Limb, tooth, beak: Three modes of development and evolutionary innovation of form

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The standard model of evolutionary change of form, deriving from Darwin's theory via the Modern Synthesis, assumes a gradualistic reshaping of anatomical structures, with major changes only occurring by many cycles of natural selection for marginal adaptive advantage. This model, with its assertion that a single mechanism underlies both micro- and macroevolutionary change, contains an implicit notion of development which is only applicable in some cases. Here we compare the embryological processes that shape the vertebrate limb bud, the mammalian tooth and the avian beak. The implied notion of development in the standard evolutionary picture is met only in the case of the vertebrate limb, a single-primordium organ with morphostatic shaping, in which cells rearrange in response to signalling centres which are essentially unchanged by cell movement. In the case of the tooth, a single-primordium organ with morphodynamic shaping in which the strengths and relationships between signalling centres is influenced by the cell and tissue movements they induce, and the beak, in which the final form is influenced by the collision and rearrangement of multiple tissue primordia, abrupt appearance of qualitatively different forms (i.e. morphological novelties) can occur with small changes in system parameters induced by a genetic change, or by an environmental factor whose effects can be subsequently canalized genetically. Bringing developmental mechanisms and, specifically, the material properties of tissues as excitable media into the evolutionary picture, demonstrates that gradualistic change for incremental adaptive advantage is only one of the possible modes of morphological evolution.

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1. Introduction

Modifications in form that occur in embryos and regenerating organs are typically driven by changes in gene expression. But gene expression changes can only elicit transitions between forms inherent to the materials within which they occur. Morphological development is, thus, not simply a phenomenon of the functioning of genes or gene networks, or even the behaviour of individual cells. Rather, it involves *groups* of cells – cell clusters and tissues (Gilbert *et al.* 1996). Gene expression changes serve mainly to mobilize the physical processes and effects characteristic of the middle-scale or 'mesoscopic' state of matter constituted by these cell collectives (Forgacs and Newman 2005).

Because cell clusters and tissues have self-organizing properties, they inherently exhibit stereotypical forms (Newman and Müller 2005). By analogy, we can think of the waves and vortices displayed by liquid water when it is agitated, or the ripples in windswept sand. Similarly, although in a more complex fashion, developing embryos generate structural features – morphological motifs (Newman 2012) – as a consequence of their chemical composition and physical state (Newman and Müller 2000; Forgacs and Newman 2005).

The existence of self-organizing and excitable cell clusters and tissues was not part of the concept of evolution by natural selection as conceived by Darwin and Wallace and carried forward by the Modern Evolutionary Synthesis. As we have argued previously, the fact that cell aggregates and tissues exhibit a finite range of intrinsic forms is antithetical to the idea of natural selection as a creative force in evolution (Linde-Medina 2010a; Newman and Linde-Medina 2013). Instead (as also noted

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by Depew and Weber 1996), the living matter of the conventional model is passive, non-intrinsically ordered: its forms would not be predictable outcomes of its material properties.

Because cells in animal tissues can be independently mobile and can slip past one another, these tissues can have the properties of highly viscous liquids, with the cells playing a role analogous to a liquid's molecules. Liquids, whether nonliving or living, can be simple or complex. If the units (molecules or cells) are nonpolar in shape and surface properties, the default morphology of the aggregate will be a sphere devoid of internal spaces ('solid' in the topological sense), like a liquid droplet. If the cell units represent two populations with sufficiently different affinities for one another, a phase-separating system, with non-mixing layers, will emerge (Steinberg 2007). Tissues can also behave analogously to a nematic liquid crystal if their cells elongate and interdigitate. In such cases, the tissue tends to elongate in the direction orthogonal to the long axis of the cells (Keller *et al.* 2008). Alternatively, if the cells are polarized with respect to their surface properties, spaces can form within the tissue, as in vesicles and micelles that assemble from polar molecules (Tsarfaty *et al.* 1992).

The cytoskeleton and some extracellular matrices (ECMs), in which the cells are embedded (if mesenchymes) or reside upon (if epithelia), give elastic properties to tissues. Furthermore, the ability of cell collectives to store chemical and mechanical energy enable developing tissues to generate gradients and strains, as well as to respond to various types of signals in an active fashion: they are what physicists term *excitable media* (Levine and Ben-Jacob 2004).

To illustrate the causal role of material properties in the generation of biological form, we will discuss three examples of increasing developmental and morphological complexity in vertebrate morphogenesis – the pre-skeletal tetrapod limb bud, the mammalian tooth and the avian beak (figure 1). Each of these organs develops from embryonic buds, i.e. parcels of mesenchyme encapsulated in epithelial tissue. However, the mechanisms by which these buds are shaped during embryogenesis differ significantly. The emergence and shaping of the limb bud has been explained principally by the properties of mechanically excitable media and the dynamics of viscous fluids (Hopyan *et al.* 2011; Zhang *et al.* 2013). Tooth three-dimensional (3D) shapes have been modelled based on the properties of chemically excitable liquid-like media (Salazar-Ciudad and Jernvall 2010). For the bird beak, we introduce the rationale of a new model (Linde-Medina *et al.*, in preparation), which includes, in addition to the viscoelastic properties of the individual buds, inter-bud interactions in the generation of the beak shape, since in contrast to the other organs, the beak develops from more than a single bud.

We then apply this perspective to the question of how morphological variation is generated in the course of evolution. The simplest morphogenetic mode considered, namely the shaping of the limb bud (before the appearance of the endoskeleton), allows only for the emergence of quantitative changes in size and shape of the same basic structure. The 'variational properties' of the limb bud (i.e. the changes it can sustain as a result of genetic change; Salazar-Ciudad *et al.* 2003; Salazar-Ciudad and Jernvall 2005) thus enable the gradual and continuous mode of evolution of the standard view. The modes of morphogenesis of the tooth and the beak, for different reasons that will be discussed below, are additionally capable of generating discontinuous, qualitative morphological changes in response to incremental genetic modifications or to environmental alterations whose effects on development can be subsequently consolidated by genetic changes. These modes of morphogenesis can, thus, promote abrupt structural transitions (or saltations) not contemplated by the standard evolutionary model. These examples provide insights into the origination of *morphological novelties* (Peterson and Müller 2013) (in the case of the tooth, new cusps; in the case of the beak, a different type), a category of phenomena that lies outside the domain of the standard evolutionary view, which focuses on the continuous remodelling of structures (Müller and Newman 2005).

2. Limb bud outgrowth and shaping: A morphostatic single-primordium system

The limb buds of birds and mammals emerge from the embryonic body wall, or flank, under the influence of a diffusible morphogen, fibroblast growth factor 8 (FGF8), secreted by a narrow strip of epithelium that runs anteroposteriorly (AP) along the limb bud tip (i.e. from thumb to little finger). In these vertebrates, the FGF8-secreting epithelium is noticeably thickened and it is called the apical ectodermal ridge (AER) (figure 2). In the chicken, FGF8 transforms the prospective limb mesenchyme into a more cohesive tissue than the flank mesenchyme from which it is derived. This process causes it to phase-separate from the adjacent flank tissue by a physical process akin to the separation of oil and water when they are present in the same container (Damon *et al.* 2008). Since the flank mesenchyme is less cohesive than the limb mesenchyme, it would be expected to engulf the latter, as occurs with immiscible liquids of different cohesivities. But the flank tissue also exhibits an active mechanical response to this tendency, causing it to expel the limb mesenchyme, which, consequently, protrudes from the body wall as a bud (Damon *et al.* 2008) (figure 2A).

