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Evolutionary novelties: the making and breaking of pleiotropic constraints

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Synopsis Body plans are remarkably well conserved, but on (very) rare occasions important novelties evolve. Such novelties involve changes at the genotypic and phenotypic level affecting both developmental and adult traits. At all levels, duplications play an important role in the evolution of novelties. Mutations for duplications, including mutations for duplications of body parts, as well as mutations for other changes in the body plan, in particular homeotic ones, occur surprisingly frequently. Hence the limitation of mutations appears to be relatively unimportant for the conservation of body plans. However, mutations for duplications of body parts and homeotic changes rarely persist in populations. We argue that the root cause of the conservation of body plans is the strong interactivity during the patterning of the embryonic axes, including the interactivity between patterning and proliferation processes. Due to this interactivity, mutations cause many negative pleiotropic effects (malformations and cancers) that dramatically lower fitness. As an example, we have shown that in humans there is extreme selection against negative pleiotropic effects of the, surprisingly frequent, mutations affecting the number of cervical vertebrae. Moreover, we argue for the relevance of relaxed selection, which temporarily allows just-arisen novelties to persist, for the effective breaking of pleiotropic constraints. We illustrate this with two empirical examples.

Evolutionary novelties involve a complex set of changes: changes at the genetic level lead to developmental changes at the phenotypic level and these developmental changes lead to changes in the adult phenotype. In addition, selection acts upon the phenotype during all stages of development and the outcome of this selective process determines whether genetic changes can persist in populations, or not. For a full understanding of the evolution of novelties one, therefore, needs to understand (1) the processes that lead to, or constrain, changes at all organizational levels and (2) the links between the levels.

The complexity of the underlying processes has slowed down progress in the understanding of evolutionary novelties. Fortunately, research over the past decades has shown that there are important similarities in the process of evolutionary change at all organizational levels. An important similarity is that duplication of units, followed by modification of one or both copies, appears singularly important as a source of evolutionary change (Serebrovsky 1938; Ohno 1970; Lynch and Force 2000). Duplication has been observed at the level of whole genomes (e.g., tetraploidy in plants), chromosomes (trisomy), genes, parts of genes, networks of genes, developmental units, and body parts and one can even argue that it plays a role at the level of populations where it facilitates speciation. We argue here that another important similarity is that mutations that provide duplications and homeotic changes are less rare than may be naively expected, but that the incipient novelties almost always fail to persist due to strong selection against many negative pleiotropic effects that are associated with them. The inference is that periods of relaxed stabilizing selection, as occur for instance after mass extinction or on the invasion of a new territory, are important in facilitating the evolutionary incorporation of novelties.

Integration and selection of duplications

There are at least two reasons that can explain why duplication, followed by modification of one or both duplicated units, is an important source for evolutionary novelties. One reason is that duplication produces new units with a ready-made and finely-tuned internal integration. For instance, a duplicated segment in an annelid will come with all the necessary elements to function as part of the

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organism. The second reason is that when there are two units that function in the same way, selection on the functioning of one, or both, copies may be relaxed if there is excess capacity for the original function(s) of the ancestral unit (Serebrovsky 1938; Ohno 1970; Arthur 2002; Kondrashov et al. 2002; Taylor and Raes 2004; Kondrashov and Kondrashov 2006). For instance, if duplicated genes code for a particular visual pigment, selection on the modification of one of the genes is expected to be relaxed, because one copy will still produce that particular visual pigment. Indeed, differentiation of duplicated visual pigment genes (opsins) that code for different pigments has happened several times independently in vertebrate evolution (Dulai et al. 1999; Parry et al. 2005; Trezise and Collin 2005).

More in general, stabilizing selection and its occasional relaxation are expected to play an important role in the evolution of novelties.

