Title
The Generation of Variation and the Developmental Basis for Evolutionary Novelty

Journal
Journal of Experimental Zoology Part B Molecular and Developmental Evolution, 318(6)

ISSN
1552-5007

Authors
HALLGRÍMSSON, BENEDIKT
JAMNICZKY, HEATHER A
YOUNG, NATHAN M
et al.

Publication Date
2012-09-01

DOI
10.1002/jez.b.22448

Peer reviewed

Published in final edited form as:
J Exp Zool B Mol Dev Evol. 2012 September ; 318(6): 501–517. doi:10.1002/jez.b.22448.

The Generation of Variation and The Developmental Basis for Evolutionary Novelty

Benedikt Hallgrímsson,
Dept. of Cell Biology & Anatomy, McCaig Bone and Joint Institute, and the Alberta Children's Hospital Foundation Institute for Child and Maternal Health, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, T2N 4N1

Heather A. Jamniczky,
Dept. of Cell Biology & Anatomy, McCaig Bone and Joint Institute, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, T2N 4N1

Nathan M. Young,
Dept. of Orthopaedic Surgery, University of California, San Francisco

Campbell Rolian,
Dept. of Cell Biology & Anatomy, McCaig Bone and Joint Institute, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, T2N 4N1

Urs Schmidt-Ott, and
Dept. of Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637, USA

Ralph S. Marcucio
Dept. of Orthopaedic Surgery, University of California, San Francisco

Abstract

Organisms exhibit an incredible diversity of form, a fact that makes the evolution of novelty seemingly self-evident. However, despite the "obvious" case for novelty, defining this concept in evolutionary terms is highly problematic, so much so that some have suggested discarding it altogether. Approaches to this problem tend to take either an adaptation or development-based perspective, but we argue here that an exclusive focus on either of these misses the original intent of the novelty concept and undermines its practical utility. We instead propose that for a feature to be novel it must have evolved both by a transition between adaptive peaks on the fitness landscape and that this transition must have overcome a previous developmental constraint. This definition focuses novelty on the explanation of apparently difficult or low probability evolutionary transitions and highlights how the integration of developmental and functional considerations is necessary to evolutionary explanation. It further reinforces that novelty is a central concern not just of evolutionary developmental biology (i.e., "evo-devo") but of evolutionary biology more generally. We explore this definition of novelty in light of four examples that range from the obvious to subtle.

Keywords

Evolutionary novelty; development and evolution; developmental constraint; integration; generation of variation; adaptive landscape

---

The evolution of features such as the tetrapod limb, bird wings/feathers, and the mammalian placenta, present some of the most interesting and challenging questions in evolutionary biology. While each of these examples can be argued to be "novel", there is much disagreement about what exactly this means, and thus whether the concept is useful at all to

evolutionary explanation (Brigandt and Love 2010). On the one hand, some who argue for the novelty concept take the view that some evolutionary explanations require the existence of variation that is different from other kinds of intraspecific variation (Love 2003; Muller and Newman 2005b; Wagner and Lynch 2005; Wagner and Lynch 2010). In this view, novelty arises from variants that are not homologous to any previously existing trait. On the other hand, those arguing against novelty point out that supposedly new traits often are not when viewed at finer temporal, phylogenetic, or developmental scales (Hall and Kerney 2012; Moczek 2008; Shubin et al. 2009).

In this essay, we propose a definition of novelty that incorporates both adaptive and developmental principles. Specifically, we argue that traits can be thought of as novel when they fulfill two criteria. The first is that their evolution involves a transition between adaptive peaks on a fitness landscape (Wright 1932; Wright 1968). The second is that to accomplish this transition, there must be a breakdown of ancestral developmental constraints such that variation is generated in a new direction or dimension.

Our definition of novelty has several advantages. The first is that it applies broadly to the kinds of transitions that are commonly classified as novel, some of which we discuss in more detail below. This is partly because our definition captures the adaptive dimension of novelty that is implicit in most peoples intuitive sense as to what constitutes novel transitions in evolution. The second is that this definition enables unambiguous tests of whether an evolutionary transition is novel when the necessary data are available. Below, we apply our definition to two cases that are fairly unambiguous. These are the evolution of asymmetry and multicellularity. However, we also discuss the applicability of the concept to two cases that are much less straightforward. The first of these is the evolution of the amnioserosa in flies while the second is the diversification of the morphology of the hominoid fore- and hind limb. We argue that this definition of novelty has the advantage that it spans the two levels at which evolutionary novelty is commonly invoked – i.e., the explanation of large scale evolutionary transitions as seen in the fossil record (Jablonski 2005) and the generation of novel variation by alterations of developmental mechanisms. We maintain that it is only when these two levels of inquiry are combined that the definition of novelty can meaningfully contribute to our understanding of some of the most intriguing questions about evolutionary history.

Defining Novelty – Why does it matter?

Evolution has clearly produced, in Darwin's words, "…endless forms most beautiful and most wonderful…" (Darwin 1859). If we label some of these forms "novel" and others not, does it matter whether we know exactly what we mean? Clearly this is not the case if we use the term simply as an informal description of an evolutionary phenomenon. However, the literature suggests that novelty is not used in this way. Evolutionary novelty is the subject of several edited works (including this one) as well as many papers and books (Jernvall 2000; Love 2006; Love and Raff 2006; Muller and Newman 2005a; Muller and Newman 2005b; Muller and Wagner 1991; Pigliucci 2008; Salazar-Ciudad 2006; Shubin et al. 2009; Stone and Hall 2004; Wagner and Lynch 2010), and is frequently used to justify the importance of work proposed in grant submissions. The origin of evolutionary novelty has even been proposed as the central question linking evolution and development (Muller and Newman 2005b; Wagner and Lynch 2010). Given its importance to such a broad spectrum of evolutionary biology, novelty must therefore either have a workable and agreed upon definition or be discarded.

The problem with providing a common definition is that opinions vary widely on what exactly is meant by evolutionary novelty. At one extreme, novelty is broadly equated with a

derived trait (Arthur 2000), while others restrict novelty to exceedingly rare events. In either case, the meaning of novelty is so diluted as to be functionally meaningless. However, proponents of novelty point out that to define away novelty leaves long-standing questions in evolutionary theory largely unaddressed (e.g., Mivart's challenge to Darwin to explain the origins of the mammalian mammary gland) (Muller and Newman 2005a; Muller and Newman 2005b; Muller and Wagner 1991; Wagner and Lynch 2005; Wagner and Lynch 2010). In this view, standard evolutionary theory primarily deals with the interplay of phenotypic variation, selection and drift and so accounts for modifications of existing structures over time (Muller and Newman 2005b), while the origin of wholly new traits is a problem that requires distinct conceptualization and a dedicated research program (e.g., "developmental evolution" (Muller and Newman 2005b; Schlosser and Wagner 2004; Wagner and Mezey 2004). Two key claims are made in this regard: that the basis for novelty is non-homology and that novel variation is qualitatively different other variation. Below we discuss each of these in turn.

Novelty and Non-Homology

The first major claim is that novelties are structures that are neither homologous to a feature in an ancestral lineage nor serially homologous to other phenotypic traits in the same organism (Muller and Wagner 1991). Brigandt (2007) takes this idea further, defining homologues as units of phenotypic evolvability. In his view, novelties arise from the appearance of new units of evolvability. Wagner (2010) makes the additional distinction between definitions of novelty that are functionally based versus those that are developmental or structural. For Wagner, the bird wing has a novel function but would not be a novel structure because it represents a modified limb. This contrasts, with Mayr's definition of novelty which emphasized the novel function and adaptive potential of an evolutionary transition (Mayr 1960; Mayr 1963). Accordingly, the bird wing would be a novelty both because flight is a novel function and because bird diversification occurred subsequent to its evolution. In Wagner and Muller's conception of novelty, the adaptive dimension of novelty, or any consideration of selection and fitness, is excluded from the definition. In Mayr's definition, by contrast, those considerations are front and center.

But when are structures truly non-homologous and thus potentially novel? Feathers are cited as an example of novelty by Wagner (2010), even though feathers are, in a continuity of information sense (Van Valen 1982), homologous with scales. Both feathers and scales initially develop in the same way, as epidermal outgrowths from a placode filled with a dermal core. However, feathers add a major and elaborate developmental step, the epidermal invagination that creates a follicle (Prum 1999; Prum 2005; Prum and Brush 2002). This invagination occurs around the base of the epidermal outgrowth, creating a tubular structure that is contained within a papilla, and enables the subsequent diversification of feather morphology.

So while feathers are clearly very different from scales, it is clear that they are also homologous at a deeper level. Indeed, difference is not a good basis on which to judge homology since many structures that clearly share a continuity of descent from an earlier predecessor are also very different (e.g., the mammalian middle ear). For most, homology rests on the *continuity* of descent or information rather than on the degree of similarity or shared information (Roth 1991). In Wagner's view, homology and novelty are sister concepts such that a novelty transition involves the creation of a new lineage of homology (Wagner 2007). If the definition of feathers as a novelty rests on their non-homology to scales, then it is clear that reasonable scholars can disagree on whether they are, in fact, a novelty.

The feather example highlights a fundamental problem with the non-homology criterion: i.e., it is very difficult to find examples of phenotypic traits that are not derived from some pre-existing features or processes (Moczek 2008). Shubin *et al.* (2009), for example, argue that on closer examination novel structures are usually homologous since they are built on pre-existing regulatory networks, thus the point at which two structures are said to be non-homologous usually becomes a matter of degree rather than a matter of kind.

To take another example to illustrate this point, in Wagner's view the mammary gland is novel because, although it is derived from sebaceous glands, it also develops independently of hair (Wagner and Lynch 2010). Clearly, the disassociation of hair and gland development in the origin of mammary glands was a major developmental step. But, what magnitude and what kind of developmental difference would be necessary to label a structure as novel (see Brigandt and Love (2010))? Again, the claim for non-homology of mammary and sebaceous glands, different as they are, rests on a matter of degree. Taking a similar approach, Hall and Kerney (this issue) argue that most evolutionary transitions that we commonly think of as evolutionary novelty or innovation fail the crucial criterion of non-homology. Important evolutionary transitions that are clearly thought of as "key innovations", such as the turtle shell or dinosaur/bird feathers, for example, fail this criterion for novelty.