Factors secreted by the AER, including FGF8, also keep the mesenchyme of the limb bud tip in a developmentally labile state, suppressing its capacity to form tight pre-skeletal aggregates, known as condensations, that would further differentiate into cartilage (Kosher *et al.* 1979). The shaping of the limb bud occurs at the tip, under the influence of the AER, the dorsal and ventral ectoderms that secrete other FGF and Wnt-family morphogens and the mesenchymally

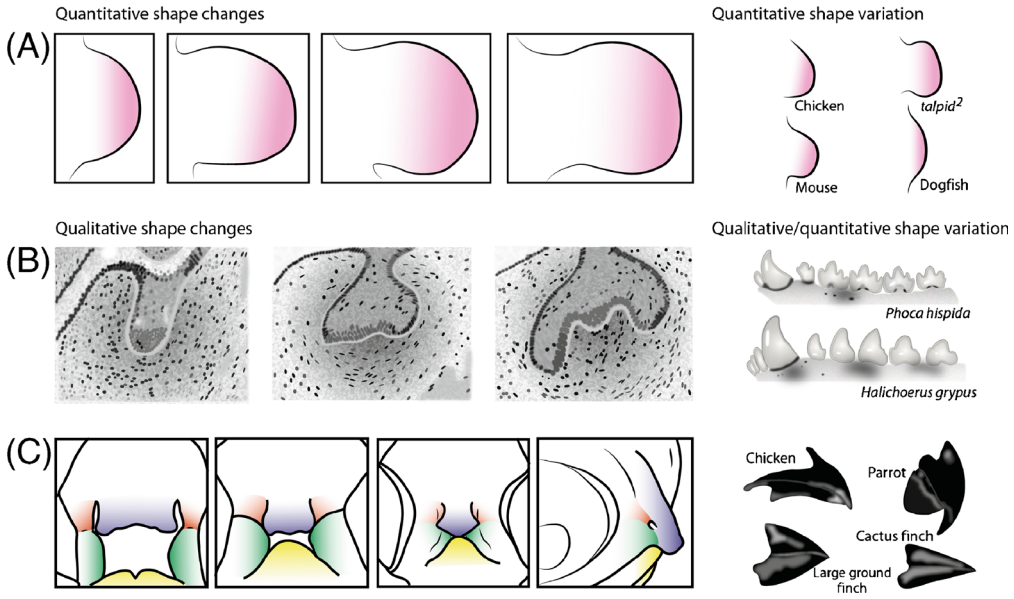

Figure 1. A sequence of different developmental stages of a single species (left) and interspecific variation (right) of embryonic or adult stages of the vertebrate limb bud (**A**), the mammalian tooth (**B**) and the bird beak (**C**). The limb bud undergoes quantitative shape changes during its morphogenesis (size and shape change of the same basic structure). The examples in **A**, left, are drawings of successive stages of mouse limb bud development. Its mode of development (figure 2) would only enable the generation of quantitative variation. The examples in **A**, right, are drawings of a normal mouse limb bud and a chicken wing bud at early embryonic stages; *talpid*^{2}, a recessive mutation in chicken which is lethal in the homozygous state (shown), where it develops a very wide bud and, later in embryogenesis, polydactyly; and the fin bud of a dogfish shark which develops a cartilaginous limb endoskeleton with similarities to that of tetrapods (see Zhu *et al.* 2010 for additional details and references). In contrast, the mammalian tooth and the bird beak undergo more complex morphological transformations during morphogenesis. The examples in **B**, left, are drawings of histological sections of successive stages in the development of mouse first molars. The examples in **B**, right, are drawings of the teeth of two different seal species, the ringed seal *Phoca hispida* (top) and the grey seal *Halichoerus grypus* (bottom). The examples in **C**, left, are successive stages (from E5 to E8) of chicken beak development. The primordia are shaded as follows: FNM, blue; LN, orange; MX, green; MD, yellow. During beak morphogenesis, FNM, LN and MX primordia progressively collide and fuse to form a unique structure, the upper beak (at E8). The collision and fusion of a pair of MD form the lower beak. The examples in **C**, right, are drawings of the beaks of a chicken, a parrot, a large ground finch and a cactus finch. The upper beak of *Geospiza* finches show the same basic type and they only differ in their size (Campàs *et al.* 2010); any other pair-wise comparison between the species depicted in the figure would represent qualitative shape changes (different beak types). The tooth and beak developmental systems are capable of generating qualitative shape variation in response to continuous genetic alterations (*morphological novelties*) (see figures 3 and 5) (the mouse limb bud sequence is based on Marcon *et al.* 2011; seal teeth are based on Salazar-Ciudad and Jernvall 2010; histological tooth sections are based on Miletich *et al.* 2011; finch beaks are based on Abzhanov *et al.* 2006).

and ectodermally sourced morphogens bone morphogenetic protein 4 (BMP4) and Sonic Hedgehog (SHH). The latter is produced in a localized region of the posterior mesoblast (the zone of polarizing activity, or ZPA), as well as a portion of the ectoderm (Bouldin *et al.* 2010).

The mouse limb bud mesoblast thus contains several gradient systems maintained by feedback loops involving the above-mentioned morphogens, and nonuniformly distributed Hoxd-class, *Gli3* and *Hand2* transcription factors. These factors regulate the formation and AP length of the AER and the localization and maintenance of the ZPA (Zeller *et al.* 2009).

In contrast to the long-held view that the limb bud is primarily shaped by a gradient of mitoses in the mesoblast in response to AER factors (i.e. by the physical mechanism of nonuniform increase of mass), the distal mesenchymal cells actually exhibit a chemotactic migratory response to FGF gradients (Li *et al.* 1996), as well as an oriented movement and growth based on cell shape polarization (Boehm *et al.* 2010; Wyngaarden *et al.* 2010). Cell orientation is dependent on Wnt signalling, while FGF signalling affects cell velocity (Gros *et al.* 2010; Hopyan *et al.* 2011) (figure 2B and C). Sonic Hedgehog, with its source in the ZPA signalling centre, does in fact act in a mitogenic capacity, indirectly influencing digit number by its control of limb bud width, in addition to its better known role, manifested later in development, in specifying digit identity (Zhu *et al.* 2008).

The shaping of the single-primordium limb bud is thus the result of mechanisms such as proliferation, chemotaxis, cell

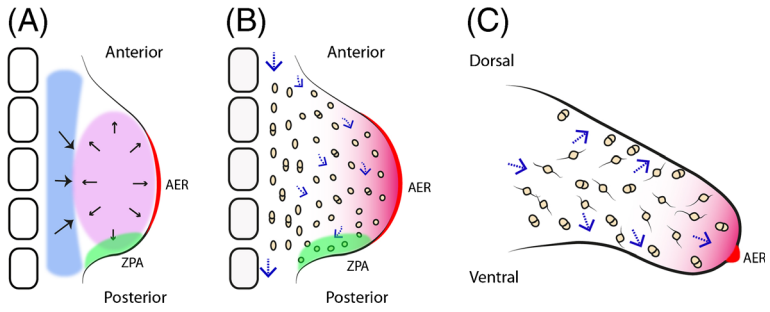

Figure 2. Tissue and cell behaviours during limb bud outgrowth. Limb initiation from the lateral plate mesoderm involves: (**A**) phase separation of limb bud and flank tissue due to acquisition of enhanced cohesivity of the prospective limb relative to the flank, and expulsion of limb bud by the flank tissue due to acquisition of active mechanical responsiveness by the latter (Damon *et al.* 2008); (**B**) loss of longitudinal cell shapes, directional changes in cell movement (blue arrows), and cell division bias (telophase separation indicated by line) (Wyngaarden *et al.* 2010); (**C**) alignment of long axes and processes in a radial manner during elongation of the bud with cell division planes and cell movements largely parallel to this orientation (Boehm *et al.* 2010; Gros *et al.* 2010). The cohesivity of the limb mesoblast (Damon *et al*. 2008), and the orientation (Boehm *et al.* 2010) and velocity (Gros *et al.* 2010) of movement of mesenchymal cells, are influenced by FGFs secreted by the AER (red). The polarity and oriented movement of some of the mesoblast cells are also regulated by Wnt proteins secreted by the dorsal and ventral ectoderm (black boundary in panel **C**; Gros *et al.* 2010; Wyngaarden *et al.* 2010). The width of the limb bud is influenced by the mitogenic effects of SHH produced at the zone of polarizing activity (ZPA) (green in panels **A** and **B**) (Zhu *et al.* 2008) (panels **B** and **C** redrawn, with changes, from Hopyan *et al.* 2011).

polarization and orientation, which can be *quantitatively* tuned (e.g. by variation in the participating genes) with no *qualitative* changes likely in morphological outcome (the same convex bud being basically conserved both during development and across species; see figure 1A).[1] The inability of the cell movement induced by these various processes to change the relation of the signalling centres in such a way as to create divergent forms is the hallmark of a *morphostatic* developmental mechanism (Salazar-Ciudad *et al.* 2003).