Initially, there may be a direct selective advantage for duplication. For instance, an extra vertebra may lead to a longer and more flexible neck that is advantageous under certain circumstances (Fig. 1) or an extra gene may lead to the advantageous production of more gene product (Kondrashov et al. 2002; Kondrashov and Kondrashov 2006). An example of the latter is the duplication of the CCL3L1 gene in humans, which provides a lower susceptibility to HIV infection (Gonzalez et al. 2005). Very often, however, there will be strong stabilizing selection against duplications. For instance, duplicated genes may lead to a suboptimal quantity of gene products. An increased gene dosage due to duplication of *Sox3* genes probably causes the perturbation of pituitary and hypothalamic development that underlies X-linked hyperthyroidism in male humans (Solomon et al. 2004). In general, stabilizing selection against duplications is expected if the duplicated unit disturbs the integration of the organization at a higher level. Thus, duplications of developmental units may disrupt developmental integration and duplications of structures may disrupt functional integration. Examples of disruptions of functional integration caused by duplicated structures are duplicated veins that lead to an enhanced chance of thrombosis (Quinlan et al. 2003) and a duplicated urethra that can cause recurrent urinary tract infections (Horie et al. 1986).

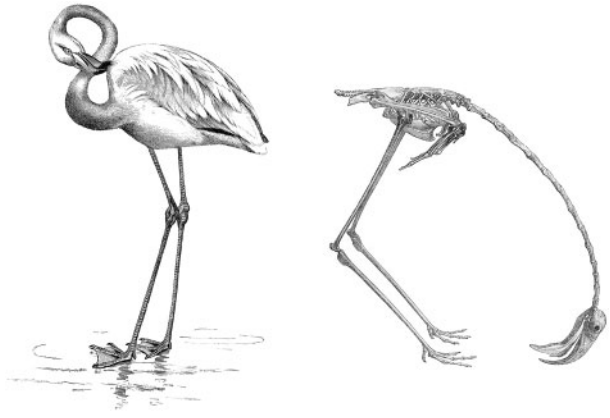

Fig. 1 A large number of vertebrae contribute to make a long and flexible neck in flamingoes. Reproduced from Evans (1900) and Owen (1866), respectively left and right.

Modification of duplicated units

Duplication followed by modification of one, or both, copies appears to have been by far the most important source of novel genes (Long et al. 2003; Taylor and Raes 2004). There is an abundance of examples among both structural and regulatory genes: crystallin genes (Wistow and Piatigorsky 1987a and b; Piatigorsky and Wistow 1991), snail and slug genes (Locascio et al. 2002), tRNA endonuclease genes in Archaea (Tocchini-Valentini et al. 2005), many plant MADS-box genes (Becker et al. 2003; Zahn et al. 2006), and the aforementioned opsin genes (Dulai et al. 1999; Parry et al. 2005; Trezise and Collin 2005), amongst others.

Duplication followed by modification seems to have been as important for the evolution of new networks of genes, i.e., the cooption of gene networks by different parts of the body. A striking example is the cooption of the developmental pathway of median fins by the lateral plate mesoderm that led to the evolution of paired fins in fishes (Freitas et al. 2006). This example shows that the duplication of gene networks can lead to duplications of developmental units and, hence, structures. Duplicated structures are also recognized as a major source of evolutionary change in body plans (Bonner 1988; Müller and Wagner 1991; Vermeij 1995; Galis 2000; Arthur 2002; Theißen 2006a and b). A beautiful example is the vertebral column. This structure with repeated (duplicated) elements has been of outstanding importance in the evolution of the large variety of body plans in vertebrates (Slijper 1946; Radinsky 1987). Arguably, even more important are the flower organs and leaves in plants (Honma and Goto 2001; Geuten et al. 2006). The earlier mentioned fins form another good example, as do the teeth (Jernval et al. 1996) and pharyngeal arches of vertebrates (Mallatt 1996, 1997),

the segments and appendages of arthropods (Minelli 2003; Arthur and Chipman 2005) among many other examples.

