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Unlocking the Black Box between Genotype and Phenotype: Cell Condensations as Morphogenetic (modular) Units

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Abstract. Embryonic development and ontogeny occupy what is often depicted as the black box between genes – the genotype – and the features (structures, functions, behaviors) of organisms – the phenotype; the phenotype is not merely a one-to-one readout of the genotype. The gene's home, context, and locus of operation is the cell. Initially, in ontogeny, that cell is the single-celled zygote. As development ensues, multicellular assemblages of like cells (modules) progressively organized as germ layers, embryonic fields, *anlage*, condensations, or blastemata, enable genes to play their roles in development and evolution. As modules, condensations are fundamental developmental and selectable units of morphology (morphogenetic units) that mediate interactions between genotype and phenotype via evolutionary developmental mechanisms. In a hierarchy of emergent processes, gene networks and gene cascades (genetic modules) link the genotype with morphogenetic units such as condensations, while epigenetic processes such as embryonic inductions, tissue interactions and functional integration, link morphogenetic units to the phenotype. To support these conclusions I distinguish units of heredity from units of transmission and discuss epigenetic inheritance by tracing the history of relationship between embryology and evolution, especially the role(s) assigned to cells or to cellular components in generating theories of morphological change in evolution. The concept of cells as modular morphogenetic units is modeled and illustrated using the mammalian dentary bone.

Key words: cell condensations, development, embryonic fields, epigenetics, evolution, evolutionary developmental biology, genotype, modularity, modules, morphogenetic units, phenotype

1. Introduction

> Or you could say that gamete was seeking gamete, genotype genotype, in order that there should be zygote and phenotype. (Byatt 1986: 175)

1.1. *Genotype and phenotype*

The terms genotype and phenotype refer, respectively, to all the genes and all the features of an individual.[1] Although gene interactions and hierarchical effects such as epistasis and pleiotropy were recognized in the first few decades of the 20th century, many assumed a one-to-one relationship between genotype and phenotype. The phenotype is not merely, however, a one-to-one readout of the genotype. Genes and the inherited activation and repression states of genes are insufficient; both a component (unit, module) and a mechanism (epigenetics, emergent properties) between genes and structures are required. The fields of developmental genetics, life history theory, morphogenesis and pattern formation, phenotypic plasticity, physiological genetics, physiology, and reaction norms, all exist because neither developmental nor evolutionary change can be explained by genes alone. So we can ask: "what components and processes lie between the inherited genotype (including the phenotype of the gene) and phenotypes?"

1.2. *Black box or treasure chest*

The short answer to this question is a simple one: development occupies what is often depicted as a black box between genotype and phenotype (Figure 1). When considered in the context of population or ecological approaches to evolution, development is the 'black box' between mutation and selection (Arthur 1997, 2000; Hall 2002a, b; Hall et al. 2003). The components in the black box are embryos, the processes' the hierarchical mechanisms of embryonic development. More inclusively, the black box is a nested box or babushka doll containing the stages and processes of ontogeny that enable genes (Hall 2001a).[2]

In this paper I address the role of condensations – groups of like cells – as structures, morphogenetic units, and as loci of processes between the genotype and the phenotype in ontogeny and phylogeny. Study of the units and processes (the evolutionary developmental mechanisms; Hall and Olson 2003) at this interface is one of the primary aims of evolutionary developmental biology or evo-devo.[3]

In order to open the black box, I begin with discussions of units or transmission and heredity, including epigenetic inheritance, and follow with an overview of relationships between embryology and evolution in the 19th and 20th centuries and the search for the cellular components of development and evolution. This leads to discussions of emergent, hierarchical interactions, the genotype-phenotype map, cell sociology cells (cells as modules) and condensations as morphogenetic units, illustrated by a model system, the mammalian dentary bone.

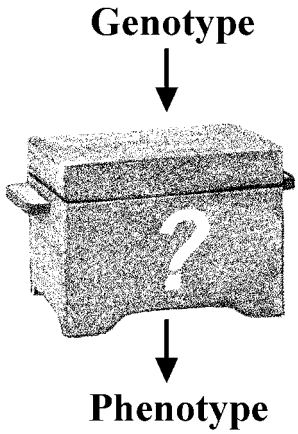

Figure 1. The mechanisms and processes of development and ontogeny, depicted as the black box between genotype and phenotype.

1.3. *Units of heredity and transmission*

By emphasizing the role of cells, I want to be very explicit and not misunderstood. I am *not* downgrading the role of genes, either in development or in evolution. Despite calls to the contrary (Jablonka and Lamb 1998; Oyama et al. 2001), genes are the hereditary units whose passage through multiple generations can be modified by mutation and selection (Hall 1999, 2001a). Genes are not, however, "law-code and executive power – or . . . architect's plan and builder's craft – in one" (Schroedinger 1944: 23). Genes provide the building blocks whose translation, elaboration, and interpretation are key to phenotypic changes in ontogeny and phylogeny, and whose products provide the raw material for ontogeny and for the sequence of ontogenies known as phylogeny. This appreciation can be shared by both evolutionary developmental biologists – for whom a gene is a heritable unit that mutates, reveals phylogenetic relationships, and provides a basis for macroevolution – and by population geneticists, for whom a gene is a heritable unit that mutates, spreads through populations, reveals the role of natural selection, and provides the basis for microevolution (Beurton et al. 2000; especially Gilbert 2000a).

Biological units other than genes *are* passed from generation to generation: the egg or ovum, mitochondria and protoplasts, structural elements of the cytoskeleton (microtubules, microfilaments), mRNA (both short- and long-lived), and proteins, precursors and enzymes, many of which are maternal gene products. Although *units of transmission* – they pass from generation to generation – these maternal products are *units of heredity*, not for the individual possessing them, but rather for the individual (mother) who

deposited them into her eggs (Maynard Smith 1989, 1990; Hall 1998a). These cellular constituents and gene products are essential: they must be present for a new generation to be initiated. Genes (as sequences of DNA) are not sufficient.

1.4. *Epigenetic inheritance*

Whether these cytoplasmic components are inherited in the same way that genes are inherited or whether there is an 'epigenetic inheritance system', has been a matter of debate among and between biologists and philosophers.[4] In simplistic (but perhaps accurate) terms, many of the components listed above are products of maternal genes and subject to mutation and selection acting on the female parent. Using the terminology introduced above, the maternal products are units of transmission, not of heredity, while the maternal genes that produced them are units of heredity, not of transmission, giving their products, but not themselves, to the next generation. Consequently, each individual at the beginning of its ontogeny is a hybrid of maternal and zygotic gene products. The old feud between epigenesis and preformation is refought in every egg as its preformed constituents epigenetically activate zygotic genes to initiate the phenotype that is a new generation (Hall 1998a, 1999; Müller and Olsson 2003). How did this modern view arise?

2. A brief overview of relationships between embryology and evolution

Although he devoted only one chapter to embryological evidence, Darwin (1859) knew that embryology would provide the strongest evidence for his theory. Almost every late 19th C embryologist signed on to and pursued embryos, not for their own sakes, but for the story they would tell about the ancestry of, and transitions between, the major groups of animals.

The broad relationships recognized today between earlier and later stages within the same embryo, and between the embryos of closely and more distantly related groups at various developmental stages of animal embryos, may be traced back to von Baer (1828), whose interest was embryological, comparative and systematic – not evolutionary, and certainly not recapitulationist. Ernst Haeckel, the arch-recapitulationist, proposed that ontogeny repeats adult ancestral stages (Haeckel 1866, 1905). If Thomas Huxley was Darwin's bulldog, Ernst Haeckel was Darwin's rotweiler. Belief in the recapitulation of adult ancestors in the ontogenetic sequences of their descendants strangled any meaningful integration of development and evolution for almost 100 years (Hall 2000a, 2002b, d). Had the analysis of embryos

to understand evolution been undertaken outside of Haeckel's recapitulationist framework, embryology would have been much more receptive to the rediscovery of Mendelian genetics in 1990, geneticists would have been receptive to the important role of embryos other than as vehicles to transport genes, paleontologists would not have been led down the destination-less path of orthogenesis, while social constructs such as eugenics, racism and segregation might well have had different outcomes in the 20th century (Punnett 1922; Provine 1971; Olby 1985; Kevles 1995; Hall 2002c, d).[5] Haeckel has much to answer for.

Even von Baer drew us away from seeking evolutionary alterations early in development. Early embryonic development was seen as stable and immune from change because that was when the broad features of animals (the body plan or *Bauplan*) were laid down (Woodger 1945; Hall 1996, 1999, 2002b, 2003a). By 1880, differences between early embryonic stages among different phyla were becoming evident to some evolutionary morphologists (Lankester, Balfour, Haeckel) who saw that natural selection could act as readily on early development as on adults (Hall 1999, 2000a). Nowadays, changes early in embryogenesis are appreciated, studied, and their existence incorporated into studies of life history evolution. Indeed, virtually all changes in any feature – structure, behavior or function – involve changes in development. If developmental processes link genotype and phenotype, as this paper will argue that they do, then we need to look for those processes at any and all developmental stages.

Even several decades ago, biologists were not seeking links between changes in development and evolutionary change, let alone how changes in development affect evolutionary change. Evo-devo exists, in large part, because we require an explanans of the causal relationships between the genotype and the phenotype, both within and between generations. Had evo-devo not arisen, genes would have remained the province of molecular biologists and developmental geneticists, cells of cell biologists and cell physiologists, and embryos of embryologists.