3. Morphogenesis of the mammalian tooth: A morphodynamic single-primordium system

While the limb bud is a mesenchyme-filled ectodermal evagination of the body wall, tooth development begins with the invagination of part of the oral ectoderm into the underlying, neural-crest derived mesenchyme. Following this, in the cap stage, the tip of this invagination stops growing and its cells become more tightly packed. This structure is called the *enamel knot* (or simply, knot) and is characterized by the same, or similar, molecular markers in all mammalian species studied. The epithelium grows more extensively at the periphery of the knot, leading to the formation of two epithelial loops (called the cervical loops) that invaginate deeper into the underlying mesenchyme. In multi-cusped teeth, other knots form at some distance from the primary knot. Over time, each knot ends up in the tip of an elevation in the epithelium while the intervening epithelium continues to proliferate and deepens into the underlying mesenchyme. This process leads to epithelial peaks and valleys, with each knot ultimately forming the tip of a cusp. As differentiation into ameloblasts (epithelium) and odontoblasts (mesenchyme) proceeds from the knots to the cervical loops, a final tooth morphology is established that corresponds to the configuration in 3D of the epithelial cusps (reviewed in Salazar-Ciudad 2012) (figures 1B and 3).

The mechanism of tooth development can be modelled by a version of a Turing-type local-activation-lateral-inhibition system (Salazar-Ciudad and Jernvall 2010), also called the Gierer–Meinhardt mechanism (Meinhardt and Gierer 2000). In such systems, it is required that at least one diffusible molecule promotes its own production, the activator, which also enhances the production of another diffusible molecule, the inhibitor, that curtails the activator's production. Depending on the strength of interaction between those molecules and their diffusion rates, patterns of spots or stripes of activator and inhibitor concentrations arise in space from initially homogeneous conditions (provided that there are small concentration fluctuations). In the tooth developmental system there is good evidence for an activatory role of both BMP2 and BMP4 in establishing the enamel knots and, in the case of BMP2, the

[1] We emphasize that our limb example only pertains to the shaping of the bud *before* the skeleton differentiates, a developmental episode in every tetrapod embryo. The skeleton itself is, of course, a set of discrete elements that emerge in a partly discontinuous fashion. Subtle changes in limb bud shape, as well as in the functioning of molecules directly involved in its formation, as described above, can have sharply divergent effects on the skeletal pattern. Specifically, due to the properties of its underlying Turing-type patterning mechanism (Turing 1952; Newman and Frisch 1979), the skeleton undergoes discontinuous jumps between numbers and sizes of elements in response to continuous changes in the shape and other parameters of the developing limb bud (Miura *et al.* 2006; Sheth *et al.* 2012).

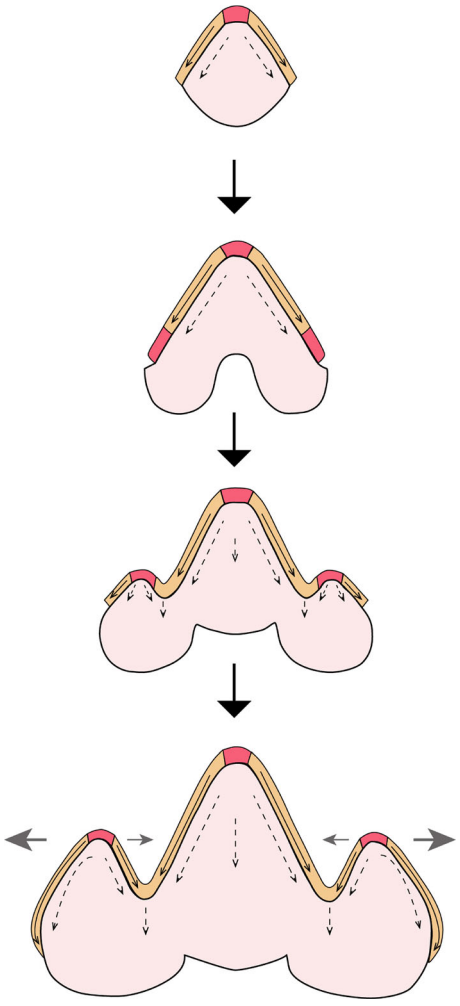

Figure 3. Schematic representation of tooth morphogenesis according to the morphodynamic hypothesis. Knots are depicted in red, the rest of the epithelium in brown and condensing mesenchyme in pink. According to this hypothesis knots inhibit each other through one or more diffusible molecules (including SHH) and also promote condensation of the underlying mesenchyme. Proliferation in the epithelium leads to the movement of cells along the plane of the epithelium, depicted as continuous black arrows, from the knots towards the cervical loops. A similar movement occurs in cells in the mesenchyme (dashed black arrows). This promotes the formation of valleys in the epithelium between knots. Since knots also promote the growth of the underlying mesenchyme, the cusps formed by each knot are pushed apart (gray horizontal arrows) while the epithelium grows to engulf the surface of the condensed mesenchyme. (Redrawn, with changes, from Salazar-Ciudad 2012.)

condensation of the underlying mesenchyme as well. BMP4, moreover, promotes its own expression through its receptors and the transcription factors *Msx1* and *Pax9* (reviewed in Salazar-Ciudad 2012).

The proposed tooth developmental mechanism exhibits some differences from the classically described reaction-diffusion systems, however, which reflect specific features of the biology (Salazar-Ciudad and Jernvall 2010). In typical reaction-diffusion models, the activator and inhibitor function simultaneously in time and space, with no threshold for the induction of either component. In the tooth model, it is stipulated that there is no inhibitor secretion until knots arise, and that it is only the knot cells that secrete the inhibitors. The implication is that cells are in a self-amplifying loop of BMP4 secretion until some of them attain sufficient levels of the morphogen and differentiate into knots. The knots then secrete inhibitors (a complex set of factors including SHH and various indirectly acting BMP inhibitors) that preclude nearby cells from reaching the same threshold.

The irreversibility of this kind of differentiation ensures that the positions of the activator peaks (i.e. the knots) do not change in space or time once they are formed. This contrasts with the behaviour of classic reaction-diffusion models in which concentration peaks continually readjust in space so that a regular spacing between them is ultimately attained. While adjacent knots are subject to lateral displacement due to growth and can lose mass due to apoptosis, knots remain stable and retain their relative heights throughout the developmental process (figure 3).

Referring back to the description of the shaping of the limb bud (section 2), we can see that the development of the tooth presents some important differences. A reaction-diffusion mechanism also underlies the process of *skeletal patterning* in the limb bud (Hentschel *et al.* 2004; Sheth *et al.* 2012; see footnote 1). In the limb, however, skeletal patterning is distinct from limb bud *shaping*, whereas in the case of the tooth the reaction-diffusion mechanism governs a process in which shaping and skeletogenesis are inextricably intertwined. This mechanism gives to the single tooth primordium the capacity to develop several independently growing signalling centres that interact with one another simultaneously to determine the tooth shape, a property that makes its development inherently *morphodynamic* (Salazar-Ciudad *et al.* 2003).

Computational simulation of the tooth model successfully predicts the range of morphological variation seen in a natural population of seals (Salazar-Ciudad and Jernvall 2010). The variational properties of the system under simulated genetic change demonstrate that *continuous* or *incremental* underlying changes can lead to *qualitatively* different tooth morphologies (figure 1B).

4. Formation of the bird beak: A multiprimordium morphogenetic system

Like the limbs, the beak develops from evaginations of the epithelium filled with mesenchymal cells. In contrast to both the limbs and the teeth, the beak is formed by more than one primordium. A total of five buds form the upper beak: one medial frontonasal mass (FNM), two lateral nasal

prominences (LN) and two maxillary prominences (MX). The lower beak is formed by the fusion of two mandibular prominences (MD) (figure 1C). At early stages of development, the skeleton of the beak consists of rods of cartilage. There is one central rod in the upper beak (the nasal cartilage) and two lateral rods in the lower beak (Meckel's cartilages). These rods represent the scaffolding of the beak; the mesenchymal cells surrounding this cartilaginous scaffold will develop into membrane bone, with the exception of the articular element of the lower jaw, which is an endochondral bone that forms by replacement of cartilage (Zusi 1993).