Mutations for duplications are common

Duplications of units are usually technically easy changes, even in cases where the duplication involves the building of entire structures. Cohn et al. (1995) showed how the mere ectopic expression of fibroblast growth factor (Fgf-8 and also Fgf-4) (Ohuchi and Noji 1999) in the lateral plate mesoderm, leads to the induction of an extra limb in chickens. Mutations for the duplication of structures are very frequent in humans, which are perhaps not surprising, given the technical ease with which they can be produced. Extra digits are among the most frequent mutations in humans (0.01–0.02% in livebirths) (Castilla et al. 1996, 1998). The medical and veterinary literature shows that many other organs are occasionally duplicated, e.g., spleens, kidneys, ureters, vaginas, penises, testicles, breasts, teeth, arteries, veins, vertebrae, ribs, rudimentary ears, and even extremely rarely additional arms and legs, although additional legs are sometimes remnants of conjoined twins (Lin et al. 2000; Brown and Schwartz 2003; Uchida et al. 2006; Lilje et al. 2007). Yet, despite their relatively high frequency of occurrence, such mutations very rarely persist in populations and, thereby do not lead to evolutionary change. Newly duplicated structures are virtually always associated with negative pleiotropic effects on functions that are under strong stabilizing selection (Wright, 1935, 1969; Grüneberg 1963; Lande 1978; Horie et al. 1986; Opitz 1987; Galis et al. 2001, 2006, Biesecker 2002; Quinlan et al. 2003; Bartram et al. 2005). Selection, thus, appears to be mainly indirect and conservation is largely due to pleiotropic constraints (Galis et al. 2006; Hansen and Houle 2004).

The previous considerations lead to two important questions: why are pleiotropic constraints so prevalent and how can such constraints be overcome, so that novelties emerge?

Duplications, homeotic changes, and early organogenesis

Most duplications of metazoan structures have their origin during the early organogenesis stage, because this is when organ primordia make their first appearance. A duplicated structure requires a duplicated organ primordium during this stage. Similarly, homeotic changes that modify the identity of a repeated structure usually have their origin during this stage, e.g., transformation of a cervical vertebra into a thoracic vertebra with rib, or an insect antenna into a limb (Galis et al. 2002, 2006). This stage is strongly conserved in at least mammals and insects (Sander, 1983; Raff 1996; Hall 1996; Galis and Metz, 2001; Galis et al. 2002; Sander and Schmidt-Ott 2004, but see Richardson et al. 1997 for an alternative view) and there is strong selection against mutations at this stage (Galis and Metz 2001; Galis et al. 2006; Ploeger et al., in press). We propose that duplications and homeotic changes are rare events in evolution because they usually require changes in the conserved early organogenesis stage (or phylotypic stage) and because the strong stabilizing selection against mutations for duplications and homeotic changes forms part of a more general stabilizing selection against changes of this stage.

Conservation of early organogenesis

Sander (1983) and Raff (1996) proposed that high interactivity between modules is the major cause of conservation in this stage. The high interactivity causes mutations affecting traits determined in this stage to have negative pleiotropic effects; these become amplified as development proceeds. Conservation is a consequence of consistently strong stabilizing selection on those pleiotropic effects. We earlier found support for the validity of this hypothesis in an analysis of teratological studies in rodents (Fig. 2) (Galis and Metz 2001). We found that chemical and other disturbances of this stage (phenocopies of mutations) lead to a considerably

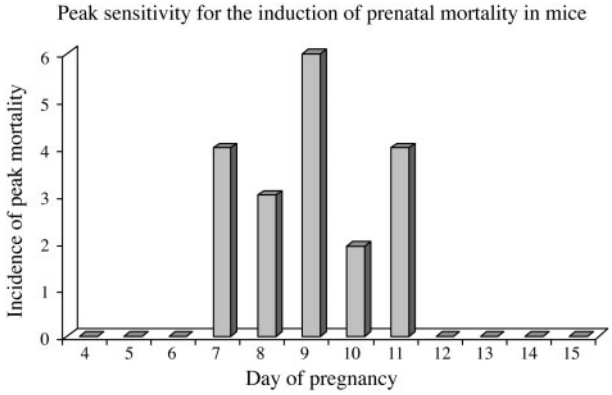

Fig. 2 The vulnerability of early organogenesis to induced changes (phenocopies of mutations). Vulnerability to teratogenic treatments in rodents is highest during embryonic day (E) 7–11 in mice. This vulnerability is caused by dependent inductive interactions. Peak sensitivity to the induction of mortality occurs on a particular day during pregnancy, always within this stage, usually on E9. (Reproduced from Galis and Metz, 2001).