3. Development, evolution, epigenetics and evo-devo

3.1. *Connections*

Beyond the long-recognized parallel that both individual development and the history of life on earth proceed from single-celled to multicellular forms, and the impression that complexity (whatever that means) increases with both gestational and generational time, there is no intuitively obvious connection between development and evolution. In a tradition going back to

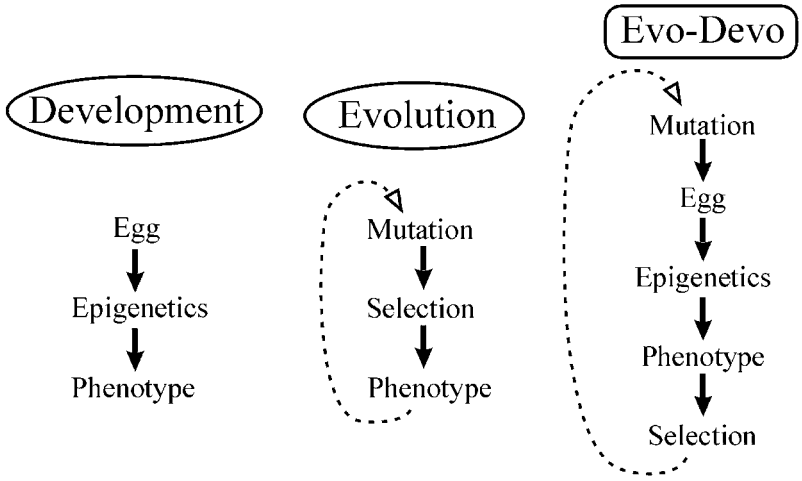

Figure 2. Depictions of development, evolution and evo-devo to show relationships between proximate and ultimate levels of causation. The solid arrows show flow within a generation, the dashed line, flow between generations. See text for details.

Aristotle, we compartmentalize development and evolution as proximate and ultimate (internal and external) causes (Mayr 1982; Hall 1999). Evo-devo seeks to integrate proximate and ultimate explanations. Separate disciplines, intellectual traditions, training, research programmes, funding organizations, even separate institutions and philosophies, guide the independent study of development and evolution. Development is the transformation from egg to phenotype under the control of *genetic and epigenetic* processes (see below), while evolution is the selection of mutations affecting phenotypic change. In this traditional view epigenetics does not impinge on evolution, selection does not impinge on development (Figure 2).

The types, if not the mechanisms, of mutation and selection are well (although not fully) understood and we have robust theories uniting the roles of mutation and selection in the evolutionary process. Although as important as selection, epigenetics is far less well understood; the term and the concept are barely peeping out from the black box (Hall 1999, 2001b), even though we need the processes of epigenetics or 'phenogenetics' (Weiss and Fullerton 2000) to understand relationships between genotype and phenotype.

With historical links to epigenesis and to preformation (Hall 1999; Müller and Olsson 2003), the term epigenetics originated with Waddington (1956), who proposed it as an alternative to Roux's *Entwicklungsmechanik* or developmental mechanics.[6] Waddington emphasized the roles of genes in development, but also that genes operate within a context; the same genes could be active and associated with a particular cell differentiation in one cell type,

associated with a different pathway of differentiation in a second cell type, and/or inactive in a third cell type. Epigenetics is "the sum of the genetic and non-genetic factors acting upon cells to control selectively the gene expression that produces increasing phenotypic complexity during development" (Hall 1999: 114).

Epigenetics does have other meanings. For geneticists, epigenetics is how genes are modified before being passed from generation to generation. Each new generation inherits the base sequences of DNA and an epigenetic code (the phenotype of the gene) which direct patterns of gene expression in early development. Such genic modification includes patterns of methylation and imprinting of DNA, random chromosome inactivation (as of one X chromosome in placental mammals), or non-random inactivation, as in chromosome diminution in the roundworm *Parascaris* (Holliday 1994; Hall 1998a, 1999, 2001a, c; Müller and Olsson 2003).

4. Relating development to evolution

Evolution occurs at at least three different levels: changes in gene frequency, the appearance of new characters, and the appearance, adaptation and radiation of new species. The common denominator of all three is genetic change through time. Change in gene frequency operates at the population level through mutation, selection, drift, migration and meiotic drive. The appearance of new characters and the appearance and adaptive radiation of species require alterations of ontogeny, even if the adaptation is behavioral or physiological (Hall 2003b; Hall et al. 2003). Changes which we think of as characterizing descent with modification – the origin of new phenotypes, gigantism, dwarfism, neoteny, paedomorphosis, mimicry, phenotypic plasticity, even speciation – are all changes that arise through the modification of developmental processes (Patterson 1983; Hall 1999; Hall and Olson 2003). The 'modification' in Darwin's descent with modification is the modulation during phylogeny of developmental stages, processes, sometimes entire ontogenies (Hall 2002a, b).

As is now well known (but came as a great surprise when discovered), there is a conservation of genes in animals with very diverse forms of morphology. This is especially true for what have been called regulatory, developmental, switching, or selection genes (MacLean and Hall 1987; Hall 1996, 1999; Carroll et al. 2001; Wilkins 2002). The 'same' genes – i.e., genes whose sequences are so similar that they are homologous (orthologous) – are used over and over again during ontogeny in all animal phyla.

The processes that allow similar genes to have different outcomes in different tissues, organs or phyla are epigenetic. Figure 2 shows how the

apparently separate mechanisms of development and evolution come together in the integrative approach of evo-devo (Hall 1999; Robert et al. 2001). As depicted: mutations introduce variation (to any stage in ontogeny); eggs transmit maternal and zygotic genetic and epigenetic information from generation to generation; as the causal control of development, epigenetics provides hierarchical developmental processes that produce embryonic, larval and adult phenotypes; upon which selection operates to spread change through populations and/or to conserve features within individuals. Selection on distinct (modular) elements of the phenotype *at any stage in ontogeny*, and mutation in subsequent generations, establishes a tight integration of development and evolution in a cycle that brings about both individual development and progressive change or stability over time.[7]

5. Whither cells?

> . . . the cells pullulated and divided, boiled and extruded, arranged genes, chromosomes, proteins, plans, patterns, another life, the same life in another form. (Byatt 1986: 237)

5.1. *Hierarchical interactions*

Genes are the units of heredity transmission and tissues the units of phenotypic construction. Where do cells and cellular processes fit?

Because of the ability of similar and dissimilar cells to interact, cells play central roles in interfacing genotype and phenotype. When Chandebois and Faber (1983) speak of "cell sociology," Maclean and Hall (1987) of "stochastic behaviour," Gurdon (1988) of "a community effect," Larsen (1992) of "tissue strategies," and Gilbert (1992) of "cells in search of community," each is dealing explicitly with the collective behavior of cells, behavior that changes during ontogeny. Mechanisticly, the emerging hierarchy of *cell-cell* (epigenetic) interactions during embryogenesis reflects a hierarchy of developmental processes at different levels of organization Interactions occur:

- within eggs, between preexisting products of genes, initially maternal and then zygotic;
- between and among individual cells during early embryogenesis (essentially during cleavage and early gastrulation);
- among groups of like cells in germ layers, and between dissimilar cells in adjacent germ-layers (embryonic inductions) beginning during gastrulation and including induction of the mesoderm as a secondary germ layer;

- among dissimilar condensations of cells from gastrulation onwards, to initiate or to maintain tissue differentiation, morphogenesis and growth; and
- among tissues, to produce and integrate organs into the functioning entity we know as an embryo. (See Maclean and Hall 1987; Hall 1983, 1999, 2002a, b; Gilbert 2000a, b for analyses.)

5.2. *The genotype-phenotype map and units of morphology*

Concepts such as cell sociology and community effects take us to a discussion of the *units of morphology* (the modules) by which cell differentiation, morphogenesis and growth are initiated in post-gastrula-stage embryos.

Morphogenetic units are the fundamental cellular component of the structural biological hierarchy of genes → cells → tissues → organs → organisms → species from which animal morphology is constructed. I will argue that cell condensations are the morphogenetic units above the level of the genes, that cell condensations are to evo-devo as genes or species are to evolution and as embryos are to development. To do this I provide a brief overview of studies on the role of cells in development and evolution; summarize and extend the arguments made by Atchley and Hall (1991) for cell condensations as morphogenetic units; discuss the example used in that model (the mammalian dentary bone, the "single" bone that, along with Meckel's cartilage, constitutes the skeleton of the lower jaw of all mammals); and evaluate how research on the molecular control of condensation formation, gene knock-out experiments in mice, and studies of quantitative trait loci (QTLs), reinforce the model of modular control of morphogenesis and allow an even stronger commitment to condensations as modular units than Bill Atchley and I supposed in 1991.

5.3. *Cells in development*

With acceptance of the universality of the cell theory, debates began over which of the cellular constituents we now know as nucleus and cytoplasm 'controlled' development. This search was not completed until the structure and role of DNA was discovered almost three hundred years after cells were recognized as such (Wilson 1896, 1925; Conklin 1905; Willmer 1960; Gilbert 1978; Harris 1999). Most embryologists in the 19th century did not regard cells as fundamental units of embryos or fundamental units of development. For them, embryos were constructed on a higher order from germ layers. Cell lineage analysis of the late 19th and early 20th centuries, along with a (reluctant) shaking off of germ-layer theory, changed all that.