Like the teeth, and in contrast to the limb bud, the beak primordia undergo complex, *qualitative* shape transformations during morphogenesis (figure 1C). Alterations of this process have led to beaks of different types (such as the beaks of chickens, parrots, or finches) or quantitative variations of the same basic beak type (e.g. the beaks of Darwin's finches) (Campàs *et al.* 2010) (figure 1C). Thus, this developmental system has produced both qualitative and quantitative shape variation in the course of evolution.

One of the most widely discussed developmental models for the bird beak is based on the patterning of facial cartilage and bone in Galapagos finches (species of the genus *Geospiz*a) (Abzhanov *et al.* 2004, 2006; Mallarino *et al.* 2011). According to this model, BMP4 and calmodulin (CaM) control beak dimensions by regulating, independently, the patterning of the prenasal cartilage. These two molecules affect different dimensions of the beak: BMP4, its depth and width, and CaM, its length. In addition, TGFβRII, β-catenin and Dickopf 3 (Dkk3) act in a coordinate fashion to further modulate the depth and length of the upper beak through their effects on the patterning of the premaxilla bone. These two sets of molecules – one acting on chondrogenesis and the other on osteogenesis – represent, in this view, two independent developmental modules that conjointly explain the *size* differences of the beaks observed in the genus *Geospiza*, which all have the same upper beak shape (Campàs *et al.* 2010).

A recent study of another group of finches, the genus *Loxigilla*, has shown that the species *L. noctis*, which shares the same regulatory network of tissue patterning described for the genus *Geospiza*, exhibits a different upper beak shape. Furthermore, *L. portoricensis* and *L. violacea*, which exhibit the same beak shape as *L. noctis*, utilize a different regulatory network (one formed by *Bmp4* and *Ihh*, whose products synergistically alter the dimensions of the premaxilla bone) (Mallarino *et al.* 2012). These observations indicate a lack of correspondence between beak morphology and the presumed underlying gene-expression-based developmental programs for tissue patterning. This implies that the evolutionary conservation of beak shape among the *Loxigilla* species, whatever assumptions are made about its presumed adaptive basis, is consistent with dramatic 'rewiring' of the skeletal patterning network. A plausible explanation for this is that beak shape may be generated not during skeletogenesis, as proposed by Mallarino *et al.* (2011), but at earlier stages of budding outgrowth (see below).

Taking into account that the adult beaks of Galapagos finches do not depart substantially from a triangular shape (Campàs *et al.* 2010), developmental models based on these species would be primary concerned with *quantitative* shape changes and, therefore, would not address the origin of other divergent and common shapes in birds, such as the curved beak of parrots and hawks.

Experiments based on BrdU staining (used for the detection of proliferating cells) have shown the existence of localized zones in the FNM mesenchyme with a higher proliferative rate. These areas have been called localized growth zones (LoGZs) (Wu *et al.* 2004, 2006). A comparative analysis showed differences in the distribution of the LoGZs between the chicken, the duck and the cockatiel. The distribution of these LoGZs, moreover, was correlated with the expression pattern of *Bmp4*. It has been suggested that the morphological diversity of the beak could be due to evolutionary changes in the spatiotemporal regulation of these LoGZs, possibly due to alterations in regulation of *Bmp4*. SHH secreted by the frontonasal ectodermal zone (FEZ), a signalling centre localized in the epithelium of the FNM, could act as an upstream molecule that regulates the pattern of LoGZs in the FNM mesenchyme (Hu and Marcucio 2009; Young *et al.* 2010). In contrast to the model described above, this would mean that the shape of the beak could be specified at early stages of budding outgrowth, prior to significant differentiation of cartilage and bone. Furthermore, based on divergent beak morphologies, this growth-based developmental model would explain the origination of both quantitative and qualitative shape changes at the adult stage.

The model relies principally on the observation that the chicken and the duck embryos possess two lateral LoGZs in the FNM (one on each of the globular processes), which at later stages of development collide at the midline to form a unique growth zone (Wu *et al.* 2006). In the duck, the two LoGZs remain independent of each other for a major period of time, leading to a broader primordium. It has been suggested, therefore, that the differences in the width of the FNM, produced by this delay in the fusion of the LoGZs, would explain the differences between the conical and the paddle-like beak of chickens and ducks, respectively (Wu *et al.* 2006). According to the model, changes in the growth rates of LN and MX primordia – which are located at the lateral sides of the FNM – would also alter the beak width, with higher growth rates leading to wider beaks and *vice versa* (figure 1C).

Sagittal sections of the FNM of the chicken, duck and cockatiel showed that the latter species has the LoGZ in a more dorsal position than the other ones (Wu *et al.* 2006). Based on this observation, the model states that beak curvature is determined by the position of the LoGZ along the

dorsoventral axis: a LoGZ located in a ventral position would lead to the formation of a straight beak, whereas in a dorsal position it would lead to a curved beak, intermediate positions leading to intermediate beak curvatures (Wu *et al.* 2006; see figure 3D–F therein).

However, this model cannot explain how a curved beak, such as the one observed in the cockatiel, can be induced in the chicken embryo (figure 4). The FEZ, a signalling centre defined by the boundary between *Fgf8* and *Shh* expression in the epithelium of the FNM, correlates with the localization of the LoGZ along the dorsoventral axis (Wu *et al.* 2006). The boundary of the *Shh* domain extends more ventrally in ducks and chickens than in the cockatiel (Wu *et al.* 2006). Thus, it has been suggested that FEZ may regulate the growth of the FNM by specifying the position of the LoGZs in the mesenchyme (Wu *et al.* 2006). Teratogens induce curved beaks in the chicken embryos when administrated at stage 23, after the FEZ and its signalling activity have been established (stage 20) (Hu *et al.* 2003). This indicates that factors other than the position of the LoGZs are involved in the generation of curved beaks.

To explore the developmental cause underlying beak curvature, we studied, by landmark-based geometric morphometrics (Rohlf and Marcus 1993), the transformations of the face during beak morphogenesis under normal and teratogenic (valproic acid exposure) conditions in the chicken embryo. Valproic acid (VPA) is a teratogen capable of interfering with the Wnt/β-catenin pathway (Wang *et al.* 2010), which promotes budding outgrowth of the facial prominences (Medio *et al.* 2012); it can also decrease embryonic growth by increasing apoptosis (Tung and Winn 2011). The treatment produced a decrease of the extension of the facial buds between embryonic days 5 to 7. Between day 8 and 9, the beak was less protruding in VPA-treated than control embryos. At later stages, VPA-treated embryos developed a curved beak similar to those observed in other bird genera (figure 5).

These results raise the question of how a reduction in budding outgrowth can generate a hooked beak. The external morphology of the embryos suggests an answer to this question. The egg tooth primordium localized in the FNM is in a vertical position at early stages of development, when the FNM is pointing downwards, but it assumes a horizontal position at later stages, when the recently formed upper beak begins to grow forward (figure 6). This indicates that the FNM does not grow forward by itself like Pinocchio's nose, but is by some means lifted. Thus, for the beak to grow forward, a mechanism capable of straightening the frontonasal mass might be at work, with the failure of such a mechanism leading to a curved beak. The fact that the maxillary buds are the most affected by the gain of function of *Mxs1*, via up-regulation of Wnt/β-catenin pathway, which leads to a curved beak (Medio *et al.* 2012), indicates that these buds may play an important role in the generation of the beak shape. (The hypothesis that the upper beak is straightened by the lower beak was ruled out by Silver 1962.)

Taking into account that the maxillary buds, in contrast to the FNM, extend principally along a proximodistal axis (McGonnell *et al.* 1998), we propose that the FNM is lifted by the maxillary buds when at embryonic day 8 these buds collide and fuse to form the upper beak. The answer to how the reduction in budding outgrowth observed in VPA-treated embryos can lead to the formation of a hooked beak is that the maxillary buds in this case do not extend far enough to lift the frontonasal mass.