Tying novelty to homology in this way has the attractive advantage of conceptual coherence. If, as Wagner (2007) argues, character identity has a basis in the continuity of core regulatory networks, this conceptual pairing of novelty and homology also dovetails with the concept of modularity, in which characters can be thought of as internally interconnected parts within the larger whole (Wagner 2001). The disadvantage of this view is that it does not accommodate (by design) functional or adaptive factors in the conception of novelty (Pigliucci 2008). Such conceptions of novelty were arguably are foremost in the minds of Darwin's critics such as Mivart, just as they are in the minds of first year biology students or non-biologists, when they ask questions such as "how did the first fish start to walk on land?" or "how did the turtle get its shell?" Adaptation also lies at the heart of Mayr's conception of novelty. Thus, the price one pays for the structuralist coherence of the developmental evolution view of novelty is that the non-homology definition restricts it to a set of rare evolutionary events that do not map to our subjective impression of what constitutes a novel evolutionary transition.

Novelty and the Generation of Variation

The second major claim of the developmental evolution program is that the origination of non-homologous entities occurs through variation that is different from the variation that generates variation in homologous structures (Muller and Newman 2005b). While we question the non-homology criterion, we agree that at the origins of novel features, variation must be generated that is outside that normally expressed. Since development either directly or indirectly structures observed variation, it also constrains evolutionary change in two fundamental ways. First, it influences the degree to which genetic or environmental variation is translated into phenotypic variation (Wagner et al. 1997). Second, development influences the correlations among traits, producing patterns of modular and integrated variation (Hallgrímsson et al. 2009; Wagner et al. 2007). Thus any discussion of novelty must explain how new variation is generated via the alteration of development.

Returning to Mivart's challenge or to those puzzled first year biology students' questions about important evolutionary transitions, a key attribute of novelty is the apparent improbability of the transformation, particularly when the "new" variation does not exist in ancestral populations. Thus, the evolution of the tetrapod limb and terrestrial locomotion is not novel because limbs arose *de novo* (they are clearly homologous to the fins of

sarcopterygian fish), but rather because the transition is conceptually difficult to imagine and seemingly improbable given the start and end points. Likewise, while feathers are homologous with scales, their transformation from one to the other is dramatic enough in both form and function to defy simple explanation utilizing existing variation in scales. In the same way, the evolution of flight in birds, bats, and pterosaurs is novel because it seems improbable and hard to explain and not because the wings of those groups are non-homologous to those of other amniotes. The crucial feature that motivates questions about novelty is not the origin of non-homologous features, but rather the explanation of transitions that have a seemingly "you can't get there from here" quality.

An Alternative Conception of Novelty

What features do these "hard to explain" evolutionary transitions have in common and how might they form the basis for an unambiguous definition of evolutionary novelty? We maintain that there are two. The first feature is that they involve a transition from one adaptive peak to another. The transition that feathers made from their original function (whether thermoregulation, display or both) to flight would be such an example. Such transitions need not produce key innovations in that they need not be associated with an explosion of diversity, rather the key feature is a shift from one adaptive context to another —the feature that makes such transitions difficult to explain.

In thinking about transitions from one adaptive peak to another it is important to remember that fitness landscapes are not static. Rather, they depend on the combination of standing variation and the dynamic environmental context in which that variation is expressed. Unfortunately, it is likely impossible to understand these dynamics fully in even the simplest evolutionary contexts. This does not mean, though, that we can't abstract a sufficient understanding to infer that such a transition has occurred. Novelties, in adaptive terms, arise when a functional context changes dramatically from the ancestral state. Fins transition to limbs when their ability to support and propel the body by pushing against the substrate becomes a more important determinant of fitness than their hydrodynamics properties in swimming. The functional context is changed and selection jumps from climbing one adaptive peak to ascending another. Similarly, the upper limb and feathers might have transitioned to a novel functional context when the ability of this combined functional complex to facilitate gliding between branches became a more important determinant of fitness than the display-related or thermoregulatory function of feathers. The ancestral function need not disappear, as it clearly has not in birds, but selection can, in this way, jump from one adaptive peak to the slopes of another.

The second feature is that these transitions involve the generation of variation that is either not present or very rare in the ancestral condition. To appear novel, variation must obviously be very unusual. We argue below that such variation often involves the breakdown of ancestral developmental constraints that limit the variation that is normally expressed. In this latter criterion, we agree with others who have argued that a key to novelty lies in the generation of variation (Brigandt In Press; Fusco 2001; Muller and Newman 2005a; Muller and Wagner 1991). Unlike some other others, however, we are not arguing that the origination of novel variation requires a different class of mechanistic changes to development.

Based on these common features, we thus propose that novel evolutionary transitions are those that involve both a transition from one adaptive peak to another and from one canalized developmental trajectory to another (Fig. 1). Selection can only drive the mean phenotype uphill on an adaptive landscape. Therefore, such jumps between peaks occur when the landscape evolves. A useful concept in thinking about this is the selection gradient,

which is a measure of the independent effect of selection on specific characters (Arnold 1983). To return to our example of the tetrapod limb, the landscape must change such that the selection gradients for characters associated with limb functions must come to exceed those for characters associated with fin functions. In our simple schematic figure, we show hypothetical adaptive landscapes for a two dimensional phenotype (x,y) at three time points. Adaptive landscapes for complex phenotypes with more dimensions are much more complicated than this. As Arnold (2003) has argued, evolution in such complex "landscapes" proceeds along lines of least resistance. For transitions to occur that appear to jump from one peak to another over time, the landscape must evolve for such "lines of least resistance" to create a path, at least transiently, from one peak to the other.

The other side of our definition of novelty involves overcoming developmental constraints. If we think of the latter issue in terms of Waddington's metaphorical epigenetic landscape of developmental trajectories, this might involve a transition from one valley to another – i.e., from one canalized developmental trajectory to another (Fig. 1B). Importantly, neither the transitions between peaks in an adaptive landscape nor between valleys in Waddington's epigenetic landscape need to involve saltational change in phenotype, *as envisioned by* Goldschmidt (1940). At the origin of a novel feature, however, variation must appear that is different from that which was present before.

A limitation of our definition of novelty is that it relies on two concepts, which although theoretically quantifiable, are most often used as metaphors. This does not mean that the definition cannot be applied in an unambiguous and meaningful way. In the next section, we discuss several examples of novel variation in light of the "breakdown of developmental constraints" and adaptive shit criteria. These examples are chosen to be both straightforward (evolution of asymmetry and multicellularity) and more problematic (the amnioserosa in flies and the diversification of hominoid positional behaviour). The reationale behind the two more marginal examples is to explore the limits and utility of this definition of evolutionary novelty.

1. Evolution of Asymmetry

Planes of symmetry can be viewed as extreme examples of developmental constraints. For symmetrical structures, for which there may often be a small amount of fluctuating asymmetry (random deviations from symmetry) there is no genetic basis for the direction of deviation from symmetry in all but a few exceptional cases (Palmer 2005). This means that for such structures there is apparently no genetic variation through which natural selection can drive the evolution of an asymmetric phenotype. Despite this, asymmetric morphologies, sometimes strikingly so, have evolved multiple times from symmetrical ancestors in at least eight different phyla (Palmer 2005). Palmer has argued that the key to this puzzle is antisymmetry. Antisymmetry occurs when one side always differs in size, shape, or some other property, from the other but the direction of the asymmetry deviation is random. Antisymmetry is fairly common in nature and when it occurs the direction of asymmetry is virtually never heritable. In the vast majority of cases, the asymmetry is heritable but the direction of deviation is not. By contrast, in species that exhibit conspicuous directional asymmetry, in which the direction of the deviation is not random, the direction of asymmetry is almost always heritable. Palmer argues that antisymmetric variation very often precedes the evolution of directional asymmetry and that the evolution of asymmetric phenotypes commonly occurs via genetic assimilation (Palmer 2004). Genetic assimilation is where environmentally induced variation becomes developmentally canalized and thus acquires a genetic basis (Palmer This Issue; Waddington 1942; Waddington 1956a; Waddington 1956b; West-Eberhard 2003). Once assimilated, the direction of asymmetry becomes heritable and can be acted on by natural selection. In this way, environmentally induced antisymmetric variation can overcome the absence of

variation in asymmetry, which is a clear developmental constraint for the evolution of asymmetric phenotypes.

Are all cases of directional asymmetry thus evolutionary novelties? Those cases in which there is a clear adaptive shift certainly meet the bar. The asymmetric body form and cranial shape of pleuronectiform fish (flatfish) are good examples of this (Fig. 2). In this case, there is a heritable overcoming of the developmental constraint to produce a left-right symmetrical body (the absence of genetic variation for directional asymmetry in symmetrical structures) and with the transition from a pelagic to a bentic lifestyle a relationship to a major adaptive shift. To a first approximation, the evolution of asymmetric phenotypes from symmetrical ancestors appears to generate a consistent definition of evolutionary novelty.

Change in Dimensionality and the Origin of Multicellularity

Perhaps the most fundamental example of overcoming developmental constraints is the generation of variation in a dimension that did not previously exist. One way to think about this is in terms of potential phenotypic spaces or morphospaces (Raup 1966; Schindel 1990). Morphospaces, of course, are defined by their axes and are blind to variation that is not captured by a dimension of the space (Polly 2008). Raup's classic shell coiling morphospace, for example, is defined by a mathematical model of a coil. Morphospaces are arbitrary in this sense because it is up to the observer to define the axes that capture variation of interest. In an interesting exception, Rice (1998) redefined Raup's morphospace mollusk shells in terms of underlying developmental determinants. Imagine that one could devise a morphospace that captured all of the parameters of phenotypic variation for some organism and that an evolutionary change might occur which added a dimension to this known and complete set of parameters. The transition from a single celled organism to a multicellular form is an example of such an addition. For single celled organisms, the parameter "cell number" would not exist. For multicellular life, however, it does.

The origin of multicellularity is a commonly accepted example of evolutionary novelty. The key developmental change underlying the transition is the appearance of cell adhesion molecules which allow cells to stick to one another (Minelli 2003). The appearance and elaboration of junctional complexes, which allow communication among cells, and the developmental mechanisms of signaling among cells occur subsequently to this fundamental prerequisite for multicellular life (Hutter et al. 2000). Interestingly, cell adhesion molecules such as integrins and cadherins are expressed in unicellular choanoflagellates (King et al. 2003) as well as cyanobacteria (Flores and Herrero 2010), which may be one reason that multicellularity has evolved several times independently (Abedin and King 2010). Nonetheless, there is a considerable developmental constraint to overcome for this transition to occur. Even if the molecular basis for cell adhesion is present in an ancestor, there must be variation in degree of cell aggregation for selection to drive the evolution of multicellularity. For aggregates of cells to produce multicellular structures with morphology, further constraints must be overcome. Minelli (2003) has argued that basic multicellular morphology such as spheres or sheets of cells can arise via spatial differentiation of expression of cell adhesion molecules on the cell membrane. The origin of multicellular morphology, if he is correct, would therefore require the appearance of genetic variation in the spatial configuration of cell adhesion on the cell surface.