At the turn of the 20th century people like Conklin and Wilson described cell lineages in embryos. Tracing the *genealogy* of a cell in a mosaic embryo into later and later stages – what begat what – was viewed as tracing the *fate* of that cell, a deterministic approach to fundamental units. Tracing family or cell trees, however, is not the same as understanding how individuals or cells take on their roles; lineage is not determination. Cell-lineage analysis can provide such information, but only when combined with specific and targeted analyses of cell fate (Wray and Raff 1989, 1990; Meinertzhagen 2002; Guralnick and Lindberg 2003).

Embryonic (developmental, morphogenetic) fields were a later concept; imaginal discs in *Drosophila* are a classic example. Fields were identified in abstract terms with reference to magnetic fields in physics (Haraway 1976) and as real identities following extirpation of regions of early stage embryos and transplantation to other embryonic regions (Harrison 1918, 1969). Remove of a small area of ectoderm and subjacent mesoderm (mesenchyme) from the region in a frog neurula from which the forearm was known to develop, transplant that region elsewhere in the embryo, and an ectopic forearm would arise. Only cells from that area would produce a limb when so grafted. Other regions, when grafted, produced hind limbs, tails, hearts, kidneys, depending on their original position in the donor embryos.

5.4. *Nucleus or cytoplasm?*

> Long ago it became evident that the key to every biological problem must finally be sought in the cell; for every living organism is, or at sometime has been, a cell. (Wilson 1925: 1)

In the first (1896) edition of *The Cell in Development and Inheritance*, Wilson made an important attempt to bring epigenesis and preformation together within the context of nuclear or cytoplasmic control of development, marshaling all the evidence for *parallel* inheritance of nuclear chromosomes and cytoplasmic organelles. He stressed interactions between nucleus and cytoplasm directing development – the cytoplasm as the context for the nucleus (Hall 2001a). Of course, in 1896, Wilson did not know where the major influence was coming from. By the third (1925) edition of *The Cell in Development and Inheritance*, twenty five years after Mendelian genetics had resurfaced (and with the new title *The Cell in Development and Heredity*), Wilson's view was one that many of us would not be uncomfortable with today:

> . . . in respect to a great number of characters *heredity is effected by the transmission of a nuclear preformation which in the course of develop-*

> *ment finds expression in a process of cytoplasmic epigenesis*. (Wilson 1925: 1112, his emphasis).

Wilson was not prepared to concede that all characters are under nuclear control ("the transmission of a nuclear preformation"). He still saw some characters as entirely under cytoplasmic (epigenetic) control.[8] Most of us today, conditioned by the old argument of epigenesis and preformation, would take the view that we inherit nuclear genes, the ovum nucleus, mitochondria (and their genes; protoplasts in plants), microfibrils and microfilaments as preformed elements present in the egg from the initiation of development – indeed present before fertilization. For those preformed elements to function, there have to be interactions, initially with maternal cytoplasmic constituents. Such interactions are often triggered by environmental factors such as temperature, pH, osmotic pressure, chemicals released from predators, and so forth (Hall et al. 2003). Once the zygote begins to cleave and becomes multicellular, the interactions are increasingly between cells, especially between epithelial and mesenchymal cells as gastrulation and cell differentiation ensues.

The discovery of the structure of the 'molecule of life' in the mid-20th century turned attention away from cells and toward DNA, apparently settling the long debate over whether the nucleus or the cytoplasm controls development. Now rather than embryos transporting abstract hereditary units from generation to generation, cells transported DNA – the molecule of life – from generation to generation. Cells remained important, but as the basis for a new cell biology, not a new biology of heredity. Cells were the power houses that drove organismal function. Elucidation of their fine structure and functioning led to Nobel Prizes for cell biologists. But cells were no longer players in fields that sought to understand how the genotype 'becomes' or 'arises from' the phenotype. Sixty-five years ago Ross Harrison was concerned that genes were being given too much importance as controllers of development:

> Already we have theories that refer the processes of development to genic action and regard the whole performance as no more than the realization of the potencies of the gene. Such theories are altogether too one-sided . . . The prestige of success enjoyed by the gene theory might easily become a hindrance to the understanding of development by directing our attention solely to the genom, whereas cell movements, differentiation and in fact all developmental processes are actually effected by the cytoplasm. (Harrison 1937: 372).

So, what *is* the link between cells and the genome?

With few exceptions, all the cells of an individual contain the entire genome.[9] Possession of all the genes as sequences of DNA, however, is

not the same as saying that all the genes are active in all cells, i.e., that all genes transcribe their DNA into mRNA and translate that mRNA into proteins. "All" cells contain all genes, but not all genes are active (switched-on, upregulated, transcribed) in all cells. Cellular properties and processes regulate selective activation and repression of the genome.

5.5. *Cells as modules*

If we examine early embryos we find cells *groups of like cells* that have coherence and identity that separates them from other groups of cells that also have identity and coherence, but with which they may be able to interact. This aggregation of like cells, coupled with the ability of cells to interact through migrating, signaling, embryonic induction, and epithelial-mesenchymal interactions, allows for the identification of morphogenetic units and the processes of epigenetics discussed above. Atchley and Hall (1991) emphasized *cell condensations* as morphogenetic units ('modules' *à la* Wagner 1996; Wagner and Altenberg 1996; Bolker 2000; Mezey et al. 2000; Gass and Bolker 2003). The concept of independent yet interactive developmental units can be traced back to Joseph Needham, who took a broadly integrative approach to embryonic organization with his discussion of what he termed "dissociability", the proposal that there are independent units within the developing embryo which, to some extent, can be disassociated from one another (Needham 1933).

Fields and condensations have distinct properties with intrinsic, spatial and temporal components. Any field exists only for a finite time. Fields can regulate (the ability of a portion of a field to produce the structures that arise from a whole field) but regulation is lost as development ensues. For example, condensations cannot regulate. Indeed, if a condensation for an individual skeletal element is too small – as can happen following mutation, surgical division of a condensation or formation of condensations *in vitro* – the resulting skeletal element will be small or may even fail to form. Alterations in the size, location, or time of appearance of condensations can result in abnormal morphology, as seen in mutations (including many that affect the mammalian skeleton), congenital anomalies and gene knock-outs (Grüneberg 1963; Hall and Miyake 1992, 1995, 2000).

Modules have been much discussed in the recent literature; see Wagner and Altenberg (1996) and Gass and Bolker (2003) for overviews. Is a gene a module? Is a DNA sequence a module? Is a sequence of genes in a gene cascade, or network, or up- and downstream pathway a module? Is a limb field a module? Is the condensation for the humerus a module? What of a condensation from which two skeletal elements arise – the common condensation for two of the middle ear ossicles, or the common condensation

for the tibia and fibula? Or, a condensation within which a cartilage and a bone arise?[10]

For me, the most useful way of thinking about modules in relation to the black box (Figure 1), is to think at the level of cell condensations whose activity enables structures to be initiated, and groups of cells to respond to selection to effect morphological change; as few as 30 cells in *Drosophila* embryos can be 'seen' by selection (Weber 1992). Both fields and condensations represent times of selective gene activation specific to the structure that will develop. We have the cell as the unit of organic structure and function, and fields and condensations as loci of gene action, serving as morphogenetic units mediating genotype-phenotype interactions.

6. Condensations as morphogenetic units

6.1. *Condensations*

Condensations may be visualized using the cell surface marker peanut agglutinin lectin and visualized using horse radish peroxidase or a flurochrome (Figure 3), methods that shows that condensations have cohesion, uniformity and a distinct boundary. They arise in one of four ways, which are not mutually exclusive: aggregation toward a center, failure to move away from a center, more rapid proliferation than surrounding cells, or slower rates of cell death than surrounding cells (Hall 1978; Fyfe and Hall 1993; Hall and Miyake 1992, 1995, 2000).

Condensations represent the initial onset of selective gene activity for the specialized molecules that 'define' or at least allow, identification of particular cell types. Expression of type II (cartilage-type) collagen at the onset of chondrogenesis is an obvious example. Before the first overt cartilage cells can be visualized, and certainly before any extracellular matrix has been synthesized or deposited, condensing cells upregulate mRNA for type II collagen as much as 100 fold (Kosher et al. 1986). Condensed cells also upregulate the core protein for the proteoglycan that will be deposited into the extracellular matrix. mRNA for type I (bone-fibroblast-type) collagen is not upregulated. Examine an osteogenic condensation and you will find upregulation of mRNA for type I collagen but not for type II. Hans Grüneberg (1963), aware that condensation represented an identifiable, critical and general step in skeletogenesis, named this stage "the membranous skeleton."