In collaboration with computational biologist colleagues, we have devised a 2D *in silico* model, based on the viscoelastic properties of the mesenchyme, which simulates the extension of the FNM and the maxillary buds at the stage in which they collide and fuse to form the upper beak (Linde-Medina *et al.*, in preparation). According to our shape analysis of the face, treatment with VPA resulted in reduced bud expansion. Therefore, we simulated the growth of VPA-treated embryos by decreasing the extension rate of the maxillary buds. Under this situation, the MXs do not extend enough to support from below the extending FNM, causing the FNM to overgrow the MXs and move downward, resulting in a curved appearance. Simulations for high and moderate extension rates lead to the generation of a straight beak like those observed in normal chicken development; only when a threshold was surpassed did the new beak shape appear. The simulations showed how changes in the extension rate of the maxillary buds could abruptly lead to the generation of beaks of different types (i.e. *qualitative* shape changes in response to a *continuous* change in the extension rate parameter), indicating the importance of mechanical interactions between the facial primordia in the process of beak morphogenesis, a feature not considered in previous models.[2]

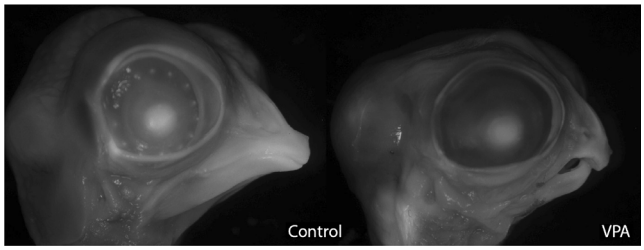

Figure 4. A control and a VPA-treated chicken embryo at embryonic day 10. Note the curved beak induced by exposure of the embryo to the teratogen.

[2] Note that the model of Mallarino *et al.* (2011) is based on a single primordium, the frontonasal mass, whereas the model of Wu *et al.* (2006) takes into account the multiprimordium nature of the bird beak. However, the latter model is exclusively based on *intrinsic* growth patterns of the facial primordia, with no mention of the *extrinsic* budding interactions in the generation of the beak form.

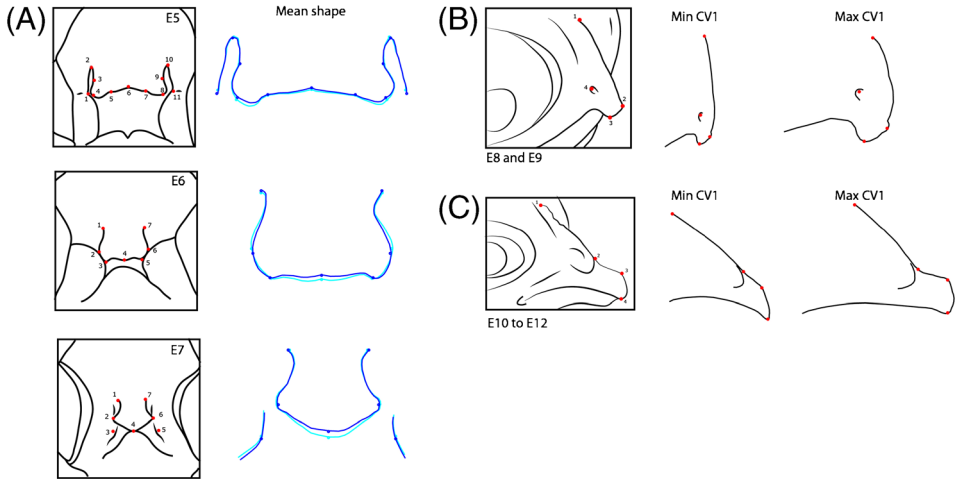

Figure 5. Mean shape of the face of a control (light blue) and a VPA-treated embryo (dark blue) from a Discriminate Analysis (embryonic day 5 to 7) (**A**). The differences between the mean shapes indicate the shape alterations induced by VPA. Shape configuration for the minimum and maximum values of the first axis of a canonical variate analysis (CV1) of control and VPA-treated embryos which summarizes the main shape differences induced by VPA at embryonic days from 8 to 9 (**B**) and 10 to 12 (**C**). The control and VPA-treated embryos presented maximum and minimum values of the first canonical axis, respectively. The panels show the localization of the landmarks (red points) that were used for the shape analysis (Linde-Medina *et al*., in preparation).

5. Discussion

Evolutionary developmental biology, at least in its internalist version (Linde-Medina 2010b), departs from evolutionary theory in the classic neo-Darwinian and Modern Synthesis modes by its focus on laws of biological form. Earlier forays in this direction, whether by Goethe, Geoffroy Saint-Hilaire, Owen, Bateson or Thompson (Russell 1916; Webster and Goodwin 1982; Lenoir 1987; Amundson 2007; Newman 2007), were all written out of the standard evolutionary theory in favour of the view that biological form has been *shaped* by natural selection. Consistent with this suppressed 'laws of form' tradition, however, it is now understood that the forms that organisms can assume over the course of evolution are neither arbitrary, nor produced exclusively by opportunistic competition for marginal advantage. They are instead generated by a limited set of physico-genetic processes inherent to developing tissues, which in turn give rise to finite arrays of kingdom- and phylum-characteristic morphological motifs (Forgacs and Newman 2005; Newman 2012; Hernández-Hernández *et al.* 2012; Heisenberg and Bellaïche 2013).

We have seen that the role of material properties in the generation of biological form is omitted in existing developmental models for the bird beak (Wu *et al.* 2006; Mallarino *et al.* 2011). However, the complex qualitative shape transformations that the facial buds undergo to generate the beak (figure 1C) cannot be understood when the physical forces that drive morphogenesis are excluded. As a consequence, the upper beak has been explicitly or implicitly conceived as

Figure 6. View of the head of a chicken embryo at day 7 (top right) and day 12 (bottom left). The arrow shows the position of the egg tooth primordium. Note that the egg tooth is in a vertical position at day 7, whereas it assumes a horizontal position at later stages. This indicates that the FNM has been lifted during the morphogenesis of the beak.

a morphostatic single-primordium organ like the limb bud. In such an idealized framework, beak shape changes are the direct consequence of *quantitative* changes at the primordium. In the model of Wu *et al.* (2006), for example, curved, longer, larger, smaller, narrowed and widened upper beaks would be the direct consequence of a curved, longer, larger, smaller, narrower and wider FNM, respectively (with wider LNs and MXs also contributing in the latter case). In the model of Mallarino *et al.* (2011), the correspondence is between upper beak morphology and the proportions of the skeletal elements; thus, a wider beak would be the consequence of a wider nasal cartilage; a larger beak, the consequence of a larger nasal cartilage and/or a larger premaxilla bone, and a deeper beak, the consequence of a deeper nasal cartilage and/or a deeper premaxilla bone (a curved beak is not contemplated in this model). Correspondingly, it was suggested that incremental changes in the expression of the regulatory genes involved in growth (Wu *et al.* 2006) or tissue patterning of the facial buds (Mallarino *et al.* 2011) would generate incremental and quantitative morphological variation along the three main growth axes of the beak, fulfilling the bases for a continuous and gradual moulding process, as required by the standard evolutionary framework.

But the mechanical lifting of the FNM that takes place during beak morphogenesis cannot be understood solely in terms of gene expression patterns. Developmental genes will contribute to this process by regulating the extension rate of the facial primordia (a system parameter), but the resulting beak shape would be ultimately due to the physical forces generated during morphogenesis. The incorporation of budding interactions in a model of beak morphogenesis reveals novel variational properties of this developmental system, which are not seen in a morphostatic single-primordium organ. Simulations of beak morphogenesis, which incorporate physical interactions, show that the lifting of the FNM would not be a gradual and continuous process, but that the system would respond abruptly to continuous alterations of its constituent parameters, generating a straight or a curved beak with apparently no intermediate beak shapes. Like the morphodynamic tooth system, this physically interactive multiprimordium system is capable of generating *qualitative* shape change in response to *continuous* variations of the system parameters.