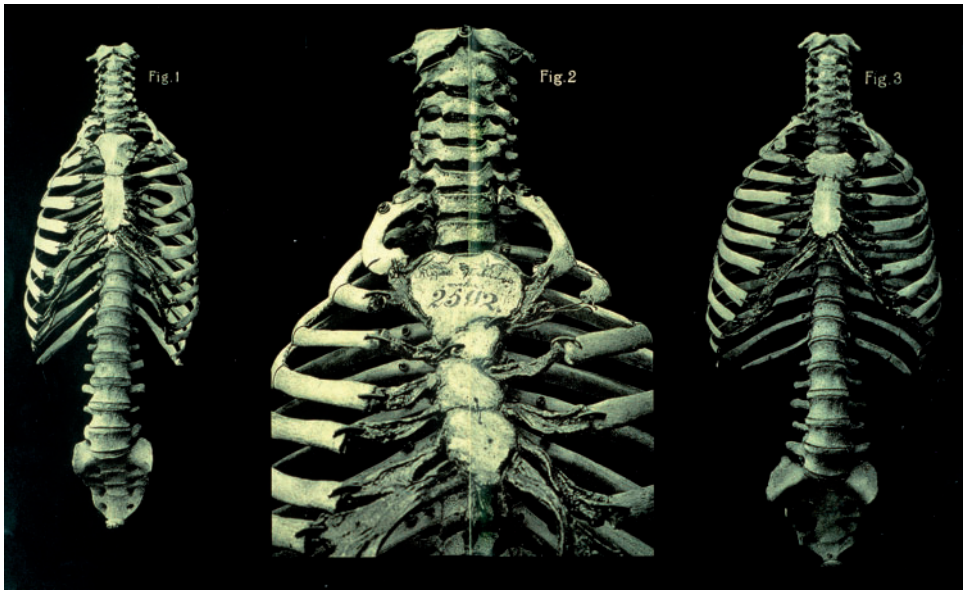

Fig. 3 Skeleton of three humans with a complete cervical rib, i.e., a rib on the seventh cervical vertebra. This change represents both the duplication of a structure, i.e., a rib, and a homeotic change, the change of identity of the seventh vertebra into that of a thoracic vertebra. Reproduced from Fishel (1906).

higher mortality than do disturbances of earlier and later developmental stages. From the pattern of multiple induced abnormalities (i.e., pleiotropic effects), we concluded that it is the high interactivity and low effective modularity that is the root cause of the vulnerability of the stage: a particular, potentially useful, change almost always will induce lethality even before the organism is exposed to external or ecological selection. Hence, this is a good example of the importance of internal or developmental selection (Whyte 1964; Arthur 2002). The importance of internal selection for the conservation of early organogenesis in insects is also in agreement with this hypothesis (Galis et al. 2002).

Selection against homeotic transformations that change the number of cervical vertebrae

Further, support for the hypothesis on the conservation of early organogenesis and the selection against duplications and homeotic changes comes from a recent study showing extremely strong selection against changes in the number of cervical vertebrae in humans (Galis et al. 2006). The number of cervical vertebrae is highly conserved and virtually always seven in mammals. This number is determined during early organogenesis. Changes of this number are extremely common and mostly seen as unilateral and bilateral ribs on the seventh vertebra, which implies both a homeotic transformation of the seventh cervical vertebra into a thoracic vertebra, as well as an increase in the number of repeated (duplicated) rib structures (Fig. 3). Rudimentary or complete cervical ribs occur in at least half of deceased fetuses and infants (cf. 0.04–1.1% in adults) and, hence in ~8% of all human conceptions. The large early mortality indicates strong selection against such changes. Selection is indirect and mutations that change the number of cervical vertebrae almost always appear to be associated with multiple, major congenital abnormalities causing mortality in fetuses and infants (Fig. 4 and Table 1). The fact that more than half of all fetal and infant deaths in this study came with cervical ribs emphasizes once again the vulnerability of early organogenesis.

Low effective modularity during the early patterning of the anterior–posterior axis leads to pleiotropic constraints

The determination of the cervico-thoracic boundary of the vertebral column is mediated by *Hox* genes and forms part of the early anterior–posterior patterning of the presomitic mesoderm (Gaunt 1994; Burke et al. 1995; Cohn and Tickle 1999; Chernoff and Rogers 2004; Stern et al. 2006). The association of cervical ribs with multiple and major abnormalities in other parts of the body suggests an interaction of early anterior–posterior patterning with many other patterning and morphogenetic processes.

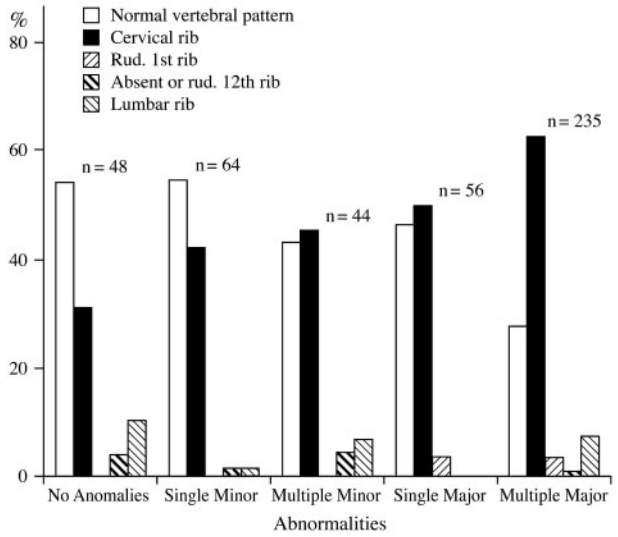

Fig. 4 Graph showing the prevalence of cervical ribs, rudimentary first ribs, rudimentary or absent twelfth ribs and lumbar ribs in fetal and infant deaths with respectively no, single minor, single major, multiple minor, and multiple major abnormalities. The incidence of cervical ribs increases with the number and severity of the abnormalities. Reproduced from Galis et al. (2006).

Corroboration for this viewpoint is, firstly, provided by grafting experiments in which the anterior–posterior position of paraxial mesoderm was altered, leading to changes in (1) the anterior–posterior patterning of the adjacent neuroepithelium (Bel-Vialar et al. 2002; Grapin-Botton et al. 1997; Ensini et al. 1998), (2) the timing of the migration of neural crest cells (Sela-Donenfeld and Kalcheim 2000) and (3) the initiation and outgrowth of the limbs (Saito et al. 2006). Second, this viewpoint is corroborated by experiments in which two processes that are involved in the determination of the anterior–posterior patterning of paraxial mesoderm were manipulated: the opposing and antagonistic gradient of the morphogens Fgfs, Wnts, and Retinoic acid, the oscillatory gene expression (somatic clock) in the paraxial mesoderm. These experiments have demonstrated couplings of the anterior–posterior patterning of paraxial mesoderm with morphogenetic processes such as proliferation and axial lengthening (Dubrulle et al. 2001; Dubrulle and Pourquié 2004), somitogenesis (Dubrulle et al. 2001; Zakany et al. 2001; Cordes et al. 2004), convergent extension (Ninomiya et al. 2004; Mathis et al. 2002) and cell migration (Yang et al. 2002), as well as with patterning along other embryonic axes, i.e. left–right and midline patterning (Raya et al. 2004; Krebs et al. 2003; Latimer et al. 2002; Yamamoto et al. 2003) and dorso-ventral patterning (Diez del Corral et al. 2003). There is thus a wealth of data supporting the precise coordination of the patterning of the three embryonic axes in the three adjacent germ-layers with a central role of the mesoderm in this process (Kumar et al. 2003) and, additionally, there is strong support for a coupling between patterning and morphogenetic processes.