While Figure 3 represents the general pattern for condensations – a condensation that makes a single cell type (chondroblast) from which a single tissue (cartilage) or a single organ (Meckel's cartilage) develops – this only hints at underlying complexity. The condensation of mesenchymal cells that

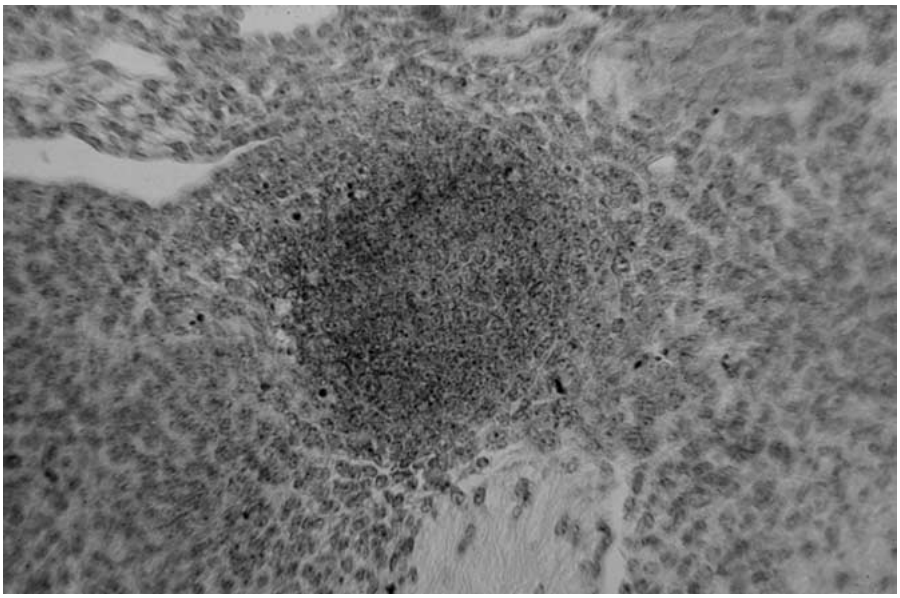

Figure 3. The condensation for the hyoid bone in a mouse embryo visualized using peanut agglutinin lectin and horse radish peroxidase. Note the clear boundary to the condensation.

contribute to the development of mammalian teeth differentiates into five cell and tissue types: odontoblasts (dentine), fibroblasts (pulp, periodontal ligament), cementoblasts (cementum) and osteoblasts (alveolar bone) (Smith and Hall 1990; Thesleff 1991).

Different individual molecules or molecular cascades initiate condensation formation, determine condensation size, set the condensation boundary, switch off condensation, and initiate the next stage in cytodifferentiation. Each step is a true threshold phenomenon. Cells will remain at the condensation stage unless the signals to transit to the next stage are present (Hall and Miyake (2000). Imaginal discs in *Drosophila* are nice examples. Future discs can be identified soon after the germ-band stage of embryogenesis as condensations of cells (Bate and Arias 1991). Cells remain as determined but undifferentiated cells within the disc until the onset of synthesis of juvenile hormone associated with molting at the third instar triggers them to differentiate along the precise path already determined. Transplant the discs from larva to larva so that they are not exposed to JH – as Hadorn (1978) did – and the cells remain determined but fail to differentiate. Transplant the discs into a larva and differentiation is initiated.[11]

Condensations for chondrogenesis anywhere in the body are controlled by similar genetic pathways. These shared pathways of gene regulation in cells that will differentiate as a particular cell type, e.g., a chondroblast, have

superimposed upon them, in later differentiation, genetic controls that are specific to specific condensations of cells, and therefore specific to particular cartilages (or bones). Furthermore, some molecular signals are used at more than one stage, cellular and temporal contexts determining whether signaling molecules such as BMPs and TGF-ßs will initiate cell division, differentiation or death.

Such ubiquity of signaling has special consequences for our understanding of the development and evolution of the phenotype. Shared initial pathways and divergent later pathways mean that those mutational changes that affect earlier stages are likely to affect many (perhaps all) skeletal elements, while later-acting mutations are more likely to affect specific (perhaps individual) cartilages. Influences early in development are likely to result in loss of elements, or major structural or functional changes. Influences later in development are likely to affect what appear to be more minor aspects of size and shape. What looks minor to our eyes, however, may have major consequences for the organism.

6.2. *A model system*

As an example of condensations as morphogenetic units, I use the morphology and morphogenesis of the dentary bone. Mammals have a single dentary bone in each of their left and right lower jaws. Evolutionarily, mammals arose from 'mammal-like reptiles' which had multiple bones in their lower jaws; the dentary persisted, while other lower jaw bones were either lost or transformed into middle ear ossicles. The mammalian dentary has a complex shape reflecting common *morphological components*: the ramus or body, alveolar bone associated with the teeth, and three bony processes (condylar, angular and coronoid) situated posteriorly (Figure 4). This morphology is recognizable irrespective of taxon-specific changes (Figure 5), even when those are dramatic, perhaps one of the most dramatically modified dentary bones being the lanceolate, almost edentate dentary of the nectar, pollen and insect-feeding honey possum or noolbender from Western Australia (Figure 6).[12]

Atchley and Hall (1991) went beyond the long-recognized evidence of morphological components of the dentary when they argued that each of these morphological components forms from a *morphogenetic unit*, identifying ramal, alveolar and process units, each of which is derived from a separate population (condensation) of cells. These morphogenetic units – all of which are derivatives of neural crest cells (Hall 1998b, 2000b) – have distinctive histogenic histories: The ramal unit forms by intramembranous ossification. The alveolar units also form by intramembranous ossification, but from a condensation of cells that also differentiates into the fibroblasts of

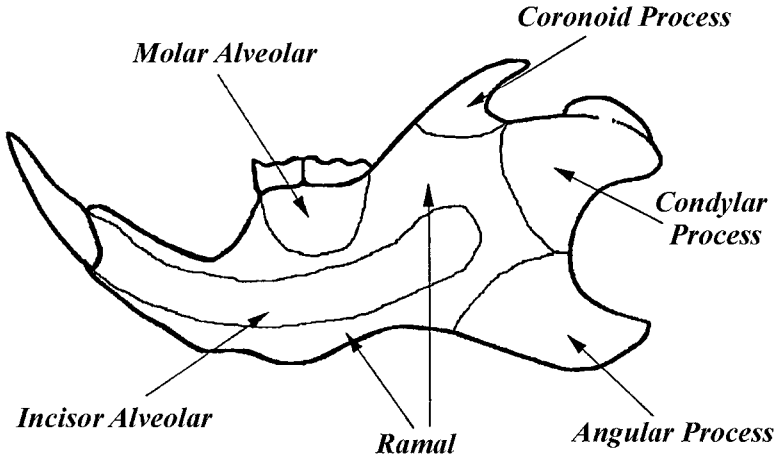

Figure 4. A diagrammatic representation of the dentary of a mouse to illustrate the morphological components/morphogenetic units.

the periodontal ligament. The units that form the posterior processes consist of cells that differentiate into secondary cartilage subsequently replaced by bone through endochondral ossification; neither ramal nor alveolar units can form bone endochondrally (Figure 4).

As already discussed, the genes and gene cascades regulating condensation formation are similar (Hall and Miyake 2000). Knock-out experiments in mice demonstrate that some genes (*goosecoid*, *msx-1*, TGFß-2, for example) affect only particular morphogenetic units (Figure 7, and see Richman and Mitchell 1996; Smith and Schneider 1998; MacDonald and Hall 2001). Mice in which *msx-1* has been knocked out have normal dentary bones, except that the teeth and associated alveolar bone fail to develop (Satokata and Maas 1994). Consequently, we conclude that *Msx-1* is required for alveolar units to form. Knocking out *Goosecoid* results in dramatic reduction in the size of the coronoid and angular processes but does not affect the condylar process (Rivera-Pérez et al. 1995). Consequently, we conclude that *goosecoid* is required for normal growth of two of the three processes. Knocking out TGFß-2 is correlated with reduced sizes of all three processes (Martin et al. 1995). Consequently, we conclude that TGFß-2 is required for growth of all three processes. It is clear that morphogenesis and growth of the morphogenetic units of the dentary are under separate genetic control.

A number of lines of evidence indicate that *goosecoid*, *Msx-1*, and TGFß-2 act *directly* on skeletogenic cells.