An earlier-studied example of an incremental change in the parameters of a developmental system leading to an abrupt morphological transition is provided by the ontogeny of cheek pouches in rodents (Brylski and Hall 1988a,b). In some species, cheek pouches open internal to the mouth cavity, while in some other species they open externally, with no connection to the mouth cavity. Both types of cheek pouches develop from an invagination of the buccal epithelium close to the corner of the mouth, with only a minimal anterior shift of the invagination site (associated with elongation of the snout in later-evolving species) resulting in the externalization of the cheek pouches (Brylski and Hall 1988a). The investigators suggested that this morphological transition took place with no intermediate phenotypes (Brylski and Hall 1988b; see the discussion in Müller 1990 p 107).

Some of the variants produced by such developmental systems are dissimilar from the incremental and arbitrary morphological variants of the morphostatic system; they appear suddenly and fully formed. These qualitative transformations (e.g. from a straight to a curved beak) would not result from a gradual evolutionary moulding process to meet functional demands, but from the properties of the underlying generative mechanisms, which may have nonlinear properties. In particular, a curved beak need not have originated by an evolutionary reshaping, through intermediate steps, of a single-primordium organ to meet some functional demands; it can instead have arisen suddenly, by a slight alteration of the physical processes involved in beak morphogenesis: it is a potential discrete morphological outcome of this developmental system. The collision and fusion of the facial buds to form the upper beak will (when certain parameters are altered) abruptly give rise to a novel form (e.g. the curved beak), the basis of which is intrinsic to the material properties of the embryonic tissues and quite independent of any subsequent functional role.[3]

By the principles of niche construction, morphological novelties, in cases like the tooth or beak, could enable new food choices and exploration of new habitats (Laland *et al.* 2008). Such examples of function following form do not conform to the notion of adaptive, gradualist natural selection as the driving force of morphological evolution.

The contrasting views of beak morphogenesis suggest that natural selection can only be imagined to be an evolutionary *shaping* mechanism if the complex morphological transformations of the face during beak morphogenesis are side-stepped. Therefore, although embryonic development is not explicitly considered by the neo-Darwinian synthesis, we suggest that the morphostatic, single-primordium paradigm, exemplified by limb bud shaping, is what is implicitly assumed in that model of evolution. Incorporating physics in the causal analysis of beak morphogenesis, permitting, in principle, the explanation of this complex morphogenetic process, concomitantly undermines the importance of natural selection in the shaping of the beak. In this view, natural selection may preserve, and thus enable, the propagation of morphological novelties which, however, are *originated* by a dynamic multiprimordium system. This exemplifies the

[3] While the early developmental events would determine the general shape of the upper beak, its adult form will also depend on processes that take place at later ontogenetic stages (patterning and growth of skeletal tissues) or during the juvenile phase (by the active use of the jaw muscles) (Genbrugge *et al.* 2011). The growth of the rhamphotheca, the sheet of cornified epithelium covering the beak, also influences the adult beak shape (Genbrugge *et al.* 2012).

assertion that when accounting for biological form 'one must not confuse the fact that a structure is used in some way… with the primary evolutionary reason for its existence and conformation' (Gould and Lewontin 1979 p 153).

An unavoidable question is: how many organs, or features of an organ, conform to the single-primordium, morphostatic paradigm which enables the Darwinian mode of evolution? The answer is that for the origination of morphological novelties and the elaboration of complex organs, they may be more of an exception than the rule. Note that while we used the limb bud as a known example of a morphostatic single-primordium system, the morphology of the limb *skeleton* is specified by a Turing-type mechanism (Hentschel *et al.* 2004; Sheth *et al.* 2012) that can generate abrupt, qualitative change in the number and arrangement of elements in response to, among other things, continuous variation in limb bud shape (Zhu *et al.* 2010).

We conclude with some comments about the role of mechanisms of abrupt (saltational) change in evolutionary theory. It might be claimed that this issue is a straw man: for evolutionists, the criticism goes, it does not matter how a phenotypic alteration arises; the only matter of importance is the contribution of its heritable determinants (typically gene variants) to the next generation. The late Stephen Jay Gould, considering the unique contribution of Darwin to the Modern Synthesis, had a different view of the role of selection in this theory:

> Darwin's theory…cannot be equated with the simple claim that natural selection operates. Nearly all his colleagues and predecessors accepted this postulate. Darwin, in his characteristic and radical way, grasped that this standard mechanism for preserving the type could be inverted, and then converted into the primary cause of evolutionary *change*. Natural selection obviously lies at the center of Darwin's theory, but we must recognize, as Darwin's second key postulate, the claim that natural selection acts as *the creative force* of evolutionary *change*…as long as change accretes in an insensibly gradual manner; and as long as the reproductive advantages of certain individuals provide the statistical source of change; then natural selection must be construed as the directional cause of evolutionary modification (Gould 2002 pp 139, 141).

Given the insistence on gradualism by both Darwin and Wallace, and the rejection by the architects and present-day advocates of the Modern Synthesis of an evolutionary role for genes of large effect (creating hopeful monsters) and of the reality (as opposed to the artifactual appearance) of punctuated equilibria in the fossil record,[4] we must give credence to Gould's characterization of the structure of the Darwinian framework. The Darwin–Wallace theory and the subsequent Synthesis have purported to explain the origin and reshaping of biological features – morphological motifs and other phenotypic characters – by incremental adaptive changes. If the effect of selection is simply to retain or discard possibilities due to inherent tissue properties, the 'creativity' lies in the laws of morphological and physiological development, not in selection itself.

Where the underlying mechanisms of phenotypic change are clear, and the presumption of gradualism is untenable, saltational change has been uncontroversial in evolutionary theory. Mutations associated with utilization of novel carbon sources in bacteria (van der Meer 1997), or with ethanol (Agarwal 2001) or lactose tolerance (Campbell *et al.* 2005), or with alterations of red blood cell morphology in humans (Mozzarelli et al. 1987), may lead to large phenotypic effects which are explicable on the basis of the discrete outcomes of enzyme chemistry or protein folding. These sudden alterations may be retained or not as a result of selection, but in such cases no one would claim that natural selection is the 'creative force' behind them. It is only in the case of morphological characters, where (until recent years) there has been no analogous physicochemical understanding of their generation, that consideration of saltational mechanisms has been anathema to the conventional theory.

We have argued elsewhere that the resistance to saltational interpretations of morphological evolution is a remnant of early 19th century concepts of the physics of materials (Linde-Medina 2010a; Newman and Bhat 2011; Newman and Linde-Medina 2013; see also Depew and Weber 1996). If living matter was not considered to be capable of organizing itself by its intrinsic physicochemical properties, it had to be moulded from without, by adaptive solutions to externally given problems. The domination of Newton's and even Aristotle's ideas, uninformed by later developments in thermodynamics, dynamical systems theory, reaction-diffusion instabilities, and so forth (which eventually incorporated abrupt transformations of organic form into the naturalistic explanatory framework), led Darwin and Wallace towards a notion of the transformation of body plans and organ forms

[4] There have been exceptions. The late John Maynard Smith, for instance, described himself as 'open-minded about the possibility that development may impose discontinuous constraints on the pattern of phenotypic variation', concluding that '[i]f so, mutations of large phenotypic effect may sometimes initiate new evolutionary departures' (Maynard Smith 1983 p 19). Although advanced three decades ago by one of the most highly regarded theorists of the Modern Synthesis, this notion, which may have even greater relevance to the less canalized forms of earlier periods of evolution than to present-day organisms (Newman 2012), has remained marginal to mainstream evolutionary theory.

that allowed for no sudden transitions: to get from 'here' (e.g. a simple form) to 'there' (a more complex one) could only be accomplished by a series of gradual changes.