Table 1 Frequent congenital abnormalities in deceased human fetuses and infants (> 10 cases) and associated changes in the number of cervical vertebrae

Congenital abnormality	No. of cases	No. with cervical rib (%)	No. with absent or rudimentary first rib (%)	No. with aberrant number of cervical vertebrae (%)
Cleft lip/palate	12	6 (50%)	3 (25%)	9 (75%)
Horseshoe kidney	10	8 (80%)	1 (10%)	9 (90%)
Bleeding disorders	98	68 (69.4%)	1 (1%)	70 (70.4%)
Oligo/polydactyly	17	9 (52.9%)	4 (23.5%)	13 (76.4%)
Spina bifida	10	4 (40%)	1 (10%)	5 (50%)
Aberrant Arteria Subclavia dextra	22	18 (81.8%)	0 (0%)	18 (81.8%)
Ventricular septum defect	31	17 (54.8%)	8 (25.9%)	25 (80.7%)
Transfusion syndrome	14	8 (57.1)	0 (0%)	8 (57.1%)
Left–right disorders	21	15 (71.4%)	1 (4.8%)	16 (76.2%)
Bilateral kidney agenesis	10	6 (60%)	1 (10%)	7 (70%)
Spina bifida	11	5 (45.5%)	1 (9.1%)	6 (54.6%)
Anal atresia	11	6 (54.5%)	3 (27.3%)	9 (81.8%)
Hydrops foetalis	22	10 (45.5%)	1 (4.5%)	11 (50%)
Dysmaturity	59	33 (55.9%)	0 (0%)	33 (55.9%)
Prematurity	68	39 (57.4%)	2 (2.9%)	41 (60.3%)
Minor (total)	103	42 (40.8%)	0 (0%)	42 (40.8%)
Major (total)	309	173 (56.0%)	14 (%)	182 (58.9%)
Single (total)	112	47 (42.0%)	2 (1.8%)	49 (43.8%)
Multiple (total)	290	159 (54.8%)	8 (2.8%)	167 (57.6%)

Reproduced from Galis et al. (2006).

Duplications of posterior vertebrae, mammae, and phalanges

Lumbar ribs and supernumerary ribs at the first lumbar vertebra occur less frequently in humans than do cervical ribs, but selection against them is not nearly as strong, so they are more frequent in the general population (Galis et al. 2006). The lower frequency of such shifts of the thoraco-lumbar boundary suggests that interference with the determination of this boundary occurs less often than is true of the cervico-thoracic boundary. Absent twelfth ribs also occur less often.

Furthermore, we found no significant association between shifts of the thoraco-lumbar boundary and congenital abnormalities. This suggests that the later stage at which this boundary is determined may be characterized by a lower overall interactivity.

The number of thoracic vertebrae varies considerably amongst mammals (from nine in the Sowerby's beaked whale, *Mesoplodon bidens* to 23 in Linnaeus' two-toed sloth, *Choloepus didactylis*), much more than does the number of cervical vertebrae, which varies from six in manatees (*Trichechus*) and two-toed sloths (*Choloepus*) to nine in three-toed sloths (*Bradypus*, Galis 1999; Narita and Kuratani 2005), and seven in all other mammals. The much weaker selection against shifts of the thoraco-lumbar boundary is, thus, in agreement with the apparently much weaker evolutionary constraint. The number of the more caudal lumbar, sacral, and coccygeal vertebrae also vary considerably among mammals and other vertebrates. The more caudal the vertebrae are, the later the number is specified. We hypothesize that duplications of structures for which the number is determined after the most vulnerable and interactive part of early organogenesis has occurred may be less evolutionarily constrained.

In mice, the period of high vulnerability resulting from global inductive interactions is from embryonic day (E) 7–11, and vulnerability sharply decreases thereafter (Fig. 2). The number of digits is determined within this vulnerable period, but the number of phalanges, carpal, and tarsal elements are determined later (Kimura and Shiota 1995; Ngo-Muller and Muneoka 2000). The number of phalanges, carpal, and tarsal elements is more variable among taxa than is the number of digits, at least as specified during organogenesis (Galis et al. 2001). Evolutionary reduction of the number of digits has happened many times and suggests high variability, but at least in amniotes evolutionary reduction proceeds by developmental arrest, usually followed by degeneration of tissue. Even horses appear to initially have five digit condensations. The strength of the apparent evolutionary constraint, thus, again appears to be in agreement with the timing of specification after the vulnerable and interactive period.