So, how does the origin of multicellularity fit with our definition of evolutionary novelty? It is clear that these transitions when they occurred involved a shift from one adaptive peak to another. The adaptive landscape of a multicellular structure with morphology is obviously very different from that of a single celled organism. To become part of a larger whole, cells must sacrifice their individual fitness and become part of a cooperative with others

(Hochberg et al. 2008). Interestingly, the gap between these adaptive peaks has been crossed several times and in both directions (Sachs 2008; Schirrmeister et al. 2011).

The developmental constraint argument is somewhat less straightforward. It is not clear that the variation that is required for cell adhesion and morphological variation is absent from single celled ancestors. Cell adhesion is certainly present in such forms (Sachs 2008). Spatial organization of cell adhesion, underlain by cell polarity, may also be present, but multicellularity requires that the spatial organization of cell adhesion is coordinated across cells (Mikhailov et al. 2009). Interestingly, functional specialization has also evolved quite rapidly in experimental evolution of multicellularity in yeast (Ratcliff et al. 2012). For morphological structures to develop, however, the polarity of the composing cells must be coordinated (Bryant and Mostov 2008). In addition, the cell division must be coordinated, requiring some form of communication among cells. In other words, although the basic parameters necessary for multicellularity may be present in a unicellular ancestor, their variation structure must change significantly. Variation must arise that involves the coordination of the building blocks of multicellularity in functional ways that are only relevant once multicellular structures have appeared. This change in variation structure, essentially opening up new dimensions of potential morphospace is a form of overcoming of developmental constraint.

The Amnioserosa in Schizophoran Flies

The examples discussed in the previous two sections would meet most investigators expectation of evolutionary novelty. The next two sections will consider examples that are more ambigupous. We consider these examples because for the novelty concept to be useful in scientific discourse, it must be possible to distinguish evolutionary transitions that result in evolutionary novelties from those that do not. The first of these is the appearance of the amnioserosa in schizophoran flies (Rafiqi et al. 2008; Rafiqi et al. 2010).

The amnioserosa is an extraembryonic epithelium. It guides important morphogenetic movements of the developing embryo (germband retraction, dorsal closure) but is resorbed in the yolk when the flanks of the germband meet along the dorsal midline. At the first glance, the amnioserosa appears to lack a clear-cut homologous tissue in other insects. Those consistently develop two distinct extraembryonic epithelia, called amnion and serosa, each of which sharing some aspect with the amnioserosa. As with other apparent evolutionary novelties, the question is how to get “from here to there”.

In close relatives of the schizophoran flies, including syrphids and phorids, the serosa abuts the inner side of the eggshell, like in other insects, and the amnion closes the germband dorsally, like an amnioserosa, rather than ventrally as in many more basal insects (primitive condition; Fig. 3A) (Panfilio 2008; Schmidt-Ott et al. 2010). Dorsal amnion and amnioserosa not only share the same topology but also a similar function in germband retraction and dorsal closure. However, the specification of dorsal amnion and amnioserosa differs. In this regard the amnioserosa resembles the serosa rather than the amnion. In Schizophora (e.g. *Drosophila*), amnioserosa specification is fully dependent on the homeobox gene *zerknüllt* (*zen*). Yet, in related species with a serosa and an amnion, *zen* strictly functions as serosa determinant (Rafiqi et al. 2008; van der Zee et al. 2005) (Fig. 3B). The critical and specific requirement of *zen* in amnioserosa on the one hand and serosa development on the other does not imply that the amnioserosa develops into a serosa, or hybrid amnion-serosa tissue. Rather, genetic data indicate that it develops into an amnion. This is not in contradiction with with the use of zen in amnioserosa specification because the expression of *zen* in the amnioserosa is downregulated after gastrulation, and the tissue is then maintained by ‘amnion genes’ (e.g. genes of the u-shaped group). In contrast, the serosa-specific expression of *zen* of non-schizophoran insects continues after gastrulation

and thereby maintains the serosal identity of this tissue (Rafiqi et al. 2010). The schizophoran amnioserosa therefore finally develops into a tissue, which by topological, functional, and genetic criteria, is comparable (and homologous) to the dorsal amnion of schizophoran outgroups, while serosal tissue does not differentiate in Schizophora.

From what has been said, one might conclude that the amnioserosa may not qualify as an evolutionary novelty. However, the origin of the amnioserosa may very well match our criteria of an evolutionary novelty by reflecting the collapse of an important developmental constraint and the shift towards a new adaptive peak. The formation of a cuticle-secreting serosa epithelium is obviously highly constrained given that nearly all insects form this tissue, but in schizophoran flies, this constraint must have been relaxed, possibly by a compensatory change in the eggshell. The collapse of the constraint that maintains the serosa in most insects, allowed a novel type of variation, the developmental trajectory of the amnioserosa, which may have rendered embryonic development more efficient in different ways. For example, the developmental trajectory of the amnioserosa reduced the extraembryonic portion of the developing egg. Furthermore, it simplified the formation of the extraembryonic tissue that supports germband retraction and dorsal closure because it neither involves the disjunction of amnion and serosa tissues nor the reorientation of the free edge of the developing amnion (Fig. 3B).

The depiction of amnioserosa development as an adaptive trait towards a new optimum in the adaptive landscape is speculative, but it is consistent with phylogenetic pattern. Within insects, schizophoran flies clearly show a pattern of elevated speciation and low extinction (Wiegmann et al. 2011). Schizophora account for the 30% of the diversity of flies and roughly 3% of all animal diversity, and the beginning of the ‘schizophoran explosion’ coincides with the origin of the amnioserosa. In summary, by our criteria the amnioserosa may well be considered an important evolutionary novelty that contributed to the diversification of Schizophora, whereas a strictly homology-based definition of evolutionary novelty would miss it as such.

Limb integration and the diversification of hominoid positional behaviour

The last case deals with an example on a much smaller evolutionary scale – the diversification of hominoid limb morphology and positional behaviour over the last 30 million years. Tetrapod fore- and hind limbs are linked in interesting ways. This is because the two pairs of limbs are serially homologous but vary widely among species in the extent to which their functions are similar. Due to the duplicated origin of limbs, an event that appears to have occurred in basal gnathostomes (Shubin et al. 1997), the basic developmental pattern of the forelimb is replicated in the hind limb. That transition, like the origins of the pectoral limb, would clearly qualify as an evolutionary novelty by our definition.

Importantly for this discussion, despite the vast evolved diversity of limb morphology since the establishment of the basic tetrapod pattern, the echo of this ancient duplication event can still be observed in the shared developmental processes and anatomical pattern of limbs (Shubin et al. 1997), and is reflected in the common limb covariation structure of highly derived tetrapods (Hallgrímsson et al. 2002; Rolian 2009; Rolian et al. 2010; Young and Hallgrímsson 2005; Young et al. 2010b). For example, covariation between serially homologous elements of fetal mouse limbs (i.e., stylopod, zeugopod, autopod) is higher than the covariation among different elements within the same limb (Hallgrímsson et al. 2002). Interestingly, this primitive covariation pattern can be modified, strengthened or weakened by selection, presumably in both directions. Mammalian species with divergent hind and forelimb function and proportions (such as bats, gibbons and humans) have lower interlimb covariances than those in which the limbs perform more similar functions (such as cursorial

quadrupeds) (Young and Hallgrímsson 2005). Nonetheless, the tendency for the serially homologous structures of the tetrapod limb to covary is a powerful developmental constraint that can drive correlated evolutionary changes in one limb as a result of selection on the other (Rolian et al. 2010).

Anthropoid primates (monkeys, apes and humans) are a particularly interesting example in this regard because hominoids (apes and humans) exhibit a dramatic diversification of divergence in limb morphology and function from a more committed quadrupedal monkey ancestor. Within hominoids, function varies from ricochetal brachiation in gibbons and siamangs, to slow, quadrumanous climbing in orangutans, to knuckle-walking in the African apes, and committed bipedalism in humans and our hominin ancestors (Fig. 4). This functional diversification was accompanied by substantial variation in both the relative proportions of the hind limb and forelimb and diversification of hand and foot morphology. Interestingly, hominoids exhibit reduced covariation between homologous elements in the forelimb and hind limb compared to old world monkeys (Young et al. 2010b) (Fig. 4). This suggests that weakened integration between limbs occurred early in hominoid evolution, perhaps as a result of selection for divergent limb function. As covariance was reduced, selection could then more readily produce changes in one limb and not the other, further facilitating diversification of differences in hind and forelimb function and morphology. In other words, the relaxation of a developmental constraint on the generation of variation precipitated later evolutionary diversification in hominoids.

By the developmental constraint criterion, the phenotypic transformations that led to the peculiar human foot, or to the morphological complexes associated with brachiation, or knuckle-walking (Tuttle 1967; Tuttle 1972) would qualify as evolutionary novelties. The phenotypic transformations of hominoid limb morphology and positional behaviour also involved significant adaptive shifts, crossing from one adaptive peak to another. Clearly the adaptive context for bipedalism, tool use, brachiation and knucklewalking are all quite different and selection on these adaptive complexes is in the direction of very different optima or adaptive peaks. So, are the unusual forelimbs and hind limbs of humans and our closest relatives examples of evolutionary novelty?

Clearly these evolutionary transformations are on a much smaller scale than the origin of the tetrapod limb or of multicellularity. That said, like those more extreme examples, they do share our two criteria for novelty – the breakdown of developmental constraint on the generation of variation and the shift to a different adaptive peak. In this case, selection must overcome the tendency for the hind and forelimb to covary rather than the complete absence of variation in the direction of the evolutionary change (as in the case of loss of symmetry). If these are examples of evolutionary novelty, that broadens the definition to include transformations at different evolutionary scales and this makes the appearance of novelty a fairly common feature of evolutionary history. Even so, most evolutionary change would not be novel since it tends to proceed within the confines of developmentally constrained morphospaces (Polly 2008).

Overcoming Constraint: What does that mean?