We now know better. Embryogenesis clearly utilizes physical processes consistent with abrupt self-organization change, including phase separation (in gastrulation; Krieg *et al.* 2008), oscillation (in segmentation; Oates *et al.* 2012), and reaction-diffusion instability (in limb skeletal development; Sheth *et al.* 2012). The examples presented here, of limb bud shaping, tooth formation and beak development, show that ostensibly similar processes of tissue morphogenesis occur by a multiplicity of effects in one or more than one primordium, that in only some, possibly exceptional cases, does this result in gradual deformation. There is no reason for the novelties produced by saltational mechanisms, such as those described here, not to have contributed to organismal changes in natural populations.[5] Because of its supposedly paradigmatic status in the genesis of Darwin's theory (van Wyhe 2011) the morphological variation of the bird's beak has particularly succumbed to gradualistic interpretations that are inconsistent with the embryological reality. With our increased knowledge of the protean dynamics of development, we can progress toward an evolutionary framework that is not tied to an obsolete concept of living matter.

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References

Abzhanov A, Kuo WP, Hartman C, Grant R, Grant P and Tabin CJ 2006 The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature* **442** 563–567

Abzhanov A, Protas M, Grant R, Grant P and Tabin CJ 2004 Bmp4 and morphological variation of beaks in Darwin's finches. *Science* **305** 1462–1465

Agarwal DP 2001 Genetic polymorphisms of alcohol metabolizing enzymes. *Pathol. Biol.* **49** 703–709

Amundson R 2007 *The changing role of the embryo in evolutionary thought* (New York: Cambridge University Press)

Boehm B, Westerberg H, Lesnicar-Pucko G, Raja S, Rautschka M, Cotterell J, Swoger J and Sharpe J 2010 The role of spatially controlled cell proliferation in limb bud morphogenesis. *PLoS Biol.* **8** e1000420

Bouldin CM, Gritli-Linde A, Ahn S and Harfe BD 2010 Shh pathway activation is present and required within the vertebrate limb bud apical ectodermal ridge for normal autopod patterning. *PNAS* **107** 5489–5494

Brylski P and Hall BK 1988a Epithelial behaviors and threshold effects in the development and evolution of internal and external cheek pouches in rodents. *J. Zool. Syst. Evol. Res.* **26** 144–154

Brylski P and Hall BK 1988b Ontogeny of a macroevolutionary phenotype: the external cheek pouches of geomyoid rodents. *Evolution* **42** 391–395

Campàs O, Mallarino R, Herrel A, Abzhanov A and Brenner MP 2010 Scaling and shear transformations capture beak shape variation in Darwin's finches. *PNAS* **107** 3356–3360

Campbell AK, Waud JP and Matthews SB 2005 The molecular basis of lactose intolerance. *Sci. Prog.* **88** 157–202

Damon BJ, Mezentseva NV, Kumaratilake JS, Forgacs G and Newman SA 2008 Limb bud and flank mesoderm have distinct 'physical phenotypes' that may contribute to limb budding. *Dev. Biol.* **321** 319–330

Darwin C 1859 *On the origin of species by means of natural selection, or, The preservation of favoured races in the struggle for life* (London: J. Murray)

Depew DJ and Weber BH 1996 *Darwinism evolving: Systems dynamics and the genealogy of natural selection* (Cambridge, MA: MIT Press)

Forgacs G and Newman SA 2005 *Biological physics of the developing embryo* (Cambridge: Cambridge University Press)

Genbrugge A, Adriaens D, Kegel B, Brabant L, Hoorebeke L, Podos J, Dirckx J, Aerts P, *et al.* 2012 Structural tissue organization in the beak of Java and Darwin's finches. *J. Anat.* **221** 383–393

Genbrugge A, Heyde AS, Adriaens D, Boone M, Van Hoorebeke L, Dirckx J, Aerts P, Podos J, *et al.* 2011 Ontogeny of the cranial skeleton in a Darwin's finch (Geospiza fortis). *J. Anat.* **219** 115–131

Gilbert S, Opitz JM and Raff RA 1996 Resynthesizing evolutionary and developmental biology. *Dev. Biol.* **173** 357–372

Gould SJ 2002 *The structure of evolutionary theory* (NY: Harvard University Press)

Gould SJ and Lewontin RC 1979 The spandrels of San Marco and the panglossian paradigm: A critique of the adaptationist programme. *P. Roy. Soc. Lond. B Bio. Sci.* **205** 581–598

Gros J, Hu J K-H, Vinegoni C, Feruglio PF, Weissleder R and Tabin CJ 2010 WNT5A/JNK and FGF/MAPK pathways regulate the cellular events shaping the vertebrate limb bud. *Curr. Biol.* **20** 1993–2002

[5] A recent study of the formation of head crests in pigeons shows this unequivocally to have occurred (Shapiro *et al.* 2013). Darwin was familiar with such abrupt morphological changes in populations of domesticated pigeons, but considered them 'sports' that, by his theory, could not have contributed to the evolution of this trait in the wild (Darwin 1859).

Heisenberg CP and Bellaïche Y 2013 Forces in tissue morphogenesis and patterning. *Cell* **153** 948–962

Hentschel HG, Glimm T, Glazier JA and Newman SA 2004 Dynamical mechanisms for skeletal pattern formation in the vertebrate limb. *P. Roy. Soc. Lond. B Bio. Sci.* **271** 1713–1722

Hernández-Hernández V, Niklas KJ, Newman SA and Benítez M 2012 Dynamical patterning modules in plant development and evolution. *Int. J. Dev. Biol.* **56** 661–674

Hopyan S, Sharpe J and Yang Y 2011 Budding behaviors: Growth of the limb as a model of morphogenesis. *Dev. Dyn.* **240** 1054–1062

Hu D and Marcucio RS 2009 A SHH-responsive signaling center in the forebrain regulates craniofacial morphogenesis via the facial ectoderm. *Development* **136** 107–116

Hu D, Marcucio RS and Helms JA 2003 A zone of frontonasal ectoderm regulates patterning and growth in the face. *Development* **130** 1749–1758

Keller R, Shook D and Skoglund P 2008 The forces that shape embryos: physical aspects of convergent extension by cell intercalation. *Phys. Biol.* **5** 15007

Kosher RA, Savage MP and Chan SC 1979 In vitro studies on the morphogenesis and differentiation of the mesoderm subjacent to the apical ectodermal ridge of the embryonic chick limb-bud. *J. Embryol. Exp. Morp.* **50** 75–97

Krieg M, Arboleda-Estudillo Y, Puech PH, Kafer J, Graner F, Muller DJ and Heisenberg CP 2008 Tensile forces govern germ-layer organization in zebrafish. *Nat. Cell Biol.* **10** 429–436

Laland KN, Odling-Smee J and Gilbert SF 2008 EvoDevo and niche construction: building bridges. *J. Exp. Zool. B Mol. Dev. Evol.* **310** 549–566

Lenoir T 1987 The eternal laws of form: morphotypes and the conditions of existence in Goethe's biological thought; in *Goethe and the sciences: A re-appraisal* (eds) F Amrine, F Zucker and H Wheeler (Dordrecht: Reidel) pp 17–28

Levine H and Ben-Jacob E 2004 Physical schemata underlying biological pattern formation-examples, issues and strategies. *Phys. Biol.* **1** 14–22

Li S, Anderson R, Reginelli AD and Muneoka K 1996 FGF-2 influences cell movements and gene expression during limb development. *J. Exp. Zool. Part A* **274** 234–247

Linde-Medina M 2010a Natural selection and self-organization: a deep dichotomy in the study of organic form. *Ludus Vitalis* **XVIII** 25–56

Linde-Medina M 2010b Two 'EvoDevos'. *Biol. Theor.* **5** 7–11

Linde-Medina M, Jeyaraman S, Bhat R and Newman SA Bird beak curvature is an emergent feature of facial bud interactions, in preparation

Mallarino R, Campàs O, Fritz JA, Burns KJ, Weeks OG, Brenner MP and Abzhanov A 2012 Closely related bird species demonstrate flexibility between beak morphology and underlying developmental programs. *PNAS* **109** 16222–16227

Mallarino R, Grant PR, Grant BR, Herrel A, Kuo WP and Abzhanov A 2011 Two developmental modules establish 3D beak-shape variation in Darwin's finches. *PNAS* **108** 4057–4062

Marcon L, Arqués CG, Torres MS and Sharpe J 2011 A computational clonal analysis of the developing mouse limb bud. *PLoS Comput. Biol.* **7** e1001071