Goosecoid acts cell autonomously in craniofacial mesenchyme. Chimeric murine embryos composed of *goosecoid*-expressing and *goosecoid*-null cells, have nasal capsule and mandibular defects equivalent to those found in

Figure 5. Medial (upper) and lateral (lower) views of the dentary of the Magdalen Isl. subspecies of the meadow vole, *Microtus pennsylvanicus madgalensis*, obtained by the author in July, 2002 with the assistance of Quest Nature Tours, Canada. Note the highly conserved morphological components when compared with the mouse (Figure 4).

goosecoid mutant embryos, the decrease in size of condylar and angular processes correlating with the proportion of *goosecoid*-null cells in those elements. The tympanic bone, which is absent from *goosecoid* mutant embryos, forms as a condensation in chimeras but has missing portions later in development, leading Rivera-Pérez et al. (1999) to conclude that *goosecoid*-expressing cells gather *goosecoid*-null cells into the condensation. Ectopic application of BMP-2 or BMP-4 in chick mandibular mesenchyme alters the expression of *Msx-1*, followed by formation of two Meckel's cartilages (Barlow and Francis-West 1997). Grafting BMP-4-producing cells into paraxial mesoderm of chick embryos upregulates *Msx-1* and *Msx-2*, resulting in formation of ectopic cartilage in the pectoral girdle (Watanabe and Le Douarin 1996). Expression of the *Msx-1* is required to maintain gap junctions between limb mesenchymal cells, while assembly and disassembly

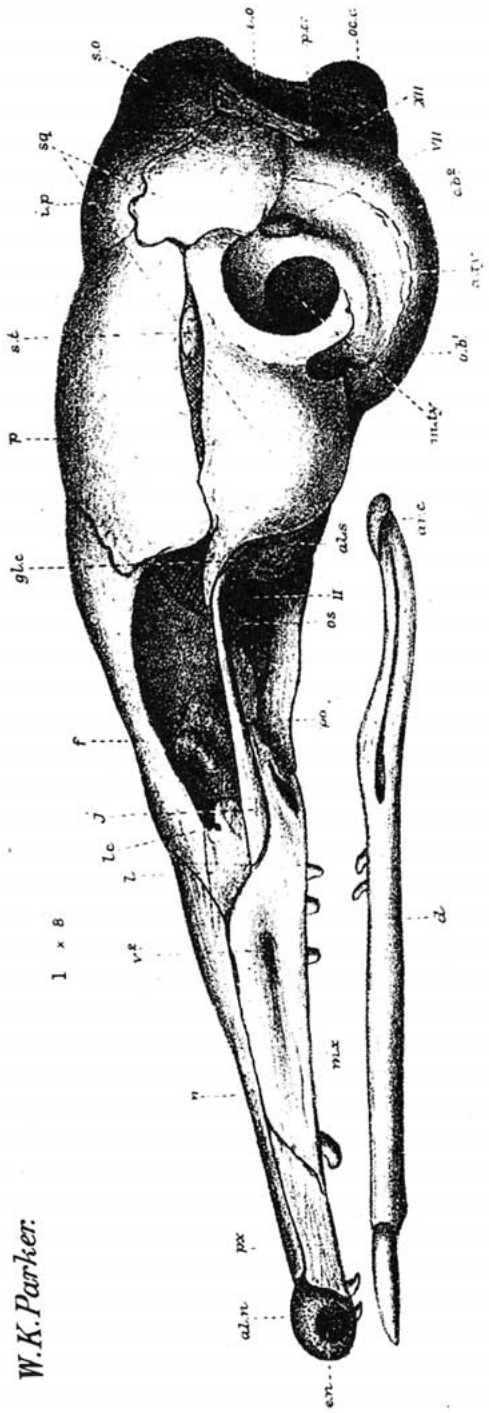

Figure 6. The Western Australian honey possum, *Tarsipes rostratus*, has a dentary (d) that is exceeding narrow, lacks any projecting posterior processes and has very reduced, peg-like molar teeth and a forward-projecting incisor (cf. Figures 4 and 5). Reproduced from Parker (1890).

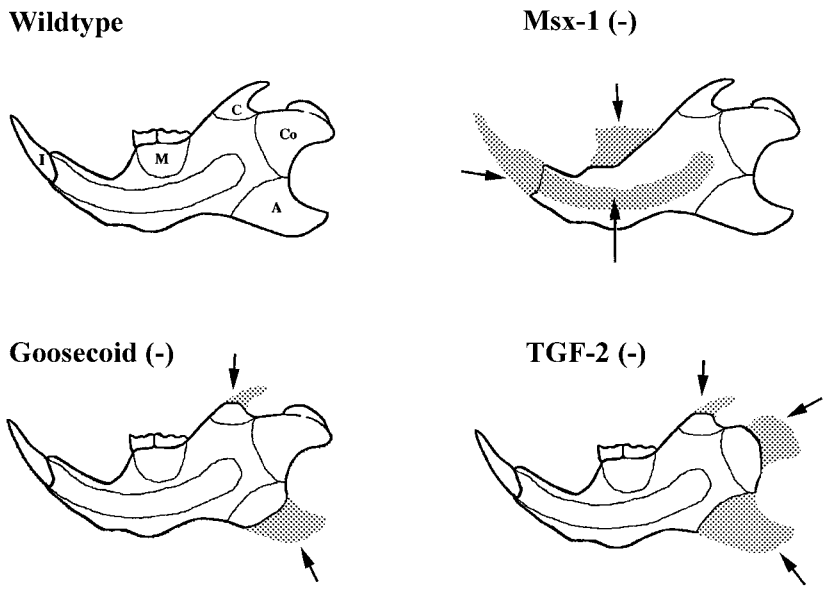

Figure 7. Outlines of mouse dentary bones to show (cross-hatched, arrows) the morphogenetic units that fail to form when the genes *Msx-1*, *Goosecoid* or *TGF-ß-2* are knocked out. See Figure 4 for the morphogenetic units, and see the text for details.

of gap junctions are part of condensation formation for the limb skeleton in mice (Zimmerman 1984; Ferrari et al. 1994; Hall and Miyake 2000)

Whether *goosecoid*, *Msx-1*, and TGFß-2 also influence skeletal morphogenetic units *indirectly* by affecting muscle action has yet to be determined. This is important, for in addition to specific *intrinsic* (but nonetheless epigenetic) genetic controls of morphogenetic units are epigenetic signals that lie external to the dentary bone or to individual condensations. One epigenetic signal is muscle action (Atchley and Hall 1991; Herring 1993).

Muscles insert preferentially onto the three posterior processes, with homologous muscles inserting onto homologous processes in different mammalian taxa. As one example, the lateral pterygoid muscle inserts onto the condylar process. Congenital absence of this muscle results in the condylar process failing to form, but has no affect on other portions of the dentary. A smaller than normal muscle or late onset of the muscle in development will result in a smaller than usual condylar process. Experimentally induced hyperactivity of the lateral pterygoid muscle leads to an unusually large condylar process; the secondary cartilage which provides the basis for the endochondral ossification of the condylar process requires mechanical stimulation to form (Hall 1978). Once initiated, lineages of cells condense and differentiate under intrinsic genetic control, while morphogenesis and

growth are under genetic and epigenetic controls that may be specific to individual condensations.

Examination of the honey possum (Figure 6) reveals a natural example of the relationship between muscle action and the initiation, morphogenesis and growth of the posterior processes, examination of which would shed light on whether genes such as *goosecoid* and *TGFß-2* act via the musculature or directly on the skeletogenic cells of the appropriate morphogenetic unit. The dentary of honey possums lacks an angular process, the coronoid process is no more than a slight elevation, while the condylar process is represented only by the upturned posterior terminus of the dentary (Figure 6). In the honey possum, the lateral pterygoid muscle (which inserts onto the condylar process in most mammals) extends from maxilla and palatine of the upper jaw to insert along the dorsal surface of the dentary (see Figure 6 in Rosenberg and Richardson 1995). Several predictions follow from the fact that the temporalis (which inserts onto the coronoid process in other mammals) inserts onto the dorsal surface of the dentary anterior to the lateral pterygoid. An analysis of embryonic development should reveal that: altered positions of muscle insertions (as a consequence of which, honey possums generate extremely low bite forces) precede failure of dentary processes to form; condensations for the processes have been lost or are present but cannot be activated and/or grow; and/or action of genes such as goosecoid or TGFß-2 (either in the muscles or in the condensations) has been downregulated.

The modularity of dentary morphogenesis and growth is reinforced by analyses using morphometrics, finite element scaling, analysis of recombinant inbred strains, microsatellite molecular markers and quantitative trait loci (QTL) mapping, which show that the posterior processes and alveolar regions of rodent dentaries are modular (Bailey 1986; Cheverud et al. 1991, 1997; Duarte et al. 2000; Mezey et al. 2000), as indeed is the skull (Hanken and Hall 1993; Dos Reis et al. 2002). In the F2 generation of a cross of two inbred mouse strains, half of 27 QTLs affected the posterior processes, 27% the alveolar processes and 23% the whole dentary (Cheverud et al. 1997).

7. Conclusion

The gene's home, context, and locus of operation is the cell. Initially, in ontogeny, that cell is the single-celled zygote. As development ensues, multicellular assemblages of like cells, progressively organized as germ layers, embryonic fields, *anlage*, condensations, or blastemata, enable genes to play their roles in development and evolution.

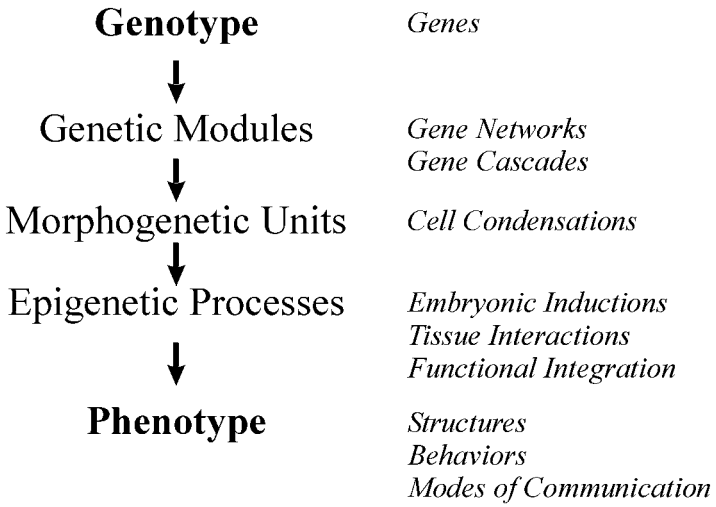

Figure 8. An integrated representation of the units and processes that lie within the black box linking genotype and phenotype (left) and the hierarchical units operating at each level (right).

