmentation (somitogenesis), and neural crest development (ten Broek et al. 2012). The authors found that segmentation defects were nearly always associated with cervical ribs and other vertebral patterning changes (∼98%), supporting the debated hypothesis that the segmentation clock influences *Hox* patterning (Dubrulle et al. 2001, Zakany et al. 2001, Cordes et al. 2004, Deschamps & Duboule 2017). In contrast, no significant relationship with the germ layer of origin of the associated abnormalities was found, supporting the primary importance of global signaling interactions during this stage.

3.5. Modularity of Later Stages

As development proceeds, progressively more and more signaling centers appear, organizing more and more localized developmental fields: Development becomes more compartmentalized. Concomitantly, the expression patterns of key signaling molecules become more restricted.

For instance, the large neural crest–related cardio-craniofacial field over time becomes subdivided into more developmental fields, including the secondary heart field, which in turn gives rise to further subdomains that each act as a developmental field (Grifone & Kelly 2007, Vincent & Buckingham 2010, Kelly 2012). Another example is the increased compartmentalization of somites (e.g., Chen et al. 2013). Within these more restricted developmental fields, intense signaling between tissues continues to occur, but the interactivity between modules becomes increasingly less important than within modules, presumably resulting in a larger developmental

robustness and higher effective modularity (Galis et al. 2002b, Opitz et al. 2002). As a result, pleiotropic constraints and stabilizing selection become gradually less important, allowing more evolutionary divergence of later stages.

3.6. Transcriptomic Support for Pleiotropy

Recently, transcriptomic studies in many different organisms, even in plants and fungi, have supported the hypothesis that conservation of gene expression tends to follow an hourglass pattern, with conservation most often strongest during mid-embryogenesis in a wide number of organisms. In particular, support for the conserved expression during mid-embryogenesis was found for regulatory genes, including expression of microRNA genes (Kalinka et al. 2010, De Mendoza et al. 2013, Stergachis et al. 2013, Ninova et al. 2014, Levin et al. 2016), genes with pleiotropic activity in other parts of the embryo (Cheng et al. 2014, Cridge et al. 2016, Hu et al. 2017), and those with pleiotropic activity at other stages during development (Levin et al. 2012, Hu et al. 2017; see also Fish et al. 2017). The spatial and temporal pleiotropy can be explained by the abovementioned global interactivity during the patterning of the three body axes and the associated downstream effects at later developmental stages. The transcriptomics studies are, thus, in good agreement with the morphological studies and support the pleiotropy hypothesis.

3.7. Physical Processes as Important Contributors to Pleiotropy

It is regularly suggested in molecular studies that the conservation of gene expression of phylotypic stages is the cause of the morphological conservation. However, this underestimates the crucial importance of (self-organizing) chemical and physical processes (Karsenti 2008). For example, physical and simple chemical processes play a major role in chemotaxis, cell division, cell migration, segmentation, and convergent extension and, as such, in all morphogenetic processes occurring during phylotypic stages (Galis et al. 2006, Salazar-Ciudad & Jernvall 2010, Newman 2011, ten Broek et al. 2012). For these processes, spatial scales are important, as diffusion and viscous coupling work over short distances. Chemical and physical processes are, thus, among the key players in the global interactiveness of the small phylotypic stages that lead to widespread pleiotropy, which, together with stabilizing selection, appears to be the root cause of the conservation of the phylotypic stage, at least in vertebrates and insects.

3.8. Robustness Cannot Cause Long-Term Conservation

Hu et al. (2017) concluded from a study on eight chordates that pleiotropic constraints are involved and that the conservation is stronger for the subphylum of vertebrates than for the chordate phylum as a whole. As an aside, they proposed that developmental robustness may be an additional cause for the conservation of the phylotypic stage in vertebrates. This hypothesis, which we have termed the robustness hypothesis (Galis et al. 2002b), was also proposed by von Dassow and colleagues (von Dassow & Munro 1999, von Dassow et al. 2000) for the conservation of the phylotypic stage of insects, the segmented germ band stage. They proposed that the robustness of the segment polarity network in each segment should provide a buffer against phenotypic effects of mutational changes of the segmented germ band stage. In robust gene networks, by definition, developmental noise and mutations do not lead to clear phenotypic effects because gene interactions neutralize perturbations and in particular make mutations recessive (Gibson & Wagner 2000, Wagner 2000). The robustness and pleiotropy hypotheses are, thus, diametrically opposed: The one proposes that the phylotypic stage is vulnerable to mutational change but that mutations have large deleterious

effects, and the other proposes that the effect of mutations will have minimal phenotypic effects and mainly produce cryptic variation. For the phylotypic stage in vertebrates and *Drosophila*, an abundance of phenotypic variation has been documented, which is for the most part strongly deleterious (Galis et al. 2001, 2002b, 2006; ten Broek et al. 2012). This strongly contradicts the robustness hypothesis (for a test of the two hypotheses in *Drosophila*, see Galis et al. 2002b). In rodents and humans, almost all major congenital abnormalities find their origin in disturbances of the phylotypic stage (Russell 1950, Shenefelt 1972, Khera 1984, Sadler 2010). Thus, although extremely strong selection for the robustness of early organogenesis undoubtedly occurs, the number of interactions involved in morphogenetic patterning is probably too limited to organize the pattern in an independent, modular way, thwarting the drive for greater robustness (Galis et al. 2002b).

Theory also does not support robustness as a cause for long-term conservation. Stabilizing selection is expected to lead to robustness to protect optimized traits against developmental noise and mutations (Wagner 2000, Galis et al. 2002b, Metz 2011, Papakostas et al. 2014, Austin 2016, Melzer & Theißen 2016). This robustness can produce short-term conservation. However, in the long term, cryptic variation will accumulate, which in high-dimensional trait space will lead to diversification of genetic backgrounds and to access to an increased number of new phenotypes (Gibson & Wagner 2000, Galis et al. 2002b, Metz 2011, Wagner 2012, Siegal & Leu 2014). Hence, during periods with drastic environmental changes that lead to strong directional selection, the robustness of development can never be sufficiently high to prevent change, owing to the accumulated cryptic variation. In fact, in the long-term, cryptic variation is expected to lead to increased evolvability (Wagner 2012, Melzer & Theißen 2016, Siegal & Leu 2014).

3.9. Convergence and Divergence of Cleavage and Gastrulation

Paradoxically, when we compare metazoan phyla, gastrulation and cleavage are sometimes remarkably similar morphologically and more so than phylotypic stages (Gilbert & Raunio 1997, Galis & Sinervo 2002). Analyses of gene expression patterns confirm this pattern (Levin et al. 2016). Without doubt, similarity is largely unavoidable, given the complete reset that occurs at the initial single-celled stage (Galis & Sinervo 2002). Only a limited number of permutations are possible in embryos with a few undifferentiated cells. Furthermore, the universal metazoan constraint that cells can divide only while undifferentiated makes an initial cleavage stage with predominantly cell division unavoidable. Further reasons for similarity are caused by convergent locomotory and nutritional adaptations and maternal attempts at dictating offspring features (Buss 1987). A good example of convergent nutritional adaptations is the development of an embryonal disk on top of the yolk in species with yolk-rich embryos, as yolk impedes cleavage (e.g., cephalopods, fishes, reptiles, and birds). Gastrulation processes, conversely, are diverse, yet again important convergence occurs, which is probably due to both the similarity in the outcome of cleavage processes and the similarity of locomotory and feeding adaptations (reviewed in Buss 1987, Galis & Sinervo 2002). Yet, within phyla and classes the process of gastrulation is far more diverse relatively, compared with the end product, as gastrulae have two or three germ layers and never more (Hall 1999) and organ systems emerging from germ layers are similarly conserved—for example, skin and nervous system arise from ectoderm, digestive tube from endoderm. A key outcome of the process of gastrulation is that sheets of cells come into contact, thus enabling the conserved embryonic inductions that are essential for the organization of the body plan during the phylotypic stage. These inductions between adjacent cell populations appear to form a stringent spatiotemporal constraint on the outcome of gastrulation, the starting point of the conserved phylotypic stage (Galis & Sinervo 2002, Zalts & Yanai 2017).

3.10. Diversity of Phylotypic Stages Among Metazoa

Phylotypic stages differ dramatically among phyla and classes (e.g., the segmented germ band stage in insects and the neurula in vertebrates). This pattern of divergence and the conservation within phyla or classes suggest an early phase of rapid diversification in the evolution of metazoans, followed by strong conservation of these discrete taxon-specific stages (Buss 1987, Galis & Sinervo 2002). Buss (1987) proposed the following ingenious hypothesis for this phenomenon. The early rapid diversification occurred during the early, chaotic phase in the evolution from unicellular to multicellular individuals (presumably during the Cambrian explosion). During this process, the level of selection shifted from individual cells to individual organisms. Early during this transition, mutations in cells that could gain access to reproduction had a chance to be maintained in future generations (as in plants). Later, when selection was firmly established at the level of the individual, heritable mutations became limited to those that occur in the germ line, or in the short period before germ line sequestration. This early rapid diversification scenario is intuitively appealing but has received surprisingly little attention, and hardly any research has been carried out to corroborate or falsify this important idea, like mutagenesis experiments with simple colonial organisms and theoretical modeling (Galis & Sinervo 2002).

3.11. Evolutionary Consequences: Influence on Regeneration Capacity

The constraint on changes of early organogenesis has important consequences for regeneration capabilities. Regeneration usually involves recapitulation of normal development, once a regeneration blastema has been formed. Galis et al. (2003) proposed that regeneration is possible only for organs that develop as a semiautonomous module and that do not require interactions with transient organs. Organ primordia typically appear during early organogenesis and hence the early development of most organs involves interactions with transiently present structures (e.g., somites, notochord, neural crest). The involvement of transient structures implies that the full process of interactions cannot be easily replicated for regeneration purposes. Galis et al. (2003) proposed that this obstacle to replication of development explains why regeneration of the limbs is not possible in amniotes. In contrast, in amphibians and lungfishes in which limb development is semi-independent, regeneration of the limbs is possible. In amphibians with metamorphosis, limb development is delayed relative to amniotes and has become decoupled from interactions with somites and other transient structures. Transplantation experiments show that limb buds develop as semi-independent, largely self-organizing modules (reviewed by Galis et al. 2003). In lungfishes, fins also develop after the phylotypic stage, and regeneration of the bony skeleton of the fins is possible. Contrastingly, amniote limb buds show no self-organizing capacity and crucially depend on the presence of transient structures such as somites. In teleosts, the bony skeletons of the pectoral fins that are homologous with tetrapod limb bones also develop early, and interactions with the transient somites are required. Regeneration of the bony skeleton is not possible (Galis et al. 2003). Correspondingly, the global interactivity of phylotypic stages probably constrains the regeneration capacity in animals with burst organogenesis.

3.12. Evolutionary Consequences: Influence on Body Plan Evolution

Most organ primordia appear during early organogenesis and, as we already discussed for digits in amniotes and cervical vertebrae in mammals, the evolutionary conservation of early organogenesis is, thus, probably implicated in the conservation of the number of repeated organs (see also Section 3.13). Other examples of conserved numbers that are determined during early

organogenesis include limbs and antennae in insects and pharyngeal arches, kidneys, eyes, ears, and limbs in vertebrates. The medical and veterinary literature shows that mutations for duplications occasionally occur—for example, spleens, kidneys, ureters, vaginas, penises, testicles, digits, and, even extremely rarely, additional arms and legs (e.g., Uchida et al. 2006, Galis & Metz 2007, Lilje et al. 2007). These duplications appear to be strongly selected against owing to associated deleterious pleiotropic effects (Grüneberg 1963, Lande 1978, Galis et al. 2006, Lilje et al. 2007, Biesecker 2011).

3.13. Evolutionary Loss of Organs

The constraint against changes of the phylotypic stage is also implicated in the constraint on the evolutionary loss of organs. The loss of organs typically occurs via the slowly continued evolution of earlier developmental arrest, followed by degeneration (Lande 1978, Bejder & Hall 2002, Galis et al. 2002a). This is presumably because the early interactions involving the organ primordia cannot easily be missed for normal development (Galis et al. 2002a, 2010). As a result, generally more primordia are developed during embryogenesis than persist. For instance, in horses and cows, initially five-digit condensations still develop (Galis et al. 2002a). Similarly, when eyes are lost in cave fishes and salamanders, development always proceeds until the lenses have been formed, after which degeneration follows (e.g., Dufton et al. 2012). Other examples of vestigial organs are wings in emus and kiwis, pelvises in whales, teeth in baleen whales, and the clavicle in canids, felids, and lagomorphs (Glover 1916, Klima 1990, Bejder & Hall 2002, Senter & Moch 2015). As a result of the slow accumulation of mutations during the loss of complex organs, re-evolution is virtually impossible, in agreement with Dollo's law (Goldberg & Igič 2008, Galis et al. 2010).

3.14. Weaker Constraint Against Late Emerging Organ Primordia

In contrast, when the final number of organs is determined after the vulnerable phylotypic stage, when development is more modular, the constraint on changes is considerably weaker. In arthropods with sequential production of segments continuing past the conserved segmented germ band stage, segment numbers are highly variable. An interesting exception to the variability is the number of segments of centipedes, which is variable but always odd (Minelli & Bortoletto 1988, Arthur & Farrow 1999). This constancy appears to be caused by the conserved oscillatory pattern generation of two segments per cycle, hence variation is caused by the number of cycles, with the cycling set up during the phylotypic stage (Chipman et al. 2004). Examples of late-determined structures are carpal and tarsal elements, phalanges, teeth, trunk and caudal vertebrae in amniotes, and nipples in mammals, which are all highly evolvable. In agreement with the weaker conservation of later-determined numbers, changes of the number of thoracic vertebrae experience considerably weaker stabilizing selection in humans than changes of cervical vertebrae and are not significantly associated with congenital abnormalities (weaker pleiotropy) (Galis et al. 2006). Similarly, in long-necked birds and reptiles, in which the cervicothoracic boundary is determined at a later stage, substantial intraspecific variation is found in the number of cervical vertebrae [e.g., 22–25 in swans (Woolfenden 1961), in contrast to amniotes with necks with 8 or fewer vertebrae (Hofstetter & Gasc 1969)].

3.15. Evolution of Novelties and Relaxed Selection

On rare occasions, pleiotropic constraints are broken and novelties evolve. This breaking of constraints is usually associated with a relaxation of the normally strong stabilizing selection (Galis

& Metz 2007, Varela-Lasheras et al. 2011). Periods of relaxed selection may be the colonization of new habitats, the disappearance of predators, or the ample availability of new types of prey. Relaxed selection allows such novelties to persist for some time, which may lead to a reduction of the pleiotropic connections through small reorganizations of the developmental pathways, so that when stabilizing selection again increases, the chance for further persistence is increased. Such periods of relaxed selection are, thus, most likely associated with the initial phase of adaptive radiation and with the emergence of key innovations (Galis 2001, Galis & Metz 2007). A good example can be found in the *Semionotus* fishes that invaded a newly formed rift lake in northeastern America in the late Triassic and early Jurassic and that radiated into a species clade (McCune 1990). McCune found that in the early history of the lake, when supposedly directional selection was strong but stabilizing selection weak, many dorsal-ridge-scale anomalies occurred. Gradually, these anomalies became less prevalent, but interestingly some of the anomalies became incorporated into new body plans (McCune 1990). Other examples can be found in pets, in which stabilizing selection is much reduced as a result of human care. As discussed above, polydactyly is strongly evolutionarily constrained among amniotes, despite the frequent occurrence of mutations for it. In contrast, polydactyly is particularly common in cats and dogs, and in some dog breeds, the breed standard even requires them to have one or two extra toes (Galis et al. 2001). Pet dogs with congenital abnormalities can breed and reproduce, although longevity is reduced (Galis & Metz 2007). At the same time, strong selection has occurred for differences in sizes and shapes (e.g., Kemp et al. 2005). The combination of strong directional selection and relaxed stabilizing selection orthogonal to it has led to the present extreme variation among dog breeds.

Internal factors can also relax stabilizing selection and this probably plays a role in the exceptional number of cervical vertebrae in sloths (six) and manatees (six to nine) (Galis 1999, Varela-Lasheras et al. 2011). Sloths and manatees frequently have congenital abnormalities, in particular skeletal abnormalities that are also common in humans with an abnormal number of cervical vertebrae (ten Broek et al. 2012). However, the abnormalities appear to have hardly any effect on fitness, presumably due to the animals' extremely low activity levels (Varela-Lasheras et al. 2011). An additional reason for the weaker constraint may be much reduced cancer rates in manatees (and probably sloths), associated with their low metabolic rates (Galis & Metz 2003). Childhood cancers are among the pleiotropic effects associated with cervical ribs in humans (Galis 1999). Lower cancer rates may also be implicated in the weaker constraint against changes of the number of cervical vertebrae in birds and reptiles. Despite their high metabolic rates, birds have particularly low cancer rates, much lower than in mammals (Galis 1999, Galis & Metz 2003).

Thus, the difficulty of breaking specific constraints varies among taxa, owing to differences in the history of selection regimes (i.e., periods of relaxed stabilizing selection) and differences in specific pleiotropic effects associated with certain traits.

4. CONCLUSIONS

The complex and highly controlled global interactivity associated with mitosis and early organogenesis leads to pleiotropic constraints against evolutionary changes of developmental interactions. This strong control, which intensifies the interactivity, is essential because of the complexity of the processes and the grave consequences of disruptions (aneuploidy, apoptosis, cancer, sterility, death). As we have shown, mutations with an effect during such highly interactive states almost unavoidably have many deleterious pleiotropic effects that drastically reduce the chance that the mutations will be successful. Conservation is a consequence of consistently strong stabilizing selection against mutations via their pleiotropic effects (Galis et al. 2001, 2002b; Hansen & Houle

2008). As the interactivity is part of developmental processes, the constraints can be considered to be developmental constraints.

We found very few examples of developmental constraints that act long-term in the literature, the only additional well-documented one being developmental constraints against parthenogenesis (Engelstädter 2008; see also Section 2.13). In this case, the tightly controlled global interactivity in the cell during meiosis probably plays a role comparable to the interactivity of mitosis and the phylotypic stage.

The strongest and most universal of the constraints is that, in metazoans, differentiated cells cannot divide by mitosis (Buss 1987). The only possibility for differentiated cells to divide appears to be first to dedifferentiate, as happens in wound healing and regeneration (and cancer). The constraint places crucial restrictions on the order and timing of proliferation and differentiation during development and consequently on the potential for body plan evolution. The constraint on evolutionary changes of phylotypic stages may be weaker. Most notably, many phyla and classes have highly diverse phylotypic stages that evolved during early metazoan evolution (Buss 1987, Galis & Sinervo 2002). Nonetheless, within many of these taxa (e.g., vertebrates and insects) evolutionary changes of this stage are highly constrained. This constraint has grave consequences for the conservation of body plans, because most organ primordia appear during this stage and, hence, the numbers and earliest development of most organs are strongly conserved.

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