Maynard Smith J 1983 The genetics of stasis and punctuation. *Annu. Rev. Genet.* **17** 11–25

McGonnell IM, Clarke JDW and Tickle C 1998 Fate map of the developing chick face: Analysis of expansion of facial primordia and establishment of the primary palate. *Dev. Dyn.* **212** 102–118

Medio M, Yeh E, Popelut A, Babajko S, Berdal A and Helms J 2012 Wnt/beta-catenin signaling and Msx1 promote outgrowth of the maxillary prominences. *Front. Physiol.* **3** 1–11

Meinhardt H and Gierer A 2000 Pattern formation by local self-activation and lateral inhibition. *BioEssays* **22** 753–760

Miletich I, Yu WY, Zhang R, Yang K, Caixeta de Andrade S, Pereira SF d, Ohazama A, Mock OB, *et al.* 2011 Developmental stalling and organ-autonomous regulation of morphogenesis. *PNAS* **108** 19270–19275

Miura T, Shiota K, Morriss-Kay G and Maini PK 2006 Mixed-mode pattern in Doublefoot mutant mouse limb–Turing reaction-diffusion model on a growing domain during limb development. *J. Theor. Biol.* **240** 562–573

Mozzarelli A, Hofrichter J and Eaton WA 1987 Delay time of hemoglobin S polymerization prevents most cells from sickling in vivo. *Science* **237** 500–506

Müller GB 1990 Developmental mechanisms at the origin of morphological novelty: A side-effect hypothesis; in *Evolutionary innovations* (ed) M Nitecki (Chicago: University of Chicago Press)

Müller GB and Newman SA 2005 The innovation triad: an EvoDevo agenda. *J. Exp. Zool. Part B* **304** 593–609

Newman SA 2007 William Bateson's physicalist ideas; in *From embryology to Evo-Devo: A history of evolutionary development* (eds) M Laubichler and J Maienschein (Cambridge, MA: MIT Press) pp 83–108

Newman SA and Bhat R 2011 Lamarck's dangerous idea; in *Transformations of Lamarckism: from subtle fluids to molecular biology* (eds) SB Gissis and E Jablonka (Cambridge, MA: MIT Press) pp 157–169

Newman SA and Frisch HL 1979 Dynamics of skeletal pattern formation in developing chick limb. *Science* **205** 662–668

Newman SA and Müller GB 2000 Epigenetic mechanisms of character origination. *J. Exp. Zool. B (Mol. Dev. Evol.)* **288** 304–317

Newman SA and Müller GB 2005 Genes and form: inherency in the evolution of developmental mechanisms; in *Genes in development: re-reading the molecular paradigm* (eds) E Neumann-Held and C Rehmann-Sutter (Durham, NC: Duke University Press) pp 38–73

Newman SA 2012 Physico-genetic determinants in the evolution of development. *Science* **338** 217–219

Newman SA and Linde-Medina M 2013 Physical determinants in the emergence and inheritance of multicellular form. *Biol. Theor.* doi:10.1007/s13752-013-0116-0

Oates AC, Morelli LG and Ares S l 2012 Patterning embryos with oscillations: structure, function and dynamics of the vertebrate segmentation clock. *Development* **139** 625–639

Peterson T and Müller GB 2013 What is evolutionary novelty? Process vs. character based definitions. *J. Exp. Zool. Part B*. doi:10.1002/jez.b.22508

Rohlf FJ and Marcus LF 1993 A revolution in morphometrics. *Trends Ecol. Evol.* **8** 129–132

Russell ES 1916 *Form and function* (Chicago: Univsity of Chicago Press)

Salazar-Ciudad I and Jernvall J 2005 Graduality and innovation in the evolution of complex phenotypes: insights from development. *J. Exp. Zool. Part B* **304** 619–631

Salazar-Ciudad I and Jernvall J 2010 A computational model of teeth and the developmental origins of morphological variation. *Nature* **464** 583–586

Salazar-Ciudad I, Jernvall J and Newman SA 2003 Mechanisms of pattern formation in development and evolution. *Development* **130** 2027–2037

Salazar-Ciudad I 2012 Tooth patterning and evolution. *Curr. Opin. Genet. Dev.* **22** 585–592

Shapiro MD, Kronenberg Z, Li C, Domyan ET, Pan H, Campbell M, Tan H, Huff CD, *et al.* 2013 Genomic diversity and evolution of the head crest in the rock pigeon. *Science* **339** 1063–1067

Sheth R, Marcon L, Bastida MFL, Junco M, Quintana L, Dahn R, Kmita M, Sharpe J, *et al.* 2012 Hox genes regulate digit patterning by controlling the wavelength of a Turing-type mechanism. *Science* **338** 1476–1480

Silver PHS 1962 In ovo experiments concerning the eye, the orbit, and certain juxta-orbital structures, in the chick embryo. *J. Embryol. Exp. Morp.* **10** 423–450

Steinberg MS 2007 Differential adhesion in morphogenesis: a modern view. *Curr. Opin. Genet. Dev.* **17** 281–286

Tsarfaty I, Resau JH, Rulong S, Keydar I, Faletto DL and Vande Woude GF 1992 The met proto-oncogene receptor and lumen formation. *Science* **257** 1258–1261

Tung EW and Winn L 2011 Valproic acid increases formation of reactive oxygen species and induces apoptosis in postimplantation embryos: a role for oxidative stress in valproic acid-induced neural tube defects. *Mol. Pharmacol.* **80** 979–987

Turing AM 1952 The chemical basis of morphogenesis. *Phil. Trans. Roy. Soc. Lond. B* **237** 37–72

van der Meer JR 1997 Evolution of novel metabolic pathways for the degradation of chloroaromatic compounds. *Antonie Van Leeuwenhoek* **71** 159–178

van Wyhe J 2011 Where do Darwin's finches come from? *Evol. Rev.* **3** 185–195

Wang Z, Xu L, Zhu X, Cui W, Sun Y, Nishijo H, Peng Y and Li R 2010 Demethylation of specific Wnt/β−Catenin pathway genes and its upregulation in rat brain induced by prenatal valproate exposure. *Anat. Rec.* **293** 1947–1953

Webster G and Goodwin B 1982 The origin of species: a structuralist approach. *J. Soc. Biol. Struct.* **5** 15–47

Wu P, Jiang TX, Shen JY, Widelitz RB and Chuong CM 2006 Morphoregulation of avian beaks: comparative mapping of growth zone activities and morphological evolution. *Dev. Dyn.* **235** 1400–1412

Wu P, Jiang TX, Suksaweang S, Widelitz RB and Chuong CM 2004 Molecular shaping of the beak. *Science* **305** 1465–1466

Wyngaarden LA, Vogeli KM, Ciruna BG, Wells M, Hadjantonakis AK and Hopyan S 2010 Oriented cell motility and division underlie early limb bud morphogenesis. *Development* **137** 2551–2558

Young NM, Chong HJ, Hu D, Hallgrímsson B and Marcucio RS 2010 Quantitative analyses link modulation of sonic hedgehog signaling to continuous variation in facial growth and shape. *Development* **137** 3405–3409

Zeller R, López-Ríos J and Zúñiga A 2009 Vertebrate limb bud development: moving towards integrative analysis of organogenesis. *Nat. Rev. Genet.* **10** 845–858

Zhang YT, Alber MS and Newman SA 2013 Mathematical modeling of vertebrate limb development. *Math. Biosci.* **243** 1–17

Zhu J, Nakamura E, Nguyen MT, Bao X, Akiyama H and Mackem S 2008 Uncoupling Sonic hedgehog control of pattern and expansion of the developing limb bud. *Dev. Cell* **14** 624–632

Zhu J, Zhang Y-T, Alber MS and Newman SA 2010 Bare bones pattern formation: a core regulatory network in varying geometries reproduces major features of vertebrate limb development and evolution. *PLoS One* **5** e10892

Zusi RL 1993 Patterns of diversity in the avian skull; in *The skull* (eds) J Hanken and BK Hall (Chicago: University of Chicago Press) pp 391–437