The examples discussed in this paper illustrate very different ways and scales in which the origin of novel traits involves the breakdown in developmental constraints. But what does that mean in developmental terms? In the examples discussed superficially here, the developmental basis for constraint is very different. Defined phenomenologically, constraint is simply the absence of the *generation* of variation in a particular direction within morphospace. Importantly, it is the direction of variation that matters here and not the unoccupied regions of space. If there is no variation in a particular direction of morphospace (e.g. deviation from symmetry), selection cannot produce evolutionary change in that

direction. A region of space that is unoccupied by phenotypic variation may be unoccupied because of functional rather than developmental constraints. The morphometric comparison of the morphospace occupied by domestic dog breeds compared to the morphospace of all carnivores illustrates this point beautifully (Drake and Klingenberg 2010). This result shows that carnivore craniofacial morphology is functionally constrained and that artificial selection can take it into large regions of morphospace that are unoccupied in nature. Some regions, however, are undoubtedly unoccupied because of developmental rather than immediate functional constraints. The amnioserosa case illustrates the complexity of distinguishing between both—the developmental path of the dorsal amnion cannot be changed to an amnioserosa path unless the constraint of making a functional serosa is relaxed. Thought of this way, a developmental change that produces generation of variation in novel direction is required for the evolution of novelty.

This conception of novelty ties the concept closely to developmental integration. Variation that follows integrated axes of variation is not novel whereas variation that breaks down such axes is. One can envision this occurring initially through a relaxation of integration-based constraints within a species, followed by movement of variation in a new direction (away from the integrated axes of variation) resulting in occupation of a new area of morphospace. This might be followed by reintegration and establishment of new axes of integrated variation in the newly occupied area of morphospace. This is shown schematically in Figure 6.

This idea is related to Wagner and Stadler's (2003) theoretical treatment of morphospaces. In their view, novel characters are new factors of variation in a factorized phenotype space. The establishment of novel integrated axes of variation corresponds formally to new factors in such a space.

Going beyond this phenomenological conception of constraint is difficult. A deeper conceptualization of constraint is certainly implicit in the Muller and Wagner non-homology based definition of novelty (Muller and Newman 2005b; Wagner and Lynch 2010). As we argued, above, this conceptualization of novelty is too restrictive to capture what is generally meant by novelty in evolution. There is, however, a weaker but somewhat related conceptualization of the developmental basis for constraint that may provide an alternative. Alberch (1982; 1989), in arguing for the need for a "theory of form", argued that developmental constraints are underlain by developmental interaction rules. This focus on regularities of interaction in complex epigenetic systems is a common thread in current thinking about the relationship of development to evolution (Newman and Muller 2000; Salazar-Ciudad 2010; Salazar-Ciudad et al. 2003). The examples we discuss above are all illustrations, at different scales, in which modification of interactions in development that alter the way in that variation is expressed. The challenge is that because any change in development will alter an interaction of some kind, if Alberch's concept is to be useful, then we must understand what is meant by the "rules" of developmental interaction.

A potential answer to that question lies in the topology of developmental systems. To the extent that complex systems exhibit deeply entrenched interactions that are both deeply embedded within networks and reoccurring throughout the network, they exhibit "rules" or "regularities." The recurrent interactions found among members of "genetic toolkits" as well as the modular nature of developmental interaction networks more generally illustrate that such regularities exist (Carroll et al. 2005; Wagner et al. 2007). Importantly, these interaction regularities reside not just at the gene level but also at higher, epigenetic, levels of developmental systems (Hallgrímsson and Hall 2011; Jamniczky et al. 2010). If such regularities represent constraints, overcoming these constraints must involve alterations to conserved features of genetic and epigenetic networks. In our conception, the developmental

basis for evolutionary novelty therefore involves overcoming entrenched regularities in developmental interaction networks to generate new variation for selection to act upon.

Discontinuities in the Generation of Variation

An intuitive aspect of evolutionary novelty as discussed in many contexts is discontinuity in the generation of phenotypic variation, i.e., the "you can't get here from there" problem. This is implicit in the non-homology criterion of Muller and Wagner (1991). Discontinuity in the generation of variation is not one of our criteria for evolutionary novelty. Given our starting point of Mivart's challenge, applying this criterion would exclude many evolutionary transitions that would be covered by the original intent of the concept such as the fin-limb transition or the origin of wings. That said, discontinuity is a common feature of evolutionary transitions that most would consider to be novel. As Polly (2008) points out, discontinuous phenotypic variation need not imply discontinuous variation in the underlying developmental parameter space. This last section reviews recent work on the developmental mechanisms that underlie the generation of phenotypic variation and what it can tell us about how discontinuous phenotypic variation can be generated from continuous variation in underlying developmental parameters.

Sonic hedgehog (Shh) signaling performs diverse functions in development. One of these myriad functions is in the regulation of the outgrowth of the vertebrate midface (Hu and Marcucio 2009a; Marcucio et al. 2005). Marcucio and colleagues have shown in a series of experiments that Shh signaling in the forebrain sets up a signaling center in the adjacent surface ectoderm, called the Frontonasal Ecodermal Zone (FEZ), which also expresses SHH (Fig. 5). The FEZ regulates gene expression patterns (Hu and Marcucio 2009b) and cell proliferation rates in the facial mesenchyme. Together these cellular processes produce outgrowth of the midface away from the brain and regulate patterning of the skeletal elements (Hu et al. 2003). Given the central role of Shh signaling in regulating outgrowth, it seems reasonable to expect that this might also be a source of phenotypic variation in the extent of facial outgrowth and thus in the shape of the face.

Young et al. (2010a) recently tested this hypothesis in an experiment that perturbed Shh signaling in the forebrain. First, Shh signaling was reduced in the forebrain by injecting the neural tube with a graded series of cells that express an immunoneutralizing anti-SHH antibody (5E1) Second, Shh signaling in the forebrain was increased by placing beads soaked in different concentrations of SHH-N protein into the forebrain. These exogenous treatments designed to influence Shh signaling from the forebrain produced remarkable results (Fig 5). Variation in Shh signaling produced a range of phenotypic variation in the face that resembled the range of phenotypes observed in humans with altered Shh signaling. These outcomes spanned varying degrees of midfacial hypoplasia and hypotelorism when the pathway was reduced to medial clefting and hypertelorism when the pathway was activated. The mid-portion of this range encompasses normal variation. Over most of the phenotypic range produced by the Shh treatments, the phenotypic variation is continuous. At the upper extreme, however, a midfacial cleft appears. This discontinuous transformation, however, is underlain by continuous variation in Shh expression in the FEZ. The discontinuity reflects the presence of a threshold in the underlying parameter space. As Shh signaling in the brain increases, the anatomical width of the Shh expression domain in the FEZ also increases until it becomes divided into two domains on either side of the face. This alters the position of the cell proliferation zones in the mesenchyme and produces growth in lateral regions of the face, which confers a mammalian-like morphology on the avian face. As a result of this altered growth the facial prominences do not come together and merge at the midline (Fig. 5). This example illustrates well Polly's (2008) point about discontinuous phenotypic variation corresponding to continuous variation in underlying developmental

parameter space at some level – in this case the amount and location of Shh expression in the forebrain.

So how does Shh signaling in a chick model relate to understanding the developmental basis for evolutionary novelty? Clearly this example is flawed in two important ways. The first is that the variation that is generated by varying Shh signaling is dysmorphic—at least at both extremes. This is less of a flaw than seems at first blush. Naturally occurring variation that occurs at extreme deviations from the mean is also often dysmorphic. In the Shh signaling example, the central portion of the range does resemble normal variation and the power of this example in that it illustrates the continuous gradation of normal to dysmorphic variation at both extremes in terms of underlying determinants. The extent to which variation in Shh signaling, size, shape and timing of the Shh domains in the brain and in the ectoderm correspond to interspecific variation in embryonic facial shape is a subject of current work in the Marcucio and Hallgrímsson labs. The other flaw is that this variation is generated by manipulating a single variable – Shh signaling in the brain. The variation on which natural selection acts to manipulate the morphology of the midface or upper jaw is undoubtedly much more complicated than this. Still the Shh pathway may have an important role here. More important, though, is the observation that manipulating the rate of a key developmental process, cell proliferation in the midface, in relation to other variables, produces a large and integrated axis of morphological variation in the face. Such key developmental processes that map onto significant integrated axes, defined here as a correlated set of changes in a suite of variables, may well be common in complex developmental systems. If so, then this reveals developmental routes through which large, potentially discontinuous and apparently novel phenotypic variation can be generated.

Detecting Evolutionary Novelty

Our definition of novelty rests on two metaphorical landscapes, the epigenetic and adaptive. Recognizing novelty in past evolutionary transitions, by this definition, requires that we infer fitness landscapes from the past and recognize developmental constraints in developmental systems that are no longer available for experimental analysis. Neither of these things can be done directly, of course. For adaptive landscapes, the first issue is one scale. Like real landscapes, fitness landscapes are likely rougher when viewed up close with multiple small peaks and valleys existing within the range of variation of a single species (Coyne et al. 1997). Evolutionary novelty, however, involves major features of the landscape and not these smaller features and this introduces an element of arbitrariness in the definition. Our suggestion is that these large-scale adaptive changes are characterized by a change in functional context. These are situations when selection related to one function (or group of functions) becomes replaced or outweighed by selection for another function. Note that this does not require that a population descends one adaptive peak and then ascends another, as is implicit in Wright's shifting balance theory (Wright 1969; Wright 1982). Our definition is completely agnostic on that important and complex theoretical issue in population genetics. It rests, rather, on the functions that are the underlying determinants of fitness landscapes. The adaptive criterion for evolutionary novelty is that a function or set of functions replaces another. The resultant shift from one adaptive peak to another is inferred from the inference about a functional shift.

The developmental constraint criterion can be tested in several ways and there is a large literature that deals with this question (Richardson and Chipman 2003' Maynard Smith, 1985 #136; Schwenk 1994). Wagner and Misof (1993) make the distinction between generative and morphostatic constraints. Generative constraints refer to constraints on the ability of developmental systems to produce variation in the first place. Morphostatic constraints are produced when there are secondary mechanisms in place that limit variation

produced by some other aspect of development. The constraint-breaking that occurs in novel evolutionary transformations involves both of these types of *constraints*. Generative constraints are presumably more difficult to break, but both represent limits on the production of variation that is due to the architecture of developmental systems. Selective constraints, even if acting early in development, are not part of the constraint-breaking criterion. Selective constraints are not produced by developmental architecture per se, but rather the functional context in which development plays out.

How can these types of constraints be detected? Most importantly, such analyses must be done in a phylogenetic context. This is because developmental constraints limit evolutionary transformations and not some abstract notion of static morphospace (Schwenk 1994). Just because a morphospace appears filled when the data from many species are plotted does not mean that the set of evolutionary transformations that connect those species and their ancestors were not influenced by developmental constraints. Those may emerge when the pattern of transformations within the morphospace is analyzed. If possible, selection experiments can be conducted within a phylogenetic context. The inability to produce a specific direction of transformation in an ancestral group when that direction of transformation is possible in a descendent is evidence that constraint-breaking has occurred. Similarly, experimental manipulation of systems to generate variation, as we have done with Shh expression and facial morphology in chicks (Marcucio et al. 2011; Young et al. 2010a), is a powerful way to explore the generative capacity and limits in developmental systems. Finally, analysis of intraspecific covariation patterns and their relationship to evolutionary transformations or the interspecific pattern of covariation is a less direct but powerful and often practically applicable method to detect the presence of developmental constraints.

In an interesting paleobiological example of this approach, Webster and Zelditch (2011) compare the integration of direct and parallel development effects in the Trilobite cranidium to evolutionary divergence in morphology. They use Klingenberg's (2005) method which uses the integration of fluctuating asymmetry to measure direct developmental effects. Although they find that both direct and indirect integration patterns in the Trilobite cranidium are not terribly conserved, their method shows how hypotheses about developmental constraints and constraint-breaking can be tested even in developmental systems as poorly understood as those of Trilobites.

Conclusion

The concept of evolutionary novelty must have a meaningful definition in order to become a tractable and worthwhile object of evolutionary inquiry. We have proposed here a definition that anchors the concept to the adaptive landscape on the one hand and developmental constraints and the epigenetic landscape on the other. In doing this, we lay out an agenda for investigating novelty in evolution. Although this agenda leaves many important questions only superficially addressed, we hope that future work will flesh out the explanatory framework proposed in this paper and attempt to apply our definition to more concrete evolutionary examples.

Unlike the more restrictive homology-based definition, ours makes the appearance of novelty a fairly common evolutionary phenomenon. This definition of novelty, for example, would allow for multiple occurrences of evolutionary novelty within mammalian evolution. Most evolutionary change, however, is not novel by this definition as it tends to not involve significant changes in functional contexts or jumps from one adaptive peak to another and it tends to fall along developmentally constrained regions of morphospace. The extent to which this claim is true, however, is an empirical question that bears directly on the relative roles of development and function as determinants of evolutionary change. Whether this

concept of novelty is useful or not will also depend on how it relates to the questions that are of key interest to evolutionary biologists. A significant advantage of this definition may well be that it contributes to a conceptual foundation for paleobiological, especially quantitative, approaches that attempt to address broader questions about the roles of intrinsic and extrinsic factors in macroevolution. Such applications would be a strong validation of the concept of novelty as proposed here. They would also move questions of evolutionary novelty back to their rightful place at the center of evolutionary inquiry.

Acknowledgments

This work is supported by National Science and Engineering Council (NSERC), Grant #238992-11and an NSERC RTI grant to BH and National Institutes of Health (USA) grants 1R01DE01963 and 1R01DE021708 to RM and BH and RM and BH and F32DE018596 to NMY. CR and HAJ were supported by the Alberta Innovates, Health Solutions post-doctoral fellowships. We thank Ingo Brigandt and Alan Love for organizing the novelty symposium that inspired us to think about this topic and which resulted in this paper. We thank Brian Hall, David Jablonski, Gunter Wagner as well as all of workshop participants, represented in this volume, for fruitful and enjoyable discussion. BH was supported to participate in the Novelty Symposium through Social Sciences and Humanities Research Council of Canada (Grant 410-2008-0400) to Ingo Brigandt.

References Cited

Abedin M, King N. Diverse evolutionary paths to cell adhesion. Trends Cell Biol. 2010; 20(12):734–742. [PubMed: 20817460]

Alberch, P. Developmental constraints in evolutionary processes. In: Bonner JT, editor. Development in Evolution. Springer-Verlag; Berlin and New York: 1982. p. 313-332.

Alberch P. The logic of monsters: Evidence for internal constraint in evolution. Geobios, memoire special. 1989; 12:21–57.

Arnold SJ. Morphology, Performance and Fitness. Am Zool. 1983; 23(2):347–361.

Arnold SJ. Performance surfaces and adaptive landscapes. Integrative and Comparative Biology. 2003; 43(3):367–375. [PubMed: 21680445]

Arthur W. Intraspecific Variation in Developmental Characters: The Origin of Evolutionary Novelties. Am Zool. 2000; 40:811–818.

Brigandt I. Typology now: homology and developmental constraints explain evolvability. Biology and Phiosophy. 2007; 22:709–725.

Brigandt, I. From developmental constraint to evolvability: how concepts figure in explanation and disciplinary identity. In: Love, AC., editor. Conceptual Change in Biology: Scientific and Philosophical Perspectives on Evolution and Development. Springer; Berlin: In Press

Brigandt I, Love A. Evolutionary Novelty and the Evo-Devo Synthesis: Field Notes. Evolutionary Biology. 2010; 37(2):93–99.

Bryant DM, Mostov KE. From cells to organs: building polarized tissue. Nat Rev Mol Cell Biol. 2008; 9(11):887–901. [PubMed: 18946477]

Carroll, SB.; Grenier, JK.; Weatherbee, SD. From DNA to Diversity. Blackwell Publishing; Malden, MA: 2005.

Coyne JA, Barton NH, Turelli M. Perspective: A critique of Sewall Wright's shifting balance theory of evolution. Evolution. 1997; 51(3):643–671.

Darwin, C. The Origin of Species (reprinted 1975). Avenel; New York: 1859.

Drake AG, Klingenberg CP. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. Am Nat. 2010; 175(3):289–301. [PubMed: 20095825]

Flores E, Herrero A. Compartmentalized function through cell differentiation in filamentous cyanobacteria. Nat Rev Microbiol. 2010; 8(1):39–50. [PubMed: 19966815]

Fusco G. How many processes are responsible for phenotypic evolution? Evol Dev. 2001; 3(4):279–286. [PubMed: 11478525]

Goldschmidth, R. The material basis of evolution. Yale University Press; New Haven and London: 1940.

Hall BK, Kerney R. Levels of biological organization and the origins of novelty. Journal of Experimental Zoology Part B-Molecular and Developmental Evolution. 2012

Hallgrímsson, B.; Hall, BK. Epigenetics: The context of development. In: Hallgrímsson, B.; Hall, BK., editors. Epigenetics: Linking Genotype and Phenotype in Development and Evolution. University of California Press; Berkeley: 2011. p. 424-438.

Hallgrímsson B, Jamniczky H, Young N, Rolian C, Parsons T, Boughner J, Marcucio R. Deciphering the Palimpsest: Studying the Relationship Between Morphological Integration and Phenotypic Covariation. Evolutionary Biology. 2009; 36(4):355–376. [PubMed: 23293400]

Hallgrímsson B, Willmore K, Hall BK. Canalization, developmental stability, and morphological integration in primate limbs. Yearbook of Physical Anthropology. 2002; 45:131–158.

Hochberg ME, Rankin DJ, Taborsky M. The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity. BMC Evol Biol. 2008; 8:238. [PubMed: 18713461]

Hu D, Marcucio RS. A SHH-responsive signaling center in the forebrain regulates craniofacial morphogenesis via the facial ectoderm. Development. 2009a; 136(1):107–116. [PubMed: 19036802]

Hu D, Marcucio RS. Unique organization of the frontonasal ectodermal zone in birds and mammals. Dev Biol. 2009b; 325(1):200–210. [PubMed: 19013147]

Hu D, Marcucio RS, Helms JA. A zone of frontonasal ectoderm regulates patterning and growth in the face. Development. 2003; 130(9):1749–1758. [PubMed: 12642481]

Hutter H, Vogel BE, Plenefisch JD, Norris CR, Proenca RB, Spieth J, Guo C, Mastwal S, Zhu X, Scheel ÄJ, et al. Conservation and Novelty in the Evolution of Cell Adhesion and Extracellular Matrix Genes. Science. 2000; 287(5455):989–994. [PubMed: 10669422]

Jablonski D. Evolutionary innovations in the fossil record: the intersection of ecology, development, and macroevolution. J Exp Zool B Mol Dev Evol. 2005; 304(6):504–519. [PubMed: 16161067]

Jamniczky HA, Boughner JC, Rolian C, Gonzalez PN, Powell CD, Schmidt EJ, Parsons TE, Bookstein FL, Hallgrímsson B. Rediscovering Waddington in the Post-Genomic Age. Bioessays. 2010

Jernvall J. Linking development with generation of novelty in mammalian teeth. Proc Natl Acad Sci U S A. 2000; 97(6):2641–2645. [PubMed: 10706636]

King N, Hittinger CT, Carroll SB. Evolution of Key Cell Signaling and Adhesion Protein Families Predates Animal Origins. Science. 2003; 301(5631):361–363. [PubMed: 12869759]

Klingenberg, C. Developmental Constraints, Modules and Evolvability. In: Hallgrímsson, B.; Hall, BK., editors. Variation: A Central Concept in Biology. Elsevier; New York: 2005. p. 219-247.

Love A. Evolutionary morphology and Evo-devo: Hierarchy and novelty. Theory in Biosciences. 2006; 124(3):317–333. [PubMed: 17046363]

Love AC. Evolutionary Morphology, Innovation, and the Synthesis of Evolutionary and Developmental Biology. Biology and Philosophy. 2003; 18(2):309–345.

Love AC, Raff RA. Larval ectoderm, organizational homology, and the origins of evolutionary novelty. J Exp Zoolog B Mol Dev Evol. 2006; 306(1):18–34.

Marcucio RS, Cordero DR, Hu D, Helms JA. Molecular interactions coordinating the development of the forebrain and face. Dev Biol. 2005; 284(1):48–61. [PubMed: 15979605]

Marcucio RS, Young NM, Hu D, Hallgrímsson B. Mechanisms that underlie covariation of the brain and face. Genesis. 2011

Mayr, E. The emergence of evolutionary novelties. In: Tax, S., editor. Evolution after Darwin, Vol 1: The Evolution of Life: Its Origin, History, and Future. The University of Chicago Press; Chicago: 1960. p. 349-380.

Mayr, E. Animal Species and Evolution. Belknap Press; Cambridge, MA: 1963.

Mikhailov KV, Konstantinova AV, Nikitin MA, Troshin PV, Rusin LY, Lyubetsky VA, Panchin YV, Mylnikov AP, Moroz LL, Kumar S, et al. The origin of Metazoa: a transition from temporal to spatial cell differentiation. Bioessays. 2009; 31(7):758–768. [PubMed: 19472368]

Minelli, A. The Development of Animal Form: Ontogeny, Morphology, and Evolution. Cambridge University Press; Cambridge: 2003.

Moczek AP. On the origins of novelty in development and evolution. Bioessays. 2008; 30(5):432–447. [PubMed: 18404691]

Muller GB, Newman SA. Editorial: evolutionary innovation and morphological novelty. J Exp Zoolog B Mol Dev Evol. 2005a; 304(6):485–486.

Muller GB, Newman SA. The innovation triad: an EvoDevo agenda. J Exp Zoolog B Mol Dev Evol. 2005b; 304(6):487–503.

Muller GB, Wagner GP. Novelty in evolution: restructuring the concept. Ann Rev Ecol Syst. 1991; 22:229–256.

Newman SA, Muller GB. Epigenetic mechanisms of character origination. J Exp Zool. 2000; 288(4): 304–317. [PubMed: 11144279]

Norman, JR. I. Psettodidae, Bothidae. Oxford University Press; London: 1934. A Systematic Monograph of the Flatfishes (Heterosomata).

Palmer AR. Asymmetry Breaking and the Evolution of Development. Science. 2004; 306(828):833.

Palmer, AR. Antisymmetry. In: Hallgrímsson, B.; Hall, BK., editors. Variation: A Central Concept in Biology. Elsevier Academic Press; New York: 2005. p. 359-397.

Palmer AR. Developmental plasticity and the origin of novel forms: unveiling of cryptic genetic variation via ‘use and disuse’. Journal of Experimental Zoology (Molecular and Developmental Evolution). This Issue.

Panfilio KA. Extraembryonic development in insects and the acrobatics of blastokinesis. Dev Biol. 2008; 313(2):471–491. [PubMed: 18082679]

Pigliucci M. What, if Anything, Is an Evolutionary Novelty? Philosophy of Science. 2008; 75:887–898.

Polly P. Developmental Dynamics and G-Matrices: Can Morphometric Spaces be Used to Model Phenotypic Evolution? Evolutionary Biology. 2008; 35(2):83–96.

Prum RO. Development and evolutionary origin of feathers. J Exp Zool. 1999; 285(4):291–306. [PubMed: 10578107]

Prum RO. Evolution of the morphological innovations of feathers. J Exp Zool B Mol Dev Evol. 2005; 304(6):570–579. [PubMed: 16208685]

Prum RO, Brush AH. The evolutionary origin and diversification of feathers. Q Rev Biol. 2002; 77(3): 261–295. [PubMed: 12365352]

Rafiqi AM, Lemke S, Ferguson S, Stauber M, Schmidt-Ott U. Evolutionary origin of the amnioserosa in cyclorrhaphan flies correlates with spatial and temporal expression changes of zen. Proc Natl Acad Sci U S A. 2008; 105(1):234–239. [PubMed: 18172205]

Rafiqi AM, Lemke S, Schmidt-Ott U. Postgastrular zen expression is required to develop distinct amniotic and serosal epithelia in the scuttle fly Megaselia. Dev Biol. 2010; 341(1):282–290. [PubMed: 20144604]

Rafiqi AM, Lemke S, Schmidt-Ott U. The scuttle fly Megaselia abdita (Phoridae): a link between Drosophila and Mosquito development. Cold Spring Harb Protoc 2011:pdb emo143. 2011

Ratcliff WC, Denison RF, Borrello M, Travisano M. Experimental evolution of multicellularity. Proceedings of the National Academy of Sciences. 2012

Raup DM. Geometric analysis of shell coiling. J Paleont. 1966; 40:1178–1190.

Rice S. The bio-geometry of mollusc shells. Paleobiology. 1998; 24:133–149.

Richardson MK, Chipman AD. Developmental constraints in a comparative framework: a test case using variations in phalanx number during amniote evolution. J Exp Zool B Mol Dev Evol. 2003; 296(1):8–22. [PubMed: 12658708]

Rolian C. Integration and Evolvability in Primate Hands and Feet. Evolutionary Biology. 2009; 36(1): 100–117.

Rolian C, Lieberman DE, Hallgrímsson B. The Co-Evolution of Human Hands and Feet. Evolution. 2010

Roth VL. Homology and hierarchies: Problems solved and unresolved. J Evol Biol. 1991; 4:167–194.

Sachs JL. Resolving the first steps to multicellularity. Trends Ecol Evol. 2008; 23(5):245–248. [PubMed: 18375012]

Salazar-Ciudad I. On the origins of morphological disparity and its diverse developmental bases. Bioessays. 2006; 28(11):1112–1122. [PubMed: 17041901]

Salazar-Ciudad I. Morphological evolution and embryonic developmental diversity in metazoa. Development. 2010; 137(4):531–539. [PubMed: 20110318]

Salazar-Ciudad I, Jernvall J, Newman SA. Mechanisms of pattern formation in development and evolution. Development. 2003; 130(10):2027–2037. [PubMed: 12668618]

Schindel, DE. Unoccupied morphospace and the coiled geometry of gastropods: Architectural constraints or geometric covariation. In: Moss, RM.; Allmon, WD., editors. Causes of Evolution: A palaeontological persepective. The University ot Chicago Press; Chicago and London: 1990. p. 270-304.

Schirrmeister BE, Antonelli A, Bagheri HC. The origin of multicellularity in cyanobacteria. BMC Evol Biol. 2011; 11:45. [PubMed: 21320320]

Schlosser, G.; Wagner, GP. Introduction: The Modularity Concept in Developmental and Evolutionary Biology. In: Schlosser, G.; Wagner, GP., editors. Modularity in Development and Evolution. The University of Chicago Press; Chicago and London: 2004. p. 1-11.

Schmidt-Ott U, Rafiqi AM, Lemke S. Hox3/zen and the evolution of extraembryonic epithelia in insects. Adv Exp Med Biol. 2010; 689:133–144. [PubMed: 20795328]

Schwenk K. A Utilitarian Approach to Evolutionary Constraint. Zoology-Analysis of Complex Systems. 1994; 98(4):251–262.

Shubin N, Tabin C, Carroll S. Fossils, genes and the evolution of animal limbs. Nature. 1997; 388(6643):639–648. [PubMed: 9262397]

Shubin N, Tabin C, Carroll S. Deep homology and the origins of evolutionary novelty. Nature. 2009; 457(7231):818–823. [PubMed: 19212399]

Stone JR, Hall BK. Latent homologues for the neural crest as an evolutionary novelty. Evol Dev. 2004; 6(2):123–129. [PubMed: 15009125]

Tuttle RH. Knuckle-Walking and the evolution of hominid hands. Am J Phys Anthropol. 1967; 26:171–206.

Tuttle, RH. Functional and evolutionary biology of Hylobatid hands and feet. Karger Basel; Gibbon and Siamang: 1972. p. 136-206.

van der Zee M, Berns N, Roth S. Distinct functions of the Tribolium zerknullt genes in serosa specification and dorsal closure. Curr Biol. 2005; 15(7):624–636. [PubMed: 15823534]

Van Valen LM. Homology and causes. J Morphol. 1982; 173(3):305–312. [PubMed: 7186550]

Waddington CH. The canalisation of development and the inheritance of acquired characters. Nature. 1942; 150:563.

Waddington CH. Genetic assimilation of the bithorax phenotype. Evolution. 1956a; 10:1–13.

Waddington CH. The genetic basis of the ‘assimilated bithorax’ stock. J Genet. 1956b; 55:241–245.

Wagner, GP. Characters, Units and Natural Kinds. In: Wagner, GP., editor. The Character Concept in Evolutionary Biology. Academic Press; New York: 2001. p. 1-13.

Wagner GP. The developmental genetics of homology. Nat Rev Genet. 2007; 8(6):473–479. [PubMed: 17486120]

Wagner GP, Booth G, Bagheri-Chaichian H. A population genetic theory of canalization. Evolution. 1997; 51(2):329–347.

Wagner GP, Lynch VJ. Molecular evolution of evolutionary novelties: the vagina and uterus of therian mammals. J Exp Zool B Mol Dev Evol. 2005; 304(6):580–592. [PubMed: 16252266]

Wagner GP, Lynch VJ. Evolutionary novelties. Curr Biol. 2010; 20(2):R48–52. [PubMed: 20129035]

Wagner, GP.; Mezey, JG. The role of genetic architecture constraints in the origin of variational modularity. In: Schlosser, G.; Wanger, GP., editors. Modularity in Development and Evolution. The University of Chicago Press; Chicago and London: 2004. p. 338-358.

Wagner GP, Misof BY. How can a character be developmentally constrained despite variation in developmental pathways. Journal of Evolutionary Biology. 1993; 6(3):449–455.

Wagner GP, Pavlicev M, Cheverud JM. The road to modularity. Nat Rev Genet. 2007; 8(12):921–931. [PubMed: 18007649]

Wagner GP, Stadler PF. Quasi-independence, homology and the unity of type: a topological theory of characters. J Theor Biol. 2003; 220(4):505–527. [PubMed: 12623283]

Webster M, Zelditch ML. Evolutionary Lability of Integration in Cambrian Ptychoparioid Trilobites. Evolutionary Biology. 2011; 38(2):144–162.

West-Eberhard, MJ. Developmental plasticity and evolution. Vol. xx. Oxford University Press; Oxford; New York: 2003. p. 794

Wiegmann BM, Trautwein MD, Winkler IS, Barr NB, Kim JW, Lambkin C, Bertone MA, Cassel BK, Bayless KM, Heimberg AM, et al. Episodic radiations in the fly tree of life. Proc Natl Acad Sci U S A. 2011; 108(14):5690–5695. [PubMed: 21402926]

Wright S. The Roles of Mutation, Inbreeding, Crossbreeding and Selection in Evolution. International Congress of Genetic. 1932; 7:356–366.

Wright, S. Evolution and the genetics of populations; a treatise. Vol. 4. University of Chicago Press; Chicago: 1968.

Wright, S. Evolution and the Genetics of Populations: Volume 2 The Theory of Gene Frequences. The University of Chicago Press; Chicago: 1969.