The weaker constraint on variation in the number of cervical vertebrae in birds, compared to mammals, may in part be due to the later stage at which the cervico-thoracic boundary is determined. The higher the number of cervical vertebrae, the later is the determination of the cervico-thoracic boundary, due to the rostro-caudad formation of the somites from which the vertebrae develop. In swans that have the highest number of cervical vertebrae among birds, there is even intraspecific variability of the number of cervical vertebrae and the number varies from 22 to 25 (Woolfenden 1961). Other examples of structures whose number appear to be determined at a relatively later stage are teeth and mammae. Indeed, the number of these structures is highly variable among taxa. It will be interesting to measure the selection strength against duplications of such structures.

On the other hand, the number of most structures (e.g., heart, eyes, ears, lungs, digits, cervical vertebrae, and kidneys, amongst others) is determined early during vulnerable early organogenetic stages and is highly conserved. Changes in numbers of most of these structures are particularly common among deceased fetuses and infants in humans (Galis et al. 2006; Wijnaends and Galis, unpublished data). This suggests that there is strong selection against duplications of these structures.

The breaking of constraints

Taxa-specific pleiotropy associations

The difficulty for the breaking of specific constraints varies among taxa. One reason for this is that the specific pleiotropic effects that are associated with a certain trait will vary for different taxa. For instance, one of the negative pleiotropic effects associated with cervical ribs in humans is childhood cancers. As a result of this association, individuals that are born with a cervical rib and no other observable abnormalities have an estimated chance of 12% to get such a cancer (Galis 1999). This provides a very high selective force. In birds, cancer rates are very low (minimal cancer risk) and much lower than in mammals, and most observed cancers are virally

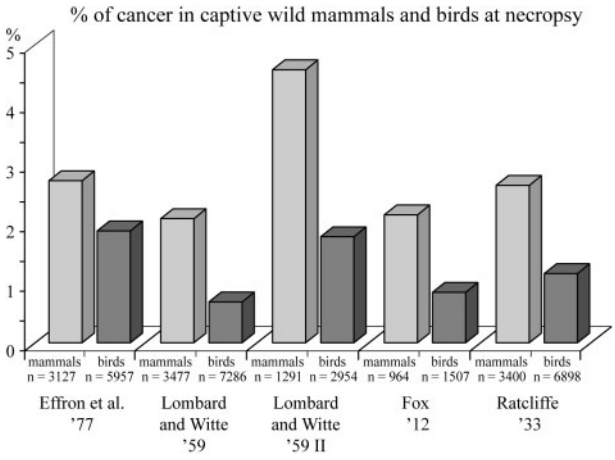

Fig. 5 Necropsy studies of animals from zoos demonstrate a higher cancer incidence in mammals compared to birds. Reproduced with permission from Galis and Metz (2003).

induced (Fig. 5) (Galis 1999; Galis and Metz 2003). We have hypothesized that the lower cancer rate in birds may be implicated in the weaker evolutionary constraint on the cervical vertebrae in birds, in addition to the above-mentioned lower cervico-thoracic boundary. A lower cancer rate may also play a role in fishes, amphibians, and reptiles and in those exceptional mammals that have an aberrant number of cervical ribs: manatees (six cervical vertebrae) and sloths (six to nine cervical vertebrae, Galis 1999). Manatees and sloths stand out among mammals as having an extremely low metabolic rate. The existence of a relation between metabolic rate and oxidative DNA damage and, thus, to cancer (Shigenaga and Ames 1993; Bajra 2004; Valko et al. 2007) suggests, combined with their very low metabolic rate, that their susceptibility for cancer may be low. For manatees, this low susceptibility for cancer has been confirmed (Galis and Metz 2003).

Relaxed selection and the emergence of novelties

Another reason why there is variation in the difficulty of breaking constraints is that there are differences in the history of selection regimes among taxa. Absence of stabilizing selection that normally acts against novelties allows such novelties to persist for some time. Such periods of persistence 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. A good example can be found in the *Semionotus* fishes that invaded newly formed rift lakes in North Eastern America in the late Triassic and early Jurassic and that radiated into a species clade (McCune 1990, 2004). McCune found that in the early history of the lake, when supposedly directional selection was strong but stabilizing selection relaxed (box 18.2, McCune 2004), many dorsal-ridge-scale anomalies occurred. Gradually, these anomalies became less prevalent, but interestingly some of the anomalies became incorporated into new body plans.

Another example that shows how the absence of stabilizing selection can lead to the persistence of characters against which there is normally strongly selection can be found in the evolution of pets. A character that is strongly evolutionarily constrained among amniotes, polydactyly, is particularly common among many dog breeds and some breeds are even required to have one or two extra toes according to the breed standard (Galis et al. 2001). Selection in dogs is relaxed due to human care and dogs with many different congenital abnormalities can breed and reproduce. Longevity is extremely reduced in many breeds, in particular in large breeds, but this does not lead to the extinction of these breeds (Galis et al. 2007). At the same time, directional selection has been very strong in dogs leading to remarkable variation in size and shape. The combination of strong directional selection (for changes in size and shape) and relaxed indirect stabilizing selection (providing food and medical care) has presumably led to the extreme variations in the body plans of dogs.

Periods of relaxed selection may be the colonization of new habitats, the disappearance of predators and the availability of new prey. Such relaxed selection may, thus, be associated with the initial phase of adaptive radiation and with the emergence of key innovations. Directional selection is also expected to be important in such circumstances, when conditions are drastically altered.

We conclude that the importance of directional selection for the evolution of novelties has been overestimated. Directional selection for novelties is important, but only in combination with relaxed selection. The latter is effectively more dominant in determining the options for the evolution of novelties, given the large availability of mutations.

Furthermore, we argue that the importance of hidden variation for the generation of evolutionary novelties has been exaggerated. Hidden variation that becomes exposed in response to severe stress can indeed lead to genetic assimilation, as Waddington has shown for the phenotype of the crossveinless and bithorax mutations in *Drosophila* in his classic experiments (Waddington 1953, 1956, 1961). Hidden directional selection is deemed important as it is usually invoked to explain the often observed differences between laboratory and field data in the effects of an imposed directional selection. It is plausible, however, that these differences will often be due to relaxed stabilizing selection in the laboratory in all directions orthogonal to that of the imposed directional selection, and strong overall stabilizing selection in the field. The above-mentioned strange shapes for which there has been selection in dogs, and also in other pets like pigeons and chickens, show how powerful the effects of directional selection in combination with an otherwise relaxed selection regime can be. Thus, without denying the evolutionary importance of phenotypic plasticity and genetic assimilation (Pigliucci et al. 2006; Chapman et al. 2000; West-Eberhard 2003), we think that for the generation of macro-evolutionary novelties the evidence for the

impact of hidden variation is, thus far, limited (Hansen and Houle 2004).

Conclusions

Duplications are an important source of novelties at all levels of organization of organisms. Despite the high frequency of mutations for duplications such mutations, nevertheless, rarely persist in populations. The persistence problems seem to stem to an important extent from a suboptimal integration of the new unit at a higher level of organization. For the duplication of developmental modules and structures, we suggest that these integration problems are probably mainly due to the interactivity of the patterning of the embryonic axes, and to the interactivity between patterning and morphogenetic processes (including proliferation). Due to this interactivity, mutations cause many negative pleiotropic effects that drastically lower fitness. We argue that this indirect stabilizing selection is the root cause of the selection against novelties and, hence, of the conservation of body plans. Furthermore, we argue that the relaxation of such indirect stabilizing selection, in combination with strong directional selection, is crucial for the evolutionary origin of novelties in body plan.

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