This modular and hierarchical cellular organization allows like cells to receive the intra- and extraorganismal environmental and epigenetic signals that allow organisms to develop, adapt to their environment, modify their development and translate the effects of mutations into phenotypic change on both developmental (including regeneration) and evolutionary (including asexual reproduction) time scales.

At the cellular level, condensations (as modules) are fundamental developmental and selectable units of morphology (morphogenetic units) that mediate interactions between genotype and phenotype via evolutionary developmental mechanisms. Both intrinsic and extrinsic developmental processes affect condensations to modulate morphological change during ontogeny and phylogeny. In a hierarchy of emergent processes (Figure 8), gene networks and gene cascades (genetic modules) link the genotype with morphogenetic units such as condensations, while epigenetic processes such as embryonic inductions, tissue interactions and functional integration, link morphogenetic units to the phenotype.

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Notes

[1] Distinctions between genes and the phenotype are much closer now. For example, we employ the phrase 'the phenotype of the gene' for inherited patterns such as DNA methylation, the structural conformation states of chromatin, and genetic imprinting (Lewontin 1974, 2000; Maclean and Hall 1987; Holliday 1994; Hall 1998a, 1999, 2001a; Keller 2000; Griffiths 2001; Malcolm and Goodship 2001).

[2] I use ontogeny for all the stages of the life cycle between egg and mature (usually reproductive) adult, although ontogeny can be equated with all stages of the life history of an individual from fertilization to death (Maclean and Hall 1987; Hall 1999). I use development for the stages of embryonic development between the zygote and birth or hatching. 'Developmental' processes are not restricted to embryos but can contribute to any stage in ontogeny (Hall and Wake 1999; Hall et al. 2003).

[3] See the preface in Hall (1992) for the origin of the term evolutionary developmental biology, and see Raff (1996), Arthur (1997), Hall (1999), Hall and Olson (2003) and Hall et al. (2003) for overviews and syntheses of evo-devo.

[4] For the various definitions of epigenetics, and for discussions of epigenetic inheritance systems, or whether there is such a thing as epigenetic inheritance, see, Hall (1983, 1998a, 1999, 2001a, c), MacLean and Hall (1987), Sapp (1987), Maynard Smith (1989, 1990); Jablonka and Lamb (1998), Holliday (1994), Wolf et al. (1998), van der Weele (1999); Beurton et al. (2000), Petronis (2001) and Müller and Olsson (2003).

[5] The close connection between embryos and evolution seen in the 19th century was lost in the first half of the 20th. Embryos, which had been so central to 'heredity' (which included transmission and development), became mere vehicles carrying genes (the 'real' hereditary units, i.e. units of transmission) from one generation to the next. Embryologists studied development, geneticists studied evolution (Gilbert 1991), although the origins of the gene theory may be found in embryology (Gilbert 1978).

[6] "The leader in . . . [the study of the causal processes of development] was Wilhelm Roux, who coined the title 'Entwicklungsmechanik' for such studies. . . . Its literal translation in English is 'developmental mechanics', a phrase which is not only rather long and clumsy as the name of a branch of science, but which carries a perhaps unfortunate suggestion that only machine-like, physical processes are being envisaged . . . Perhaps the most satisfactory expression would be 'epigenetics'. This is derived from the Greek word epigenesis, which Aristotle used for the theory that development is brought about through a series of causal interactions between the various parts; it also reminds one that genetic factors are among the most important determinants of development. It is, however, not yet in common use" (Waddington 1956: 10).

[7] 'At any stage in ontogeny' includes the maternal cytoplasmic constituents inherited by each egg, these having been deposited during oogenesis, i.e., selection on products of the *maternal genome*, along with cell lineages and patterns of cleavage, which, along with mutations in the zygotic genes, influence the next generation. Most refer to such patterns as 'maternal inheritance,' the phenotypic effects of maternal genes being delayed by a generation, some-

times by two. Sturtevant and Beadle (1939: 329–331) caution against using the term, given the potential confusion with 'cytoplasmic inheritance.' However, the term is entrenched, and, I think, defensible.

[8] At the same time, Morgan had separated transmission from development in his considerations of heredity: ". . . [we should] keep apart the phenomenon of heredity, that deals with the transmission of the hereditary units, and the phenomena of embryonic development that take place almost exclusively by changes in the cytoplasm" (Morgan 1926: 490). Lewontin (1992: 33) referred to such views as. ". . . an artifact of another error of vulgar biology, that it is only the genes that are passed from parent to offspring . . . We inherit not only genes made of DNA but an intricate structure of cellular machinery made up of proteins."

[9] As is always the case in biology, the few exceptions are interesting and instructive. They include: loss of the nucleus at the terminal stage in the differentiation of mammalian red blood cells; random inactivation of one of the X-chromosomes in female placental mammals (otherwise, females would have a double dose of the genes on the X chromosome, males having one X and one Y chromosome); chromosome diminution in round worms and in some midges (in which cells are determined as germ cell or somatic on the basis of retention of all or elimination of most chromosomes. See MacLean and Hall (1987), Sapp (1987) and Hall (1999) for further discussion of these examples and their significance for our understanding of gene regulation and cell commitment.

[10] For genes or gene cascades as modules, see Abouheif (1997), Carroll et al. (2001), Wilkins (2002), Gass and Bolker (2003), and Hall and Olson (2003). For multiple skeletal elements arising in single condensations, see Dunlop and Hall (1995), Miyake et al. (1996), Smith and Schneider (1998), Hall (1999) and Hall and Miyake (2000). For morphogenetic fields, see Van Valen (1970), Haraway (1976) and Gilbert et al. (1996).

[11] 'Transdetermination' was discovered in the imaginal disc-transplant experiments carried out by Hadorn (1978). After residing in larvae for many generations, and then being allowed to develop in the presence of JH, an occasional imaginal disc produced a structure (usually an entire appendage) characteristic of a different imaginal disc. An eye disc would produce an antenna, for example. Clearly, an alternate state of determination could exist in individual discs. Ernst Hadorn should have received a Nobel Prize for this work, which sits (temporally and conceptually) midway Bateson's naming of homeosis and the discovery of the genes responsible for such homeotic transformations.

[12] The honey possum has the smallest weight at birth (<5 mg), the longest sperm (360 mm), and the largest testes relative to body weight, of any mammal.

References

Abouheif, E.: 1997. 'Developmental Genetics and Homology: A Hierarchical Approach', *Trends in Ecology and Evolution* **12**, 405–408.

Arthur, W.: 1997, *The Origin of Animal Body Plans: A Study in Evolutionary developmental Biology*, Cambridge University Press, Cambridge.

Arthur, W.: 2000, 'The Concept of Developmental Reprogramming and the Quest for an Inclusive Theory of Evolutionary Mechanisms', *Evolution and Development* **2**, 49–57.

Atchley, W.R. and Hall, B.K.: 1991, 'A Model for Development and Evolution of Complex Morphological Structures and Its Application to the Mammalian Mandible', *Biological Reviews of the Cambridge Philosophical Society* **66**, 101–157.

Baer, K.E. von.: 1828. *Über Entwicklungsgeschichte der Thiere. Beobachtung und Reflexion*, Gebrüder, Bornträger, Königsberg.

Bailey, D.: 1986, 'Genes that Affect Morphogenesis of the Murine Mandible, Recombinant Inbred Strain Analysis', *Journal of Heredity* **77**, 17–25.

Barlow, A.J. and Francis-West, P.H.: 1997, 'Ectopic Application of Recombinant BMP-2 and BMP-4 can Change Patterning of Developing Chick Facial Primordia', *Development* **124**, 391–398.

Bate, M. and Arias, A.M.: 1991. 'The Embryonic Origin of Imaginal Discs in *Drosophila*', *Development* **112**, 755–761.

Beurton, P.J., Falk, R. and Rheinberger, H.-J. (eds): 2000, *The Concept of the Gene in Development and Evolution*, Cambridge University Press, Cambridge.

Bolker, J.A.: 2000, 'Modularity in Development and Why it Matters to Evo-Devo', *American Zoologist* **40**, 770–776.

Byatt, A.S.: 1986. *Still Life*, Penguin Books, London.

Carroll, S.B., Grenier, J.K. and Weatherbee, S.D.: 2001, *From DNA to Diversity. Molecular Genetics and the Evolution of Animal Design*, Blackwell Science, Malden, MA.

Chandebois, R. and Faber, J.: 1983, *Automation in Animal Development. A New Theory Derived from the Concept of Cell Sociology*, Monographs in Developmental Biology, Volume 16, S. Karger AG, Basle.

Cheverud, J.M., Hartman, S.E., Richtsmeier, J.T. and Atchley, W.R.: 1991, '1A Quantitative Aenetic Analysis of Localized Morphology in Mandibles of Inbred Mice Using Finite Element Scaling Analysis', *Journal of Craniofacial Genetics and Developmental Biology* **11**, 122–137.

Cheverud, J.M., Routman, E.J. and Irschick, D.J.: 1997, 'Pleiotropic Effects of Individual Gene Loci on Mandibular Morphology', *Evolution* **51**, 2006–2016.

Conklin, E.G.: 1905, 'The Mutation Theory from the Standpoint of Cytology', *Science* **21**, 525–529.

Darwin, C.: 1859, *The Origin of Species by Means of Natural Selection*, John Murray, London.

Dos Reis, S.F., Duarte, L.C., Monteiro, R. and von Zuben, F.J.: 2002, 'Geographic Variation in Cranial Morphology in *Thrichomys apereoides* (Rodentia: Echimyidae). I. Geometric Descriptors and Patterns of Variation in Shape', *Journal of Mammalogy* **83**, 333–344.

Duarte, L.C., Monteiro, L.R., von Zuben, F.J. and Dos Reis, S.F.: 2000, 'Variation in Mandible Shape in *Thrichomys apereoides* (Mammalia: Rodentia): Geometric Analysis of a Complex Morphological Structure', *Systematic Biology* **49**, 563–578.

Dunlop, L.-L.T. and Hall, B.K.: 1995, 'Relationships between Cellular Condensation, Preosteoblast Formation and Epithelial-Mesenchymal Interactions in Initiation of Osteogenesis', *International Journal of Developmental Biology* **39**, 357–371.

Ferrari, D., Kosher, R.A. and Dealy, C.N.: 1994, 'Limb Mesenchymal Cells Inhibited from Undergoing Cartilage Differentiation by a Tumor Promoting Phorbol Ester Maintain Expression of the Homeobox-Containing Gene Msx1 and Fail to Exhibit Gap Junctional Communication', *Biochemical and Biophysical Research Communications* **205**, 429–434.

Fyfe, D.M. and Hall, B.K.: 1993, 'The origin of Ectomesenchymal Condensations which Precede Development of the Bony Scleral Ossicles in the Eyes of Embryonic Chicks', *J. Embryology and experimental Morphology* **73**, 69–86.

Gass, G.L. and Bolker, J.A.: 2003, 'Modularity', in B.K. Hall and W.M. Olson (eds), *Keywords and Concepts in Evolutionary Developmental Biology*, Harvard University Press, Cambridge, MA, pp. 260–267.

Gilbert, S.F.: 1978. 'The Embryological Origins of the Gene Theory', *J. History of Biology* **11**, 307–351.

Gilbert, S.F. (ed.): 1991, *Developmental Biology: A Comprehensive Synthesis. Volume 7, A Conceptual History of Modern Embryology*, Plenum Press, New York.

Gilbert, S.F.: 1992, 'Cells in Search of Community: Critiques of Weismannism and Selectable Units in Ontogeny', *Biology and Philosophy* **7**, 473–487.

Gilbert, S.F.: 2000a, 'Genes Classical and Genes Developmental: The Different Use of Genes in Evolutionary Syntheses', in P.J. Beurton, R. Falk and H.-J. Rheinberger (eds), *The Concept of the Gene in Development and Evolution*, Cambridge University Press, Cambridge, pp. 178–192.

Gilbert, S.F.: 2000b, *Developmental Biology* (6th edn.), Sinauer Associates, Sunderland, MA.

Gilbert, S.F., Opitz, J.M. and Raff, R.A.: 1996, 'Resynthesizing Evolutionary and Developmental Biology', *Developmental Biology* **173**, 357–372.

Griffiths, P.E.: 2001, 'Genetic Information: A Metaphor in Search of a Theory', *Philosophy of Science* **68**, 394–412.

Grüneberg, H.: 1963, *The Pathology of Development*, Blackwell Scientific Publications, Oxford.

Guralnick, R. and Lindberg, D.R.: 2003. 'Lineages: Cell and Phyletic', in B.K. Hall and W.M. Olson (eds), *Keywords and Concepts in Evolutionary Developmental Biology*, Harvard University Press, Cambridge MA, pp. 243–249.

Gurdon, J.B.: 1988. 'A Community Effect in Animal Development', *Nature* **366**, 772–774.

Hadorn, E.: 1978, 'Transdetermination', in M. Ashburner and T.R.F. Wright (eds), *Genetics and Biology of Drosophila*, Academic Press, New York, Vol. 2C, pp. 556–617.

Haeckel, E.: 1866. *Generelle Morphologie der Organismen*, Vols. 1 & 2, Berlin.

Haeckel, E.: 1905, *The Riddle of the Universe at the Close of the Nineteenth Century* (Joseph McCabe, trans.), Harper & Brothers Publishers, New York and London.

Hall, B.K.: 1978, *Developmental and Cellular Skeletal Biology*, Academic Press, New York.

Hall, B.K.: 1983, 'Epigenetic Control in Development and Evolution', in B.C. Goodwin, N.J. Holder and C.C. Wylie (eds), *Development and Evolution*, British Society for Developmental Biology Symposium 6, Cambridge University Press, London and Cambridge, pp. 353–379.

Hall, B.K.: 1992, *Evolutionary Developmental Biology*, Chapman & Hall, London.

Hall, B.K.: 1996, 'Baupläne, Phylotypic Stages and Constraint: Why There are so Few Types of Animals', *Evolutionary Biology* **29**, 215–261.

Hall, B.K.: 1998a, 'Epigenetics: Regulation not Replication', *J. Evolutionary Biology* **11**, 201–205.

Hall, B.K.: 1998b, 'Germ Layers and Germ-Layer Theory Revisited. Primary and Secondary Germ Layers, Neural Crest as a Fourth Germ Layer, Homology, and Demise of the Germ-Layer Theory', *Evolutionary Biology* **30**, 121–186.

Hall, B.K.: 1999, *Evolutionary Developmental Biology*, (2nd edn.), Kluwer Academic Publishers, Dordrecht, Netherlands.

Hall, B.K.: 2000a, 'Balfour, Garstang and de Beer: The First Century of Evolutionary Morphology', *American Zoologist* **40**, 718–728.

Hall, B.K.: 2000b, 'The Evolution of the Neural Crest in Vertebrates', in L. Olsson and C.-O. Jacobson (eds), *Regulatory Processes in Development*, Wenner-Gren International Series Volume 76, The Portland Press, London, pp. 101–113.

Hall, B.K.: 2001a, 'The Gene is Not Dead, Merely Orphaned and Seeking a Home', *Evolution and Development* **3**, 225–228.

Hall, B.K.: 2001b, 'Foreword', in M. Zelditch (ed.), *Beyond Heterochrony: The Evolution of Development*, John Wiley & Sons, Inc., New York, pp. vii–ix.

Hall, B.K.: 2001c, 'A Review of *Images of Development: Environmental Causes in Ontogeny* by Cor Van der Weele', *Evolution and Development* **3**, 366–368.

Hall, B.K.: 2002a, 'Homoplasy and Homology: Dichotomy or Continuum?', in C. Lockwood and J. Fleagle (eds), *Homoplasy in Primate and Human Evolution*, Cambridge University Press, Cambridge, in press.

Hall, B.K.: 2002b, 'Descent with modification: The Unity Underlying Homology and Homoplasy as Seen Through an Analysis of Development and Evolution', *Biological Reviews of the Cambridge Philosophical Society* (in press).

Hall, B.K.: 2002c, 'Evolutionary Developmental Biology: Where Embryos and Fossils Meet', in N. Minugh-Purves and K.J. McNamara (eds), *Human Evolution Through Developmental Change*, Johns Hopkins University Press, Baltimore, pp. 7–29.

Hall, B.K.: 2002d, 'Palaeontology and Evolutionary Developmental Biology: A Science of the 19th and 21st Centuries', *Palaeontology* **45**, 647–669.

Hall, B.K.: 2003a, 'Woodger, Joseph Henry (1894–1981)', *Oxford Dictionary of National Biography, in association with the British Academy*, Oxford University Press, Oxford (in press).

Hall, B.K.: 2003b, 'Evolution as the Control of Development by Ecology', in B.K. Hall, R. Pearson and G.B. Müller (eds), *Environment, Evolution and Development: Towards a Synthesis*, MIT Press, Cambridge MA. (in press).

Hall, B.K. and Miyake, T., 1992, 'The Membranous Skeleton: The Role of Cell Condensations in Vertebrate Skeletogenesis', *Anatomy and Embryology* **186**, 107–124.

Hall, B.K. and Miyake, T.: 1995, 'Divide, Accumulate, Differentiate: Cell Condensation in Skeletal Development Revisited', *International Journal of Developmental Biology* **39**, 881–893.

Hall, B.K. and Miyake, T.: 2000, 'All for One and One for All: Condensations and the Initiation of Skeletal Development', *BioEssays* **22**, 138–147.

Hall, B.K. and Olson, W.M. (eds): 2003, *Keywords and Concepts in Evolutionary Developmental Biology*, Harvard University Press, Cambridge, MA.

Hall, B.K., Pearson, R.D. and Müller, G.B. (eds): 2003 *Environment, Development and Evolution; Toward a Synthesis*, MIT Press, Cambridge MA (in press).

Hall, B.K. and Wake, M.H. (eds.: 1999, *The Origin and Evolution of Larval Forms*, Academic Press, San Diego, CA.

Hanken, J. and Hall, B.K.: 1993. *The Skull Volume 1, Development*, The University of Chicago Press, Chicago.

Haraway, D.J.: 1976, *Crystals, Fabrics, and Fields. Metaphors of Organicism in Twentieth-Century Developmental Biology*, Yale University Press, New Haven.

Harris, H.: 1999, *The Birth of the Cell*, Yale University Press, New Haven.

Harrison, R.G.: 1918, 'Experiments on the Development of the Forelimb of *Amblystoma*, a Self-Differentiating, Equipotential System', *J. Experimental Zoology* **25**, 413–462.

Harrison, R.G.: 1937, 'Embryology and Its Relations', *Science* **85**, 370–374.

Harrison, R.G.: 1969. *Organization and Development of the Embryo* (S. Wilens, ed.), Yale University Press, New Haven and London.

Herring, S.W.: 1993, 'Epigenetic and Functional Influences on Skull Growth', in J. Hanken and B.K. Hall (eds), *The Skull Volume 1, Development*, The University of Chicago Press, Chicago, pp. 153–206.

Holliday, R.: 1994, 'Epigenetics: An Overview', *Developmental Genetics* **15**, 453–457.

Jablonka, E. and Lamb, M.J.: 1998, 'Epigenetic Inheritance in Evolution', *J. Evolutionary Biology* **11**, 159–183.

Keller, E.F.: 2000, *The Century of the Gene*, Harvard University Press, Cambridge, MA.

Kemp, T.S.: 1982, *Mammal-Like Reptiles and the Origin of Mammals'*, Academic Press, London.

Kevles, D.J.: 1995, *in the Name of Eugenics: Genetics and the Uses of Human Heredity*, with a new preface by the author, Harvard University Press, Cambridge, MA.

Kosher, R.A., Kulyk, W.M. and Gay, S.W.: 1986, 'Collagen Gene Expression During Limb Cartilage Differentiation', *J. Cell Biology* **102**, 1151–1156.

Larsen, E.W.: 1992, 'Tissue Strategies as Developmental Constraints: Implications for Animal Evolution', *Trends in Ecology and Evolution* **7**, 414–417.

Lewontin, R.: 1974, *The Genetic Basis of Evolutionary Change*, Columbia University Press, New York.

Lewontin, R.: 1992, 'The Dream of the Human Genome,' *New York Review of Books*, May 28, 31–40.

Lewontin, R.: 2000, *The Triple Helix: Gene, Organism, and Environment*, Harvard University Press, Cambridge, MA.

Maclean, N.M. and Hall, B.K.: 1987, *Cell Determination and Differentiation*, Cambridge University Press, Cambridge.

MacDonald, M.E. and Hall, B.K.: 2001, 'Altered Timing of the Extracellular-Matrix-Mediated Epithelial-Mesenchymal Interaction that Initiates Mandibular Skeletogenesis in Three Inbred Strains of Mice: Development, Heterochrony, and Evolutionary Change in Morphology', *J. Experimental Zoology* **291**, 258–273.

Malcolm, S. and Goodship, J.: 2001. *Genotype to Phenotype* (2nd edn.), Academic Press, San Diego.

Martin, J.F., Bradley, A. and Olson, E.N.: 1995, 'The Paired-Like Homeobox Gene *MHox* is Required for Early Events of Skeletogenesis in Multiple Lineages', *Genes and Development* **9**, 1237–1249.

Maynard Smith, J.: 1989, 'Weismann and Modern Biology', in P.H. Harvey and L. Partridge (eds), *Oxford Surveys in Evolutionary Biology*, Volume 6, Oxford University Press, Oxford, pp. 1–12.

Maynard Smith, J.: 1990, 'Models of a Dual Inheritance System', *J. Theoretical Biology* **143**, 41–53.

Mayr, E.: 1982, *The Growth of Biological Thought: Diversity, Evolution, and Inheritance*, The Belknap Press of Harvard University Press, Cambridge, MA.

Meinertzhagen, I.A.: 2002, 'Cell Lineage', in M. Pagel (ed.-in-chief), *Encyclopedia of Evolution*, Vol. 1, Oxford University Press, New York, NY, pp. 142–144.

Mezey, J.G., Cheverud, J.M. and Wagner, G.P.: 2000, 'Is the Genotype-Phenotype Map Modular? A Statistical Approach Using Mouse Quantitative Trait Loci Data', *Genetics* **156**, 305–311.

Miyake, T., Cameron, A.M. and Hall, B.K.: 1996. 'Stage-Specific Onset of Condensation and Matrix Deposition for Meckel's and other First Arch Cartilages in Inbred C57BL/6 Mice', *J. Craniofacial Genetics and Developmental Biology* **16**, 32–47.

Morgan, T.H. (1926).: 'Genetics and the Physiology of Development', *American Naturalist* **60**, 489–515.

Müller, G.B. and Olsson, L.: 2003, 'Epigenesis and Epigenetics', in B.K. Hall and W.M. Olson (eds), *Keywords and Concepts in Evolutionary Developmental Biology*, Harvard University Press, Cambridge, MA, pp. 114–123.

Needham, J.: 1933, 'On the Dissociability of the Fundamental Processes in Ontogenesis', *Biological Reviews of the Cambridge Philosophical Society* **8**, 180–223.

Olby, R.: 1985; *Origins of Mendelism* (2nd edn.), The University of Chicago Press, Chicago.

Oyama, S., Griffiths, P.E. and Gray, R.D. (eds): 2001, *Cycles of Contingency. Developmental Systems and Evolution*, The MIT Press, Cambridge, MA.

Parker, W.K.: 1890, 'On the Skull of *Tarsipes rostratus*', *Studies from the Museum of Zoology, University College Dundee*, 79–83 + plate.

Patterson, C.: 1983, 'How Does Phylogeny Differ from Ontogeny', in B.C. Goodwin, N.J. Holder and C.C. Wylie (eds), *Development and Evolution*, British Society for Developmental Biology Symposium 6 Cambridge University Press, London and Cambridge, pp. 1–31.

Petronis, A.: 2001, 'Human Morbid Genetics Revisited: Relevance of Epigenetics', *Trends in Genetics* **17**, 142–146.

Provine, W.: 1971, *The Origins of Theoretical Population Genetics*, The University of Chicago Press, Chicago.

Punnett, R.C.: 1922, *Mendelism*, MacMillan and Co., London.

Raff, R.A.: 1996, *The Shape of Life: Genes. Development, and the Evolution of Animal Form*, University of Chicago Press, Chicago.

Richman, J. and Mitchell, P.J.: 1996, 'Craniofacial Development: Knockout Mice Take One on the Chin', *Current Biology* **6**, 364–367.

Rivera-Pérez, J.A., Mallo, M., Gendron-Maguire, M., Gridley, T. and Behringer, R.B.: 1995, '*Goosecoid* is Not an Essential Component of the Mouse Gastrula Organizer but is Required for Craniofacial and Rib Development', *Development* **121**, 3005–3012.

Rivera-Pérez, J.A., Wakamiya, M. and Behringer, R.R.: 1999, '*Goosecoid* Acts Cell Autonomously in Mesenchyme-Derived Tissues During Craniofacial Development', *Development* **126**, 3811–3821.

Robert, J.S., Hall, B.K. and Olson, W.M.: 2001, 'Bridging the Gap between Developmental Systems Theory and Evolutionary Developmental Biology', *BioEssays* **23**, 954–962.

Rosenberg, H.I. and Richardson, K.C.: 1995, 'Cephalic Morphology of the Honey Possum, *Tarsipes rostratus* (Marsupialia: Tarsipedidae): An Obligate Nectarivore', *Journal of Morphology* **233**, 303–323.

Sapp, J.: 1987, *Beyond the Gene: Cytoplasmic Inheritance and the Struggle for Authority in Genetics*, Oxford University Press, Oxford.

Satokata, I. and Maas, R.: 1994, '*Msx*1 Deficient Mice Exhibit Cleft Palate and Abnormalities of Craniofacial and Tooth Development', *Nature Genetics* **6**, 348–356.

Schroedinger, E.: 1944, *What is Life*, Cambridge University Press, Cambridge.

Smith, K.K. and Schneider, R.A.: 1998, 'Have Gene Knockouts Caused Evolutionary Reversals in the Mammalian First Arch?' *BioEssays* **20**, 245–255.

Smith, M.M. and Hall, B.K.: 1990, 'Developmental and Evolutionary Origins of Vertebrate Skeletogenic and Odontogenic Tissues', *Biological Reviews of the Cambridge Philosophical Society* **65**, 277–374.

Sturtevant, A.H. and Beadle, G.W.: 1939, *An Introduction to Genetics*.

Thesleff, I.: 1991, 'Tooth Development,' *Dental Update* November, 382–387.

van der Weele, C.: 1999, *Images of Development. Environmental Causes in Ontogeny*, State University of New York Press, Albany, NY.

Van Valen, L.: 1970, 'An Analysis of Developmental Fields', *Developmental Biology* **23**, 456–477.

Waddington, C.H.: 1956, *Principles of Embryology*, George Allen & Unwin Ltd., London.

Wagner, G.P.: 1996, 'Homologues, Natural Kinds and the Evolution of Modularity', *American Zoologist* **36**, 36–43.

Wagner, G.P. and Altenberg, L.: 1996, 'Perspective: Complex Adaptations and the Evolution of Evolvability', *Evolution* **50**, 967–976.

Watanabe, Y. and Le Douarin, N.M.: 1996, 'A Role for BMP-4 in the Development of Subcutaneous Cartilage', *Mechanisms of Development* