Evolvability

A Unifying Concept in Evolutionary Biology?

Edited by: Thomas F. Hansen, David Houle, Mihaela Pavličev, Christophe Pélabon

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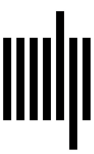

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16 Evolvability of Body Plans: On Phylotypic Stages, Developmental Modularity, and an Ancient Metazoan Constraint

Frietson Galis

The evolvability of animal body plans is limited. For instance, strong constraints exist against evolutionary change of early organogenesis, also called the phylotypic stage. Most of the body plan is usually laid out during this stage, and as a consequence, its conservation is implicated in the conservation of body plans. Two hypotheses have been proposed to explain the strong conservation of the phylotypic stage. One states that the conservation reflects a strong interactivity between developmental modules, so that mutations would have many pleiotropic effects, resulting in stabilizing selection against the mutations. The other states that, at least in insects, the conservation is caused by the robustness of a centrally important organizer gene network against mutational changes. I describe how the empirical and theoretical support for the robustness hypothesis is weak, but it is strong for the pleiotropy hypothesis. This highlights the importance of developmental modularity for evolvability. Finally, I discuss how an ancient metazoan constraint on the division of differentiated cells causes the early loss of pluripotentiality of cells. Consequently, the layout of the body plan occurs early, when the embryo is small, and the number of inductive interactions is too limited to allow for effective developmental modularity. Hence, this constraint on simultaneous cell division and differentiation causes another constraint: The one against changes of the phylotypic stage and of the body plan traits that are determined at these stages.

16.1 Introduction: Limited Evolvability of Phylotypic Stages and Body Plans

Evolution has produced an astonishing array of organisms, yet early stages of organogenesis in animals have been remarkably conserved across many higher taxa (figures 16.1 and 16.2; Medawar 1954; Seidel 1960; Ballard 1981; Sander 1983; Raff 1994; Gilbert 1997; Hall 1997). Furthermore, most of the body plan traits that are determined during these stages have been conserved. Evolution, thus, appears to be subject to constraints and there are limits to the evolvability of body plans. These conserved early organogenesis stages, at which the morphological and genetic similarity appears to be greater than at earlier or later stages, are usually called phylotypic stages. During these phylotypic stages most of the body plan is laid out. Sander (1983) introduced the term phylotypic stage as an alternative to the terms Körpergrundgestalt of Seidel (1960) and phyletic stage of Cohen (1977). Since then, the term has not only been applied to phyla, but also to other higher taxa, for example, to the class of insects (Sander 1983; Sander and Schmidt-Ott 2004).

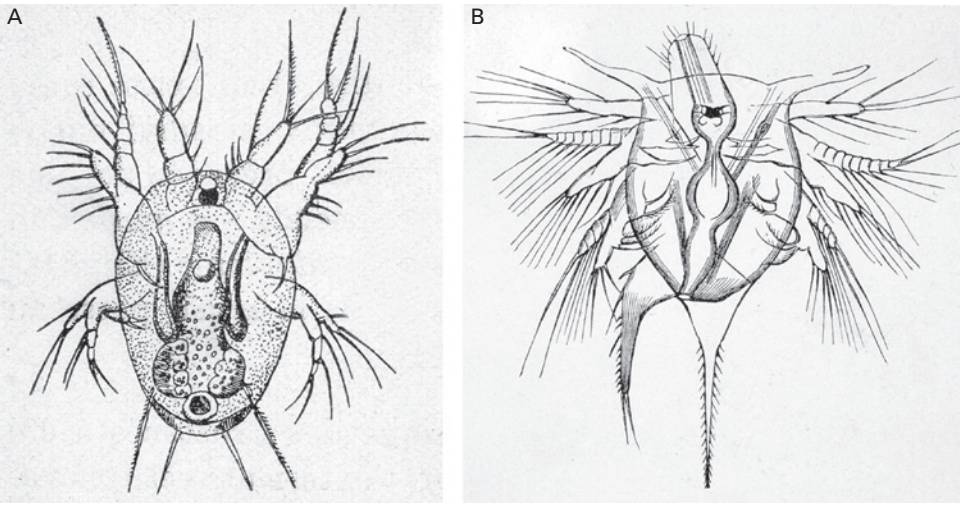

Figure 16.1
Early organogenesis stages are very similar in crustaceans. Nauplius stages in (A) *Cyclops*, (B) Cirripedia species (from Claus and Grobben 1917).

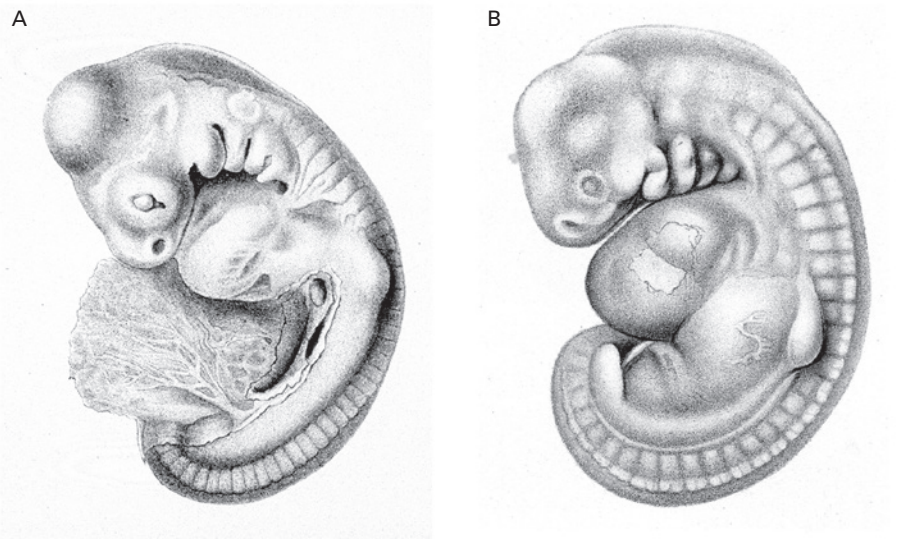

Figure 16.2
Early organogenesis stages in vertebrates are more similar than earlier or later stages. Especially in amniotes, the similarity is striking. Pharyngula stage in (A) *Lacerta* and (B) human (from Keibel 1904 and 1908).

Von Baer (1828) was the first to hypothesize that the constraint against evolutionary changes of these stages might be caused by the negative cascading consequences of early changes (pleiotropic effects), with later stages having fewer cascading consequences. Although this proposed constraint is undoubtedly real (Buss 1987), its importance is challenged by the existence of considerable variation in the embryonic stages before early organogenesis, cleavage, and gastrulation (Seidel 1960; Sander 1983; Gilbert and Raunio 1997; Galis and Sinervo 2002). Sander (1983) already pointed out that the stages preceding the phylotypic stage are highly variable, but that thereafter, the developmental pathways converge (see also Seidel 1960). The larger variability of the earlier stages is not always immediately apparent, as there are striking cases of morphological similarity that result from convergent or parallel evolution (Buss 1987; Gilbert and Raunio 1997; Hall 1999; Galis and Sinervo 2002). The morphological similarity is reflected in similarity of gene expression patterns (Levin et al. 2016). Similarity is almost unavoidable in the early developmental stages, because of the complete reset of the organism at the initial single-celled stage (Galis and Sinervo 2002, Galis et al. 2018). Only a limited number of permutations is possible in embryos with a few, not yet differentiated cells. Further reasons for similarity of cleavage and gastrulation are caused by convergent locomotory and nutritional adaptations plus maternal attempts at dictating offspring features (reviewed in Buss 1987). A good example of remarkable convergence is cleavage and gastrulation in the yolk-rich embryos of cephalopods, fishes, reptiles, and birds, because yolk impedes cleavage and as a result, the embryo develops as a disk on top of the yolk (figure 16.3). Yet, despite often remarkable similarity, within phyla and classes the processes of cleavage and gastrulation are far more diverse than is the end product of gastrulation: the beginning of the phylotypic stage. For instance, cnidarians have seven different types of gastrulation (Gilbert and Raunio 1997). In insects, there are drastic differences in gastrulation between short, intermediate, and long-germ-band insects, poly-embryonic wasps being even more derived (Grbič 2000). Within teleosts and mammals, gastrulation is also highly variable (Collazo et al. 1994; Viebahn 1999). Yet, at the end of gastrulation, the developmental pathways converge,

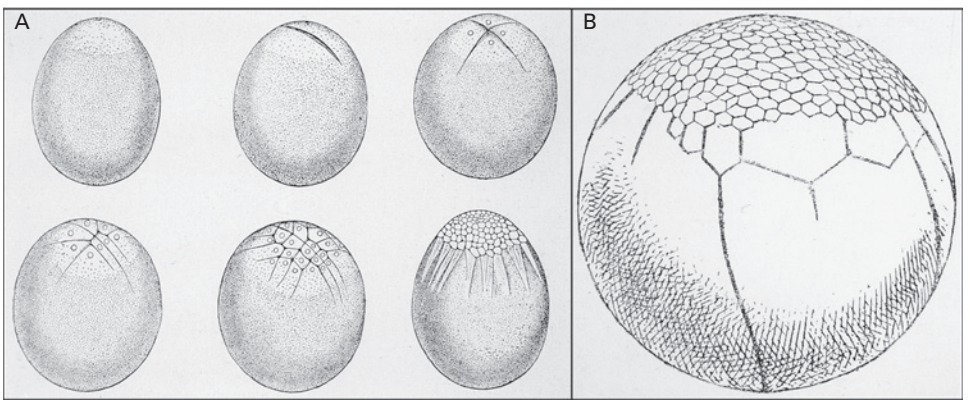

Figure 16.3
Convergence of cleavage stages. Cleavage of the embryo on top of the yolk, as yolk impedes cell division (meroblastic cleavage), in (A) the cephalopod *Loligo pelalei* (after Claus and Grobben 1917) and (B) in the longnose gar *Lepisosteus osseus* (after Balfour 1881).

gastrulae invariably have no more than two or three germ layers, and the organ systems emerging from the germ layers are similarly conserved (e.g., skin and nervous system arise from ectoderm and the digestive system from endoderm; Hall 1999). A key outcome of the process of gastrulation is that sheets of cells come into contact with each other, enabling the conserved embryonic inductions that are essential for the organization of the body plan at the phylotypic stage. These inductions between adjacent cell populations appear to form a severe spatiotemporal constraint on the outcome of gastrulation, which is the starting point of the conserved phylotypic stage (Galis and Sinervo 2002; Zalts and Yanai 2017).

In contrast to the strong conservation of the phylotypic stages within phyla or classes, these stages differ dramatically between phyla and classes (see below for a discussion). The segmented germ-band stage in insects, the nauplius stage of crustaceans, and the neurula/pharyngula stage in vertebrates are examples of this diversification (cf. figures 16.1 and 16.2). Nonetheless, what all these stages have in common is that they start at the end of gastrulation and that most of the body plan is being laid out during this part of development.

16.2 Pleiotropy Proposed as the Cause of the Conservation of Phylotypic Stages

Sander (1983) hypothesized that the evolutionary conservation of the phylotypic stages in animals is caused by pleiotropic effects resulting from strong interactivity between developmental modules. Raff (1994, 1996) proposed a similar hypothesis to explain the presumed strong stabilizing selection against evolutionary changes influencing the phylotypic stage: The web of intense interactions among organ primordia of the embryo at this stage causes any small mutational change to lead to many pleiotropic effects elsewhere in the embryo, thus reducing the chance of a favorable mutation. Raff further argued that at earlier stages, fewer inductive interactions occur, as there are not yet organ primordia, and thus fewer pleiotropic effects (although changes may still affect the entire embryo). At later, larger, stages there are many more inductive interactions, but they take place within semi-independent modules (e.g., limbs, the heart); hence changes will usually be limited to a smaller part of the embryo. The hypotheses of Sander (1983) and Raff (1994, 1996) both see the high connectivity between developmental modules as the major cause for conservation. This high connectivity causes mutations to have many pleiotropic effects, which will have cascading consequences as development proceeds (von Baer 1828; Buss 1987). The strong connectedness of modules implies an easily destabilized network of inductive events, with low effective robustness and low effective modularity. Because pleiotropic effects during embryogenesis are generally disadvantageous (Hadorn 1961; Wright 1970), strong stabilizing selection against mutational variation ensues. In this scenario, conservation is a consequence of consistently strong selection against mutations via their pleiotropic effects (pleiotropic constraints; Hansen and Houle 2004; Galis et al. 2002, 2018). These hypotheses support ideas about the importance of modularity (the existence of semi-independent units in organisms as a condition for evolutionary change; e.g., Lewontin, 1978; Bonner 1988; Galis 1996; G. Wagner and Altenberg 1996; Galis and Metz 2001; Galis et al. 2002; Schlosser 2002; Mitteröcker 2009; Armbruster et al. 2014). Developmental modularity limits the effects of mutational changes to only part of the organism, thereby greatly reducing the probability that advantageous changes are associated with adverse pleiotropic

effects elsewhere. It is thus the lack of developmental modularity that is hypothesized to be the cause of the limited evolvability of phylotypic stages.

16.3 Robustness as an Alternative Cause for the Conservation of the Phylotypic Stage

Von Dassow and Munro (1999) proposed an alternative hypothesis to explain the conservation of the phylotypic stage in insects (the segmented/extended germ-band stage) that is diametrically opposite to the pleiotropy hypothesis from Sander and Raff (Galis et al. 2002). Here, the robustness of a centrally important organizer gene network and module, the segment polarity gene network, is hypothesized to be causally involved in the conservation (figure 16.4). Hu et al (2017) also mention the robustness of gene networks as a possible cause for the conservation of phylotypic stages in vertebrates, although their major conclusion is that pleiotropic constraints appear to be involved.

This robustness hypothesis implies strikingly different roles for modularity in evolution than does the pleiotropy hypothesis of Sander and Raff (see table 16.1), that is, constraining rather than facilitating evolutionary change (Galis et al. 2002).

Von Dassow and Munro (1999) proposed that conservation of the segment polarity network occurs despite accumulation of genetic changes, as these changes have little phenotypic effect and mainly lead to hidden variation (von Dassow et al. 2000; von Dassow and Odell 2002). Hence, the robustness of the segment polarity network in each segment is proposed to 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 and G. Wagner 2000; A. Wagner 2000).

16.4 Evaluating Pleiotropic Constraints and Robustness

The modeling of the robustness of the network by von Dassow and colleagues is valuable, but what matters here is whether robustness can cause long-term conservation. Although it is not possible to directly test these hypotheses about the evolutionary past, they can be tested indirectly, as they lead to very different predictions for mutations affecting the phylotypic stage (table 16.1). The robustness hypothesis proposes that mutations will have minimal phenotypic effects and mainly produce hidden, or cryptic, variation (i.e., phenotypically similar, but genotypically different), whereas the pleiotropy hypothesis proposes that the

Table 16.1
Predictions of the robustness and pleiotropy hypotheses

	Pleiotropy hypothesis	Robustness hypothesis
Genetic mutational variation	Visible at the phenotypic level	Hidden
Direct phenotypic effects	Potentially large	Small
Dominance of direct effects	Haplo-insufficiency possible	Recessivity, or near recessivity
Pleiotropic effects	Many	Few

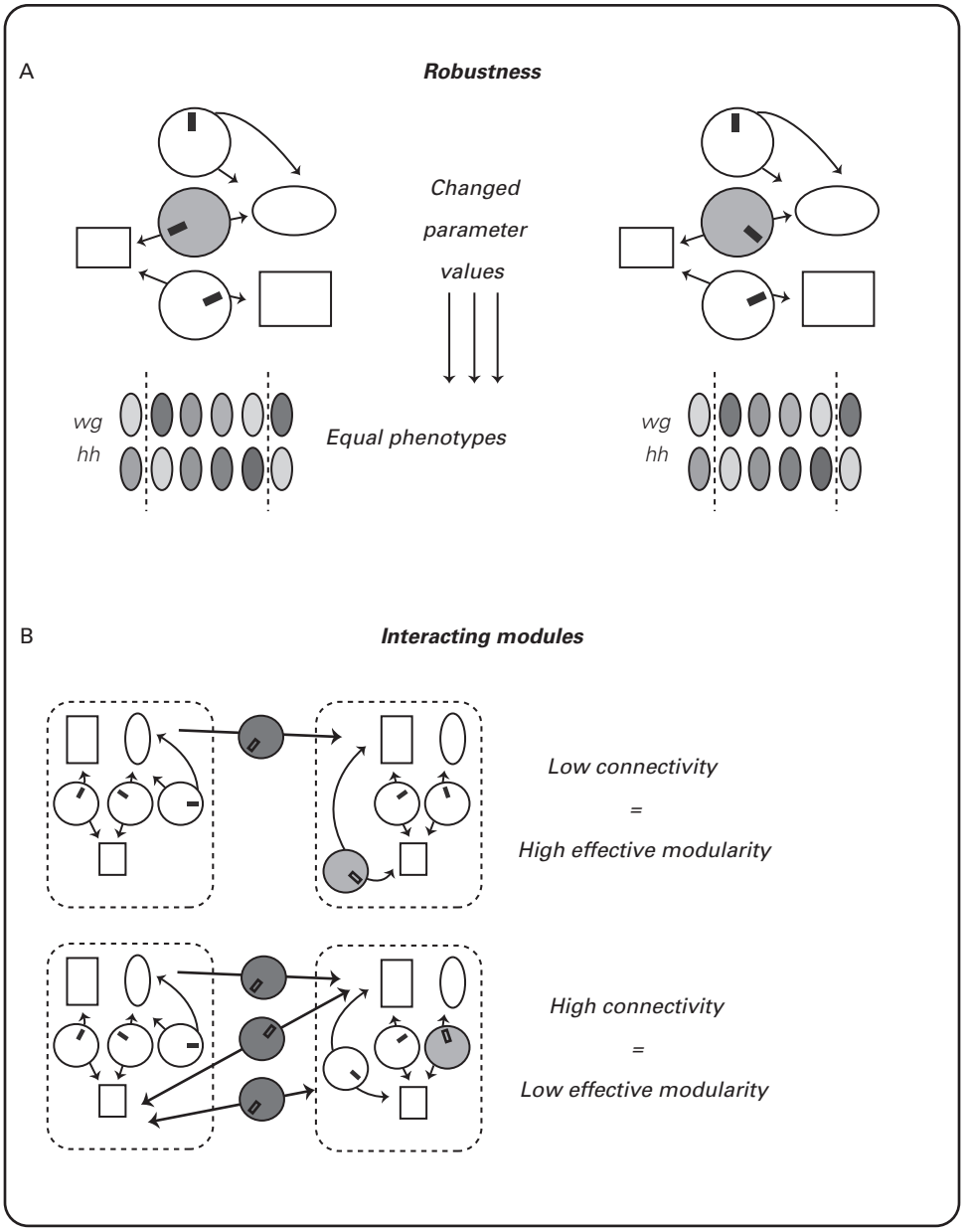

Figure 16.4
Robustness and effective modularity. (A) Robustness. When a parameter is changed in a robust genetic network, the resulting phenotype does not change (in this case illustrated with the concentration of the organizing morphogens *Wingless* (WG) and *Hedgehog* (HH) in the cells of the ectoderm of *Drosophila* during the segmented germ-band stage.) (B) Effective modularity. Modules are discernible and discrete units in large genetic networks that have some autonomy and a clear physical location (Raff 1996). These modules can differ in the amount of connectedness. Low connectivity (i.e., few connections having small effects) implies high effective modularity. High connectivity implies low effective modularity (from Galis et al. 2002).

phylotypic stage is vulnerable to mutational change and that mutations will have large deleterious effects.

16.4.1 Phylotypic Stage in *Drosophila:* Importance of Pleiotropy

When evaluating the empirical evidence for these hypotheses in *Drosophila,* Galis et al. (2002) found little support for effective robustness of the segment polarity gene network (or other gene networks active during the stage) against mutational change acting on the phylotypic stage. Small changes in the position, shape, and intensity of segment polarity stripes lead to dramatic phenotypic effects, so that the system does not appear robust. The organizer function of segment polarity and other regulatory genes causes mutations in these genes generally to result in a cascade of pleiotropic effects. This pleiotropy is not surprising, as during this stage, the segment polarity genes are, together with *Hox* and other genes, involved in the specification and early differentiation of virtually all organ primordia and the patterning of drastic morphogenetic events (e.g., germband retraction, dorsal closure, and head involution). Many auto-regulatory and cross-regulatory interactions provide feedback on the input of the segment polarity gene network, further lowering the robustness and modularity of this gene network. Consequently, phenotypic effects of mutations with an effect on the segment polarity network (and more generally on the phylotypic stage) are severe and include many cascading pleiotropic effects. The severe phenotypic effects of even weakly hypo-morphic mutations illustrate the observation of Lande et al. (1994) that mutations of small, nearly additive effects are usually expressed relatively late in development, whereas lethal mutations are usually expressed early (see also Hadorn 1961; Wright 1970).

Although extremely strong selection for the robustness of early organogenesis undoubtedly occurs, given the deleteriousness of most mutations affecting that stage, the number of interactions involved in the morphogenetic patterning is probably too limited to prevent substantial and global interactivity between developmental modules (Galis et al. 2002).

16.4.2 Phylotypic Stage in Mammals: Importance of Pleiotropy

Teratological data on rodents strongly support the pleiotropy hypothesis for the vertebrate phylotypic stage: Early organogenesis was found to be more vulnerable to disturbances than earlier or later stages, as disturbances caused more abnormalities and lethality (Galis and Metz 2001). The interdependent pattern of the numerous induced abnormalities (pleiotropic effects) indicates that the high interactivity and low effective modularity is the root cause of the vulnerability of this stage. The vulnerability, thus, is not due to a single vulnerable process (e.g., neural tube closure), as is the case for the vulnerability of cell divisions during the cleavage stage (Galis et al. 2018); instead, it is due to the global interactivity during the stage. Indeed, in rodents and humans, almost all major congenital abnormalities find their origin in disturbances of the phylotypic stage (Russell 1950; Shenefelt 1972; Sadler 2010). The global interactivity implies that a particular, potentially useful change of this stage (e.g., a change in the number of limbs and antennae in insects or the number of kidneys, lungs, spleens, eyes, ears, long bones, and digits in vertebrates) will nearly always be accompanied by other abnormalities and by early lethality.

For example, in humans, changes to the number of (normally) 7 cervical vertebrae are induced at the phylotypic stage and are associated with a wide variety of abnormalities and early lethality (Galis et al. 2006; Furtado et al. 2011; Varela-Lasheras et al. 2011; Ten

Broek et al. 2012; Schut et al. 2020a–c; Galis et al. 2021). Changes of the cervical number are usually manifested as cervical ribs (rudimentary or full ribs on the seventh vertebra) or rudimentary or absent first ribs. Approximately 90% of individuals with such changes are dead at birth, and a strong association is seen with cardiovascular, nervous, urogenital, and other congenital abnormalities (op. cit.). After birth, pleiotropic effects increase the risk for embryonal tumors (Schumacher and Gutjahr 1992; Galis 1999; Galis and Metz 2003; Merks et al. 2005) and miscarriages (Schut et al. 2020b). The increased risk for miscarriages in humans is in agreement with fertility problems in thoroughbred horses either with rudimentary and absent first ribs, or cervical ribs (termed flared ribs and bifid first rib; May-Davis 2017).

Although the deleterious pleiotropic effects and the resulting strong selection against changes of the number of cervical vertebrae have been best investigated in humans, further support comes from studies on a wide variety of mammals, including afrotherians and xenarthrans (Varela-Lasheras et al. 2011), thoroughbred horses (May-Davis 2017), extinct woolly mammoths and rhinoceroses (Reumer et al. 2014; van der Geer and Galis 2017), and domesticated dogs (Brocal et al. 2018).

Further support for the importance of the disturbance of global interactivity for the induction of cervical ribs comes from the large heterogeneity of genetic and environmental causes of cervical ribs. Many different genetic abnormalities can disrupt the patterning of early organogenesis and lead to the induction of cervical ribs, including single gene disorders, large copy number variations and most aneuploidies (Keeling and Kjaer 1999; Galis et al. 2006; Furtado et al. 2011; Schut et al. 2019, 2020a). Moreover, a wide variety of teratogenic disruptions can lead to the development of cervical ribs in rodents (Li and Shiota 1999; Kawanishi et al. 2003; Wéry et al. 2003). The timing and duration of the disruption of the patterning matters more than the specific nature of the disruption. The many different possible disruptions leading to cervical ribs probably explain their extraordinarily frequent occurrence in humans. Approximately half of all deceased fetuses and infants have cervical ribs (McNally et al. 1990; Furtado et al. 2011; Ten Broek et al. 2012; Schut et al 2019). Assuming that ~15% of clinically recognized pregnancies end in a miscarriage (Forbes 1997), and approximately half of these fetuses have cervical ribs, it follows that almost 8% of all human conceptions experience a disturbed early phylotypic stage and develop cervical ribs. This makes cervical ribs one of the most common congenital abnormalities and emphasizes the vulnerability of the phylotypic stage.

As with changes in the number of cervical vertebrae, ~90% of human individuals with an extra digit are dead at birth (Opitz et al. 1987, Galis et al. 2010), and at least 290 syndromes are associated with extra digits (Biesecker 2011).

The medical and veterinary literature shows that increases in the number of replicated organs, which is determined during the phylotypic stage, are observed very rarely—for example, spleens, kidneys, ureters, vaginas, penises, testicles, long bones, and (even if extremely rarely) additional arms and legs (e.g., J. Uchida et al. 2006; Galis and 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. 2001, 2010; Lilje et al. 2007; Biesecker 2011). Without doubt, extra organs, including digits, can have strong selective advantages. For instance, extra digits are advantageous in digging and swimming. Yet, despite the potential functional advantages and the extremely frequent

occurrence of mutations for polydactyly, extra digits in amniotes are extremely rare, whereas extra digit-like structures (from modified carpal or tarsal bones, or sesamoid bones) are common across a range of animals (e.g., the panda's thumb, the mole's thumb, and an extra digit-like structure in the sea turtle *Chelone;* Galis et al. 2001).

Similarly, a higher number of cervical vertebrae would most likely be advantageous in long-necked mammals. Even giraffes have only 7 cervical vertebrae, whereas swans have 23–25 (Woolfenden 1961). A giraffe's neck is quite stiff, requires substantial force to lift it, and it is too short to reach the ground unless the front legs are spread wide apart (figure 16.5). This awkward position renders giraffes vulnerable to predators while drinking (Valeix et al. 2009). Hence, absence of directional selection for change is often not a plausible alternative explanation of the strong conservation.

Correspondingly, Grüneberg found that loss of digits (oligodactyly) is associated with a multitude of pleiotropic effects in both the appendicular and axial skeleton in mice. Presumably, due to this pleiotropic constraint, loss of organs typically occurs via the slow and continued evolution of an earlier stop of development, followed by partial or complete degeneration of the organ (Lande 1978; Raynaud and Brabet 1994; Galis et al. 2001, 2002; Bejder and Hall 2002). Hence, construction is followed by destruction. For instance, in horses and cows, 5-digit condensations initially still develop. The developmental interactions with these digit condensations apparently cannot be easily discarded evolutionarily. Similarly, in blind cave fishes and salamanders, eye development always proceeds until the lenses have been formed, after which degeneration follows (e.g., Dufton et al. 2012). Other examples of vestigial organs that have evolutionarily lost functionality but not yet fully disappeared during early development are teeth in baleen whales; the clavicle in canids, felids, and lagomorphs; wings in emus and kiwis; and pelvises in whales (Glover 1916; Klima 1990; Bejder and Hall 2002; Senter and Moch 2015). Generally, more of these structures are seen during early development than later on, due to their subsequent degeneration. 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 and Igić 2008; Galis et al. 2010).

16.4.3 Transcriptomic Data: Importance of Pleiotropy

Further support for the pleiotropy hypothesis comes from transcriptomic studies on insects and vertebrates, which show conserved expression of regulatory genes during the phylotypic stage, 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; 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).

16.4.4 No Theoretical Support for Robustness as a Cause of Long-Term Conservation

Theory supports robustness as a cause of short-term—but not long-term—conservation. Stabilizing selection is expected to lead to robustness to protect optimized traits against developmental noise and mutations, potentially leading to short-term conservation (A. Wagner 2000; Metz 2011; Papakostas et al. 2014; Austin 2016; Melzer and Theißen

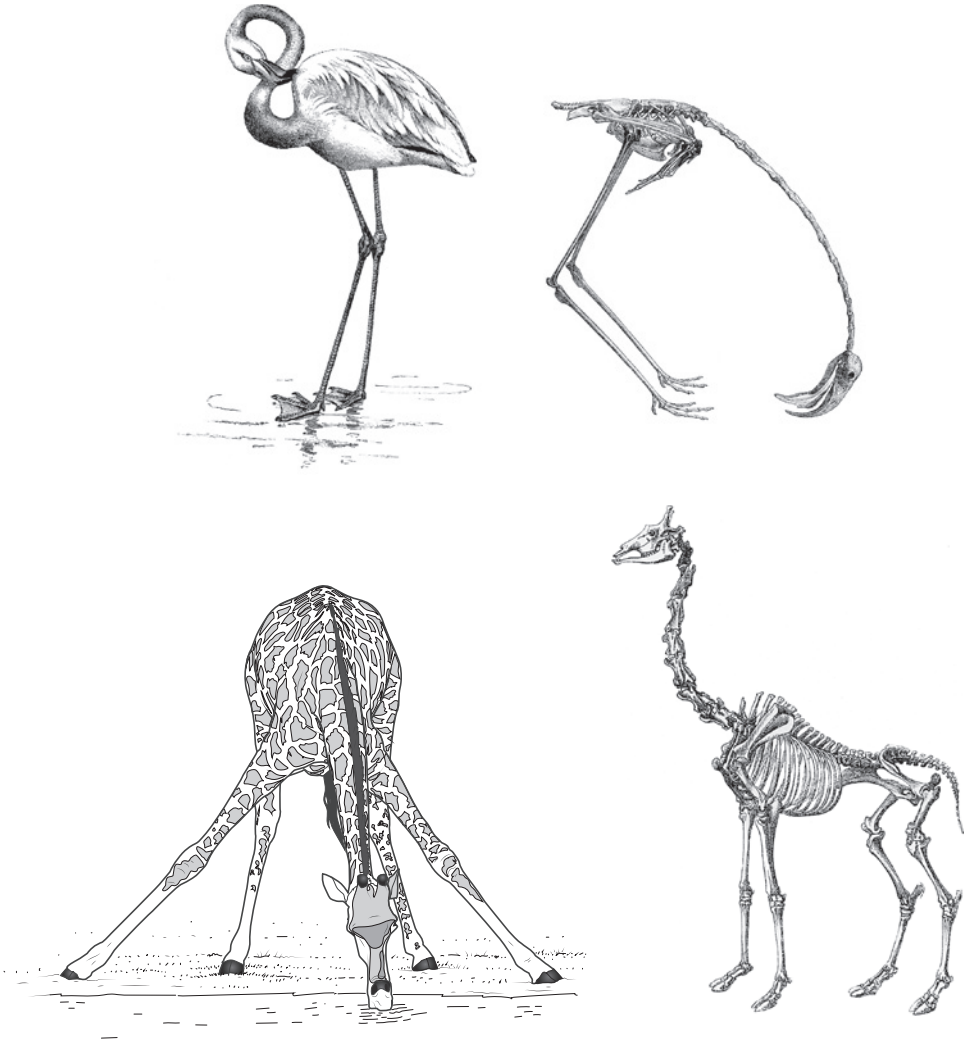

Figure 16.5
The long neck of the giraffe has only 7 vertebrae, which makes it rather stiff, so that lifting it costs considerable force. Despite the length of each cervical vertebra, the neck is not long enough to reach the ground unless the front legs are spread wide apart, which is an awkward position that makes the giraffe vulnerable to predators. Many vertebrae make a long and flexible neck in flamingoes. Top-left and bottom-right figures reproduced from Owen (1866), top right from Evans (1900), bottom left drawing by Erik-Jan Bosch (Naturalis).

2016). However, over long evolutionary scales, hidden variations will continue to accumulate, leading to a diversification of genetic backgrounds for new mutations (Gibson and G. Wagner 2000; Metz 2011; A. Wagner 2012; Siegal and Leu 2014). Hence, during periods with drastic environmental changes that lead to strong directional selection, the robustness of development alone cannot be sufficient to prevent change, because of the accumulated cryptic variation. In fact, in the long-term, cryptic variation is expected to lead to increased evolvability (A. Wagner 2012; Siegal and Leu 2014; Melzer and Theißen 2016).

In conclusion, empirical and theoretical support for the robustness hypothesis as an explanation for the conservation of the phylotypic stages is weak, and for the pleiotropy hypothesis it is strong, emphasizing the importance of modularity of developmental pathways for evolvability. The high interactivity between the developmental pathways not only constrains the evolvability of the phylotypic stages but also the evolvability of body plans, as the layout of most of the body plans occurs during the phylotypic stages.

16.5 Increased Modularity and Evolvability of Later Stages

When the final number of organs is determined after the vulnerable phylotypic stage, and development becomes 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 vary strongly. Another instructive case is the probable polydactyly in frogs (Galis et al. 2001; Hayashi et al. 2015). Anterior to the first digit, a prehallux or prepollux is present in various species of frogs, most likely representing a rudimentary sixth digit. In amphibians with aquatic larvae, limb development occurs later than in amniotes and generally occurs after the phylotypic stage (Galis et al. 2001; Galis et al. 2003). Limb development is especially late in anurans with an extreme mode of metamorphosis. Thus almost all limb development is unaffected by the interactivity of the phylotypic stage, a drastic difference compared with amniotes. This result is in agreement with the extremely high self-organizing capacity of the limb buds in many amphibians compared with those in amniotes (Balinsky 1970). Amphibian limb buds can be grafted to very different places, such as the head, and still successfully develop into limbs, which are even capable of movement (Detwiler 1930). After the initiation of the limb field, limb development can proceed almost as an independent module, with few interactions with other parts of the body (Galis et al. 2003). This independence reduces the number of pleiotropic effects of mutations that affect limb development. Hence, the probable polydactyly in frogs represents another example of relaxed selection against evolutionary changes due to the reduced pleiotropy of later developmental stages.

In agreement with the weaker conservation of later-determined numbers, changes of the number of thoracic vertebrae are not significantly associated with congenital abnormalities in humans (weaker pleiotropy), and stabilizing selection is considerably weaker compared to that of cervical vertebrae changes (Galis et al. 2006).

The late determination of the number of thoracic vertebrae is comparable to that of cervical vertebrae in birds and many reptiles. The number of cervical vertebrae is highly variable across bird species, with 12 in pigeons and up to 25 in swans (Woolfenden 1961). Even in the shortest necks, the number of vertebrae is considerably larger than in mammals.

Also in reptiles, the number of vertebrae can be highly variable; however, this is mainly the case in reptiles with long necks (e.g., many plesiosaurs and dinosaurs), whereas the number is quite constant in families with 9 or fewer cervical vertebrae, such as pterosaurs, crocodiles, turtles, geckos, and many other lizards (Hofstetter and Gasc 1969; Bennett 2014). The later determination of cervical vertebrae when there are many of them, as in birds and reptiles, should weaken the constraint, because it is expected that number changes will be associated with fewer pleiotropic effects, as is the case for thoracic and lumbar vertebrae in humans (Galis et al. 2006; Ten Broek et al. 2012). In agreement with this, the largest intraspecific variability in number is found in birds and reptiles with the longest necks (e.g., swans can have 21–25 vertebrae; Woolfenden 1961). Hence, the constraint on changes in the number of cervical vertebrae in mammals is less exceptional than it seems, it is just somewhat stronger than in short-necked reptiles with limbs, presumably because of a combination of the generally greater mobility and higher metabolic rates (Galis et al. 2021).

Other examples of late-determined structures are the number of segments in insects, carpal and tarsal elements, phalanges, teeth, trunk, and caudal vertebrae in amniotes, and nipples in mammals, which are all highly evolvable.

16.6 Relaxation of Stabilizing Selection Increases Evolvability

The conservation of the phylotypic stages should not be taken overly strictly. What matters is that in many higher taxa, development is remarkably conserved during phylotypic stage, more strongly than during earlier or later stages (Sander and Schmidt-Ott 2004). As argued above, stabilizing selection against the pleiotropic effects of mutations appears to be the reason that mutations affecting the phylotypic stage hardly ever persist in populations. Thus, when external or internal conditions relax the normally strong stabilizing selection, evolutionary changes may occur on rare occasions. In the wild, such breaking of constraints is often associated with the start of adaptive radiations and the emergence of key innovations, when for instance many empty niches suddenly become available, or a massive extinction of predators has taken place (Galis 2001; Galis and Metz 2021). Arguably, relaxed selection allows novelties to persist for some time, despite associated pleiotropic effects. This longer persistence in the population most likely leads to selection against the most deleterious pleiotropic effects, such that when stabilizing selection increases again, the chance for persistence of the novelties is increased (McCune 1990).

The effect of relaxation of stabilizing selection is most clearly illustrated in domesticated animals, where human care keeps those individuals alive that would likely not survive in nature. Extra digits in chickens, cats, and dogs are good examples of persistence of traits that are strongly selected against in nature (Galis et al. 2001). Dog and horse breeds with unusually high frequencies of cervical ribs and rudimentary first ribs provide further examples, where human care allows the survival of traits despite their frequent co-occurrence with congenital abnormalities or fertility problems (May-Davis 2017; Brocal et al. 2018). Sloths and manatees, carrying exceptional numbers of cervical vertebrae, provide good natural examples of the effect of relaxed stabilizing selection. Sloths and manatees commonly have skeletal and other congenital abnormalities that are associated with cervical ribs in deceased human fetuses (Varela-Lasheras et al. 2011). These abnormalities would probably be fatal

in most more active mammals. Their extremely slow metabolic rates, combined with weak locomotory constraints, appear to provide a relaxation of stabilizing selection against some of the associated skeletal and congenital abnormalities (Varela-Lasheras et al. 2011). Interestingly, sloths and manatees also have extremely low cancer rates (Galis and Metz 2003; Tollis et al 2020). Several other species show the association between cervical ribs and low metabolic and activity rates, providing support that these reduced rates may be involved in breaking the constraint. Slow loris and pottos (primates with extremely low metabolic and activity rates) often have cervical ribs (Galis et al. 2022). Whales and dolphins are also exceptional in regularly having cervical ribs. They also often have skeletal abnormalities. Relaxed selection against skeletal abnormalities is probably also involved; in this case, the relaxation is thought to be caused by the supporting effect of water (Galis et al. 2021). Furthermore, whales and dolphins also have low cancer rates, which probably also weakens the stabilizing selection against cervical ribs.

Thus, the difficulty of breaking specific constraints varies among taxa, due to differences in the selection regimes experienced and in the specific pleiotropic effects associated with trait changes (Galis and Metz 2018).

16.7 Evolvability of Vulnerable Early Cleavage and Gastrulation Stages

Raff (1994, 1996) proposed that the larger evolvability of the earlier developmental stages of cleavage and gastrulation may be due to the lower number of inductive interactions in the embryo, which should lead to fewer pleiotropic effects. Yet effective modularity is low, and cleavage is a vulnerable stage, particularly with respect to high doses of toxicants and radiation (e.g., Russell 1950; Shenefelt 1972; Galis and Metz 2001; Jacquet 2004). This vulnerability of cleavage has been used to argue that the vulnerability of organogenesis can, therefore, not be involved in the conservation of the phylotypic stage, as cleavage is evolvable, despite its vulnerability (Y. Uchida et al. 2018). However, in contrast to the phylotypic stage, the vulnerability in cleavage is mainly due to one vulnerable process: cell division. Furthermore, dividing cleavage cells are greatly similar (not yet differentiated) and are capable of self-renewal. The high capacity for self-renewal of the cleavage cells implies that, either too many cells are killed and the embryo dies, or the damage is reversible and development proceeds largely normally without adverse embryonic outcome (Russell 1950; Shenefelt 1972; Jacquet 2004; Adam 2012). In medicine, this is known as the all-or-none phenomenon, which has been extensively used in genetic counseling of pregnant women, who have inadvertently undergone an exposure to teratogenic substances in the early stages of pregnancy, frequently before the pregnancy has been recognized (Jacquet 2004; Adam 2012). The vulnerability of the subsequent phylotypic stage differs critically in that the strong global interactivity restricts the potential for reversal of damage. And mutations that affect cleavage and gastrulation presumably have a greater probability of being successful, as it is more difficult to destabilize a simple pattern than a more complicated one (Galis and Sinervo 2002; Galis et al. 2018). Hence, the weaker conservation of cleavage and gastrulation may well be due to the greater simplicity of the early forms.

16.8 Diversity of Phylotypic Stages among Metazoans

The remarkable diversity of phylotypic stages among metazoans (e.g., the Nauplius stage in crustaceans and the neurula in vertebrates; figures 16.1 and 16.2) seemingly contradicts the explanation of conservation caused by interactivity of the phylotypic stage. But the pattern of divergence suggests an early rapid phase of diversification in the evolution of metazoans during the Ediacaran Cambrian times, followed by strong conservation of discrete taxon-specific phylotypic stages and body plans (Buss 1987). The cause for this pattern of early rapid diversification of body plans followed by stasis is not well understood. Davidson and Erwin (2006) proposed that the evolution of more hierarchical and interconnected gene regulatory networks and their increased cooption for other developmental functions may be involved.

One hypothesis that did not receive much attention was by Buss (1987), who suggested that the initial 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, somatic 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 hypothesis thus assumes that during the early chaotic transition, when control was not yet at the level of the individual, the lack of integration increased evolvability. This early diversification scenario is intuitively appealing, but it has received surprisingly little attention, and hardly any research has been carried out to investigate this important question in evolutionary biology. Mutagenesis experiments with simple colonial organisms and theoretical modeling could probably contribute to a better understanding of this possibility (Galis and Sinervo 2002).

16.9 An Ancient Metazoan Constraint Causes the Early Layout of the Body Plan

In animals, most of the body plan traits are initiated early, during a highly interactive stage limiting evolvability of the body plans. In complex animals, most flexibility is provided by the changes in the number of segments, which allow the multiplication of legs and other organs of the segment. Another solution to the problem of evolvability 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). In contrast, the body plan in plants is not laid out early, and new organs can be initiated throughout life (Heidstra and Sabatini 2014; Cridge et al. 2016). Why does the layout of body plans occur so early in animals? Even in animals with metamorphosis, the organ primordia emerge early, with the adult fate already determined, like imaginal discs in insects (Held 2005). A major difference between plants and animals concerns an ancient animal constraint: Differentiated cells cannot divide by mitosis unless they first dedifferentiate (which does occur in regeneration and in cancer; Buss 1987; Galis et al. 2018). The conflict between differentiation and mitosis stems from the presence in cells of a single centrosome, which is necessary for both processes in

animals. Plants do not have centrosomes and use other structures to organize their microtubuli during cell division, and cilia (discussed later in this chapter) only occur in sperm cells of certain taxa (Schmit 2002). Even the cells outside the stem cell niches are able to return to a proliferative pluripotent state (Heidstra and Sabatini 2014).

16.9.1 Single Centrosome Precludes Simultaneous Cell Division and Differentiation

Centrosomes change dynamically during the cell cycle. During mitosis in animals, centrosomes are duplicated, precisely once per cell cycle. The duplicated centrosomes form the bipolar spindles that precisely segregate the duplicated chromosomes, producing an equal distribution of chromosomes between daughter cells (Sir et al. 2013; Meraldi 2016). Although centrosomes are not absolutely required for mitotic spindle formation and division in many cells, mitosis in the absence of centrosomes is an error-prone process that leads to chromosomal instability (Bonaccorsi et al. 2000; Basto et al. 2008; Sir et al. 2013; Meraldi 2016). When cells stop dividing, they form a primary cilium, for which one of the centrioles of the centrosome (the mother centriole) is necessary. This centriole (the mother centriole) converts into a basal body and migrates to the cell surface, where it organizes the primary cilium. Primary cilia were long thought to be vestigial organelles, not present in many cells. It was not until the 1990s, owing to technical improvements of visualization techniques, that it was discovered that primary cilia are present as antennae on almost all metazoan cells, including quiescent stem cells (Wheatley 1995). Furthermore, they do not function only as sensory organelles but also have a key function in intercellular signaling (Dawe et al. 2007; 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.

When cell division resumes, re-entry of the cell cycle begins with the resorption of the primary cilium, detachment of the basal body (the mother-centriole) from the cell surface, and migration of the centrosome to near the nucleus. Recent studies have 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 (Walz 2017). For example, the physical 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, which in turn influences cell-fate decisions (Walz 2017). Hence, these discoveries, which began in the 1990s and continue today, allow us to understand the metazoan constraint on simultaneous cell division and differentiation.

16.9.2 Metazoan Constraint Already Proposed in 1898

At the end of the 19th century, the constraint on cell division by ciliated cells had already been independently proposed by Henneguy (1898) and Lenhossék (1898). Buss could not have known in 1987 that virtually all differentiated metazoan cells have primary cilia and thus, the constraint that he proposed on the incompatibility of cell division and differentiation, equates in essence to the constraint proposed by Henneguy (1898) and Lenhossék (1898). Incidentally, Buss (1987) erroneously attributed the proposal of the constraint on

cell division by ciliated cells to Margulis (1981), instead of to Henneguy and Lenhossék. The hypothesis of Henneguy and Lenhossék remains uncontested for metazoans (for a review of the constraint, including rare exceptions, see Galis et al. 2018). Even lymphocytes, which were long thought to be exceptional in 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 and 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; Afelik and Rovira 2017). For a detailed review of the constraint, including rare exceptions, see Galis et al. (2018).

16.9.3 Extra Centrosomes Cannot Break the Evolutionary Constraint

Exceptionally, de novo generation of extra centrosomes occurs. But it is not a viable evolutionary road to the breaking of the constraint. 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; Basto et al. 2008; Godinho and Pelman 2014; Gönczy 2015), and perhaps cancer, as already proposed by Theodor Boveri (1902). 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 and Stearns 2012). 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 instead of one (Boveri 1901; Bell 1989; Manandhar et al. 2005). The cost of centriole elimination is that meiotic divisions in egg cells are less reliable. We conclude that supernumerary centrosomes are usually seriously disadvantageous for the individual and will be strongly selected against. Therefore, it is unlikely that the inability of ciliated cells to form proper mitotic spindles could be compensated for by the evolution of extra centrosomes.

16.9.4 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). However, the pathways used to produce these centrioles are different from those involved in the duplication of centrioles during the cell division cycle (op. cit.). Furthermore, multiciliated cells are terminally differentiated, and for cell renewal, unciliated progenitor cells are employed (Bird et al. 2014). Hence, multiciliated cells are not an exception to the rule that differentiated cells cannot divide.

16.9.5 Pluripotent Stem Cells Only Present During Early Development

Without a doubt, the incompatibility of simultaneous cell division and ciliation has crucially shaped development and evolution of metazoan body plans. The body plan is mostly defined during early embryonic development, when there are still zones that produce pluripotent stem cell colonies that subsequently migrate to other places in the embryo to start their paths of differentiation. Thus, the ancient metazoan constraint generally causes most of the layout of the body plans to occur early, when the number of inductive interactions does not yet provide sufficient effective modularity to prevent major pleiotropy. Later

in life, pluripotent cells are absent, which prevents the initiation of organ primordia. As already mentioned, also in animals with metamorphosis, organ primordia emerge early during embryogenesis (Held 2005). Adults generally only have multipotent, tissue-specific, stem cells that function in cell renewal, wound healing, and regeneration (Tanaka and Reddien 2011).

16.9.6 Constraint Inherited from Unicellular Metazoan Ancestors

Buss (1987) argued that metazoans inherited the possession of only one organizing center for microtubules, the centrosome, from their unicellular protist ancestors and that, in contrast, other unicellular groups (e.g., euglenophytes, cryptophytes, and chlorophytes) with multiple of such organizing centers 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.

16.10 Conclusion

The ancient metazoan constraint on simultaneous cell division and differentiation (Buss 1987), thus causes the layout of body plans to occur early, when the embryo is small and there are still sufficient pluripotential cells that can differentiate into a large number of different types of cells. The limited number of inductive interactions in the small embryo does not yet allow developmental modularity, and so the interactivity is global. As a result, mutations affecting this stage will almost always have many pleiotropic effects and will, therefore, be selected against. Thus, the ancient metazoan constraint causes another developmental constraint, the one on changes at the early organogenesis stage: the conserved phylotypic stage. Furthermore, as most of the body plan is laid out during the phylotypic stage, the minimal early developmental modularity not only constrains the evolvability of the stage itself but also of animal body plans in general. Together, these appear to be two of the rare hard developmental constraints that prevent evolvability at macro-evolutionary scales and have had a major influence on animal evolution (Galis et al 2018; Galis and Metz 2021).

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