Wright S. The Shifting Balance Theory and Macroevolution. Annu Rev Genet. 1982; 16:1–19. [PubMed: 6760797]

Young NM, Chong HJ, Hu D, Hallgrímsson B, Marcucio RS. Quantitative analyses link modulation of sonic hedgehog signaling to continuous variation in facial growth and shape. Development. 2010a; 137(20):3405–3409. [PubMed: 20826528]

Young NM, Hallgrímsson B. Serial homology and the evolution of mammalian limb covariation structure. Evolution. 2005; 59(12):2691–2704. [PubMed: 16526515]

Young NM, Wagner GP, Hallgrímsson B. Development and the evolvability of human limbs. Proc Natl Acad Sci U S A. 2010b; 107(8):3400–3405. [PubMed: 20133636]

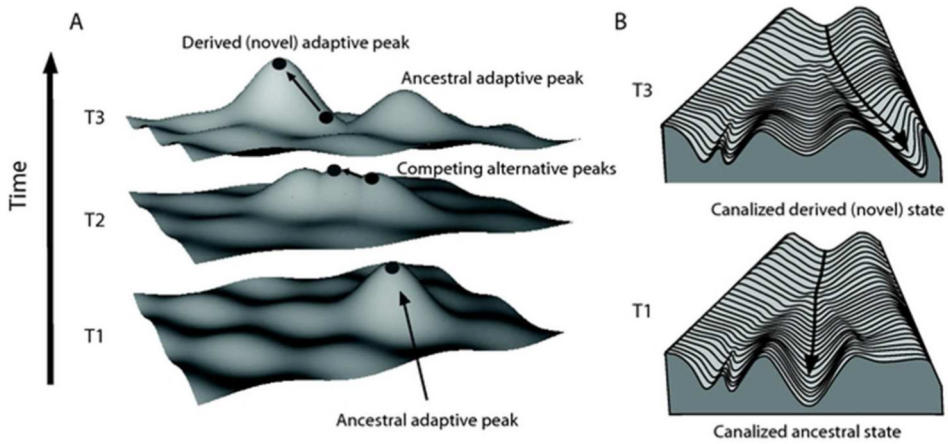

Figure 1.

Evolutionary novelty involves both a transition from one adaptive peak to another and a transition from one canalized state to another. A shows three hypothetical adaptive landscapes at different times. In T1, an optimum has been reached within a particular functional context. In T2, an alternative functional context has arisen which is an equally important determinant of fitness. The ridge between the two peaks at this time point is intended to capture a "path of least resistance" between the two functional contexts. In T3, the alternative functional context has become a more important determinant of fitness than the ancestral functional context, producing a new adaptive peak. The transition from one adaptive peak to another is shown here with an intermediate stage, but this does not necessarily occur (see text). B shows two alternative epigenetic landscapes corresponding to T1 and T3. The transitions between these states can be gradual or discontinuous depending on the particular developmental context. The landscape evolves between T1 and T3 such that the constraint preventing the derived state is removed. The derived state in T3 is also canalized (the valley is deepened), although this would presumably occur secondary to the breakdown of the constraint (the ridge between the two states).

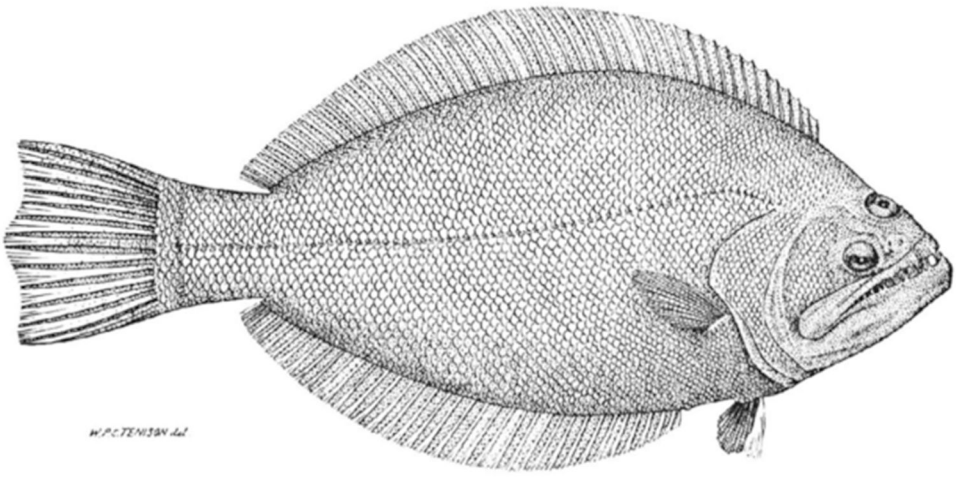

Figure 2.
Drawing of *Psettodes erumei* illustrating directional asymmetry. From Norman, (1934).

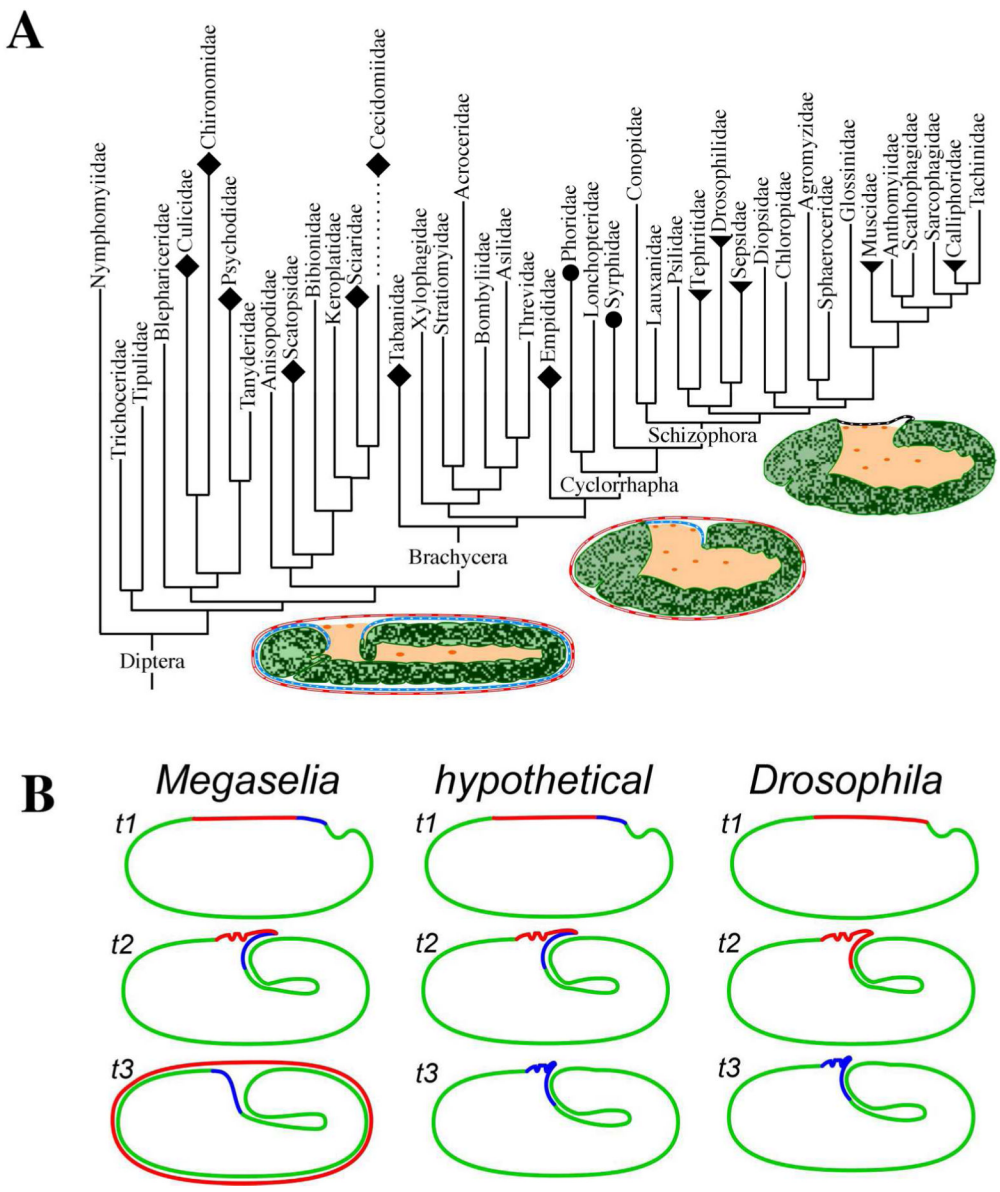

Figure 3.

Amnioserosa evolution in flies. **(A)** Phylogenetic occurrence of ventral amnion closure (squares), dorsal amnion closure (circles), and amnioserosa (triangles) are shown together with embryo sketches depicting the embryos in green with serosa (red) and amnion (blue), or amnioserosa (black) (modified after Rafiqi et al. (2011)). The phylogram is based on Bayesian likelihood analysis of 14 concatenated nuclear genes, full mitochondrial genomes, and 371 morphological features (Wiegmann et al. 2011). (**B**) Evolution of the amnioserosa in relation to expression of the serosa-determining gene *zen*. Sketches of fly embryos with dorsal amnion closure (*Megaselia*) or amnioserosa (*Drosophila*) are shown at consecutive developmental stages with anterior to the left and dorsal up. *zen* positive extraembryonic tissue is depicted in red, *zen* negative extraembryonic tissue in blue, and embryonic tissue in green. The hypothetical intermediate type depicts a developmental path in which serosa formation is suppressed at the expense of an enlarged amnion in response to the repression of late *zen* expression, while leaving serosa (*zen* positive, red) and amnion specification (*zen*

negative, blue) during early development unaffected (modified after Rafiqi et al. 2008 and Rafiqi et al. 2010).

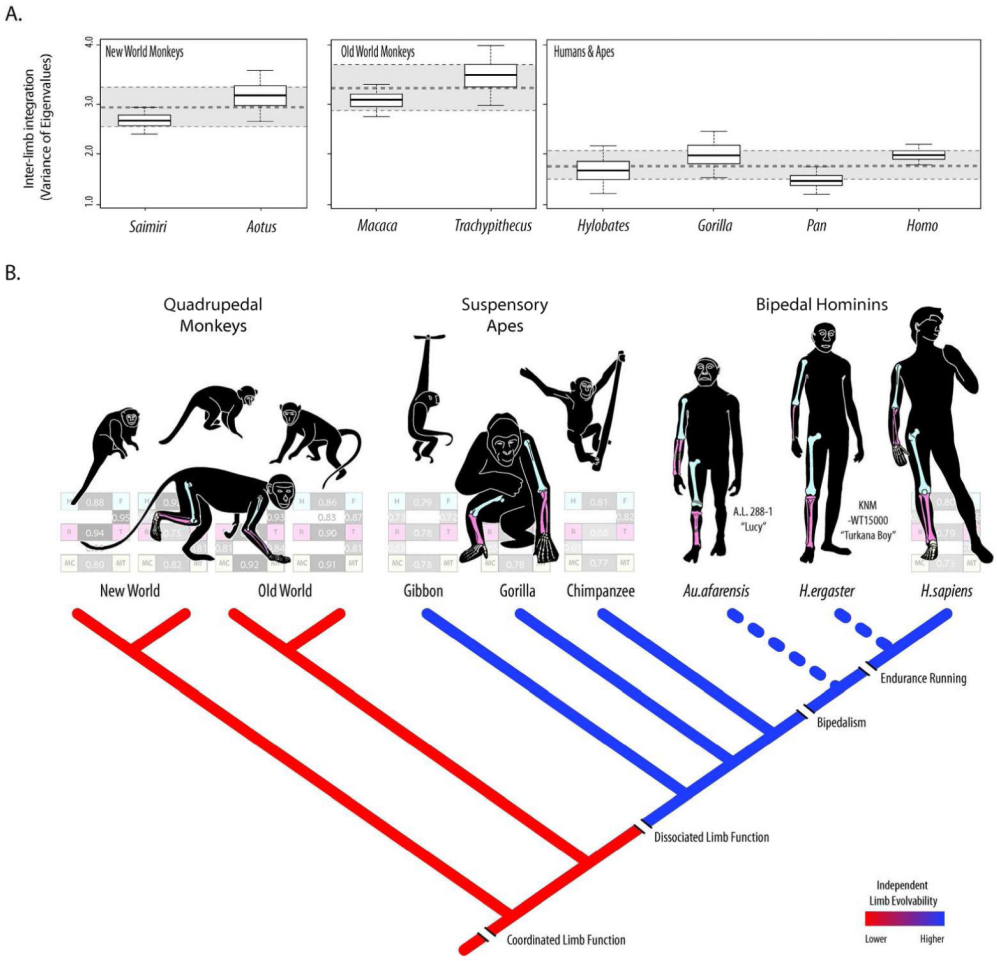

Figure 4.

Interlimb morphological integration in select hominoids, old world monkeys and new world monkeys. After correction for the variance-dependence of integration, hominoids exhibit significantly reduced interlimb integration compared to old and new world monkeys (Young et al. 2010b). The upper portion of the figure shows the scaled variances of eigenvalues for the species shown. The lower portion shows schematic representations of the positional behaviour of these species as well as the commonly accepted phylogeny. The long legs and short arms of humans are distinctive among anthropoid primates and reflect adaptations for bipedalism and endurance running. Although developmental constraints can cause serially homologous structures like limbs to evolve in concert, the fossil record shows that these traits evolved mosaically in fossil hominins. Evidence for a developmental shift from monkeys to apes indicates that this divergent evolution of modern human limb proportions was likely facilitated by selection to reduce shared genetic effects across homologous limb elements, thus increasing their independent evolvability (Illustration by Nathan M. Young).

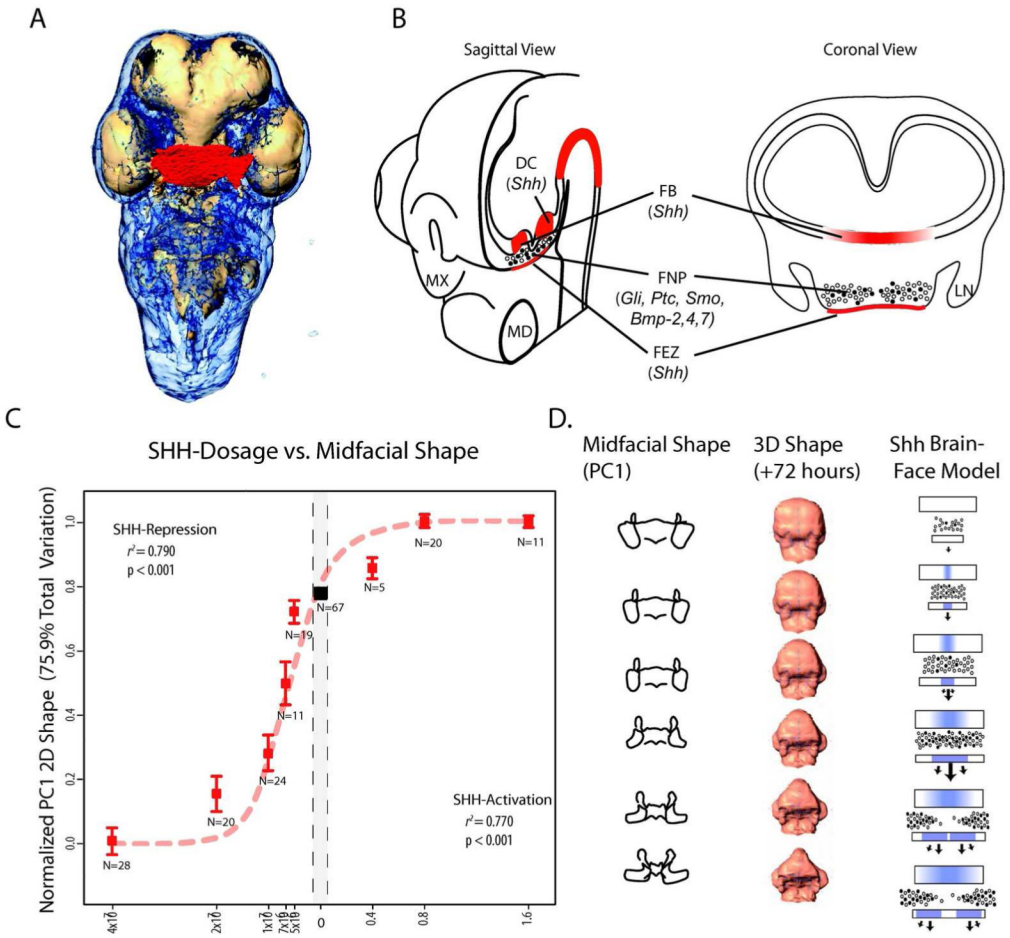

Figure 5.

Manipulation of Sonic hedgehog (Shh) expression in the chick and the generation of continuous and discontinuous morphological variation. A shows an optical projection tomography scan of a chick wholemount *in situ* preparation for Shh expression. Shh expression has been cropped to show only the FEZ region. B shows a schematic representation of Shh expression at this developmental time, illustrating the regions of expression in the forebrain and the FEZ. C and D show the results of the experiment reported in Young *et al.* (2010a), which is discussed in the text. D shows 2D wireframe deformations and 3D morphs for the first principal component from a Principal Components Analysis of the geometric morphometric dataset generated in this experiment. E shows a schematic representation of the model that is discussed in this text which explains the generation of both continuous variation and the threshold at which the discontinuous midfacial cleft is produced.

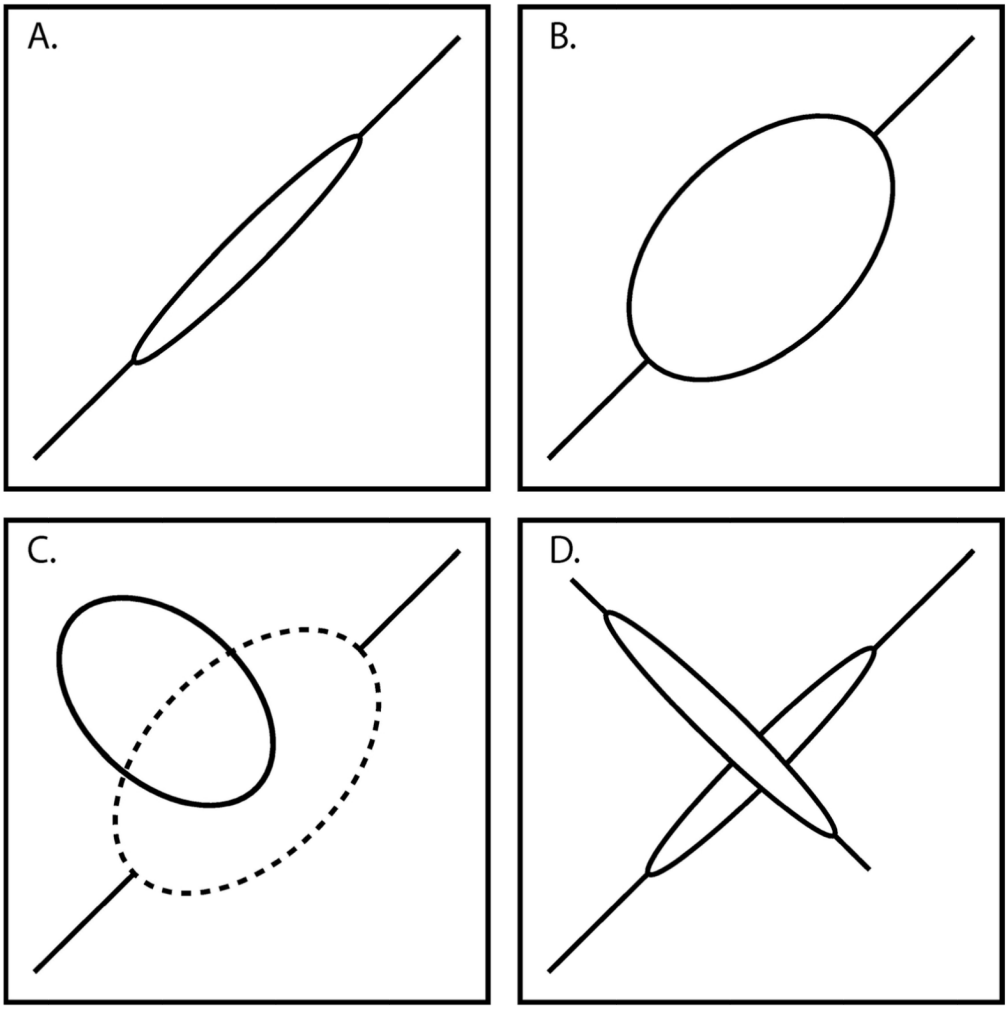

Figure 6.

Integration and Evolutionary Novelty. Breakdown of developmental constraints may usually involve the following steps, starting from an integrated axis of variation as shown in A for a two dimensional trait. In B, there is relaxation of integration, possibly resulting from selection in a direction orthogonal to the integrated axis. In C, this selection has shifted the mean into a new area of morphospace and broken down the earlier pattern of covariation. In D, variation is reintegrated in the new area, in this case orthogonal to the original axis of integration, although that need not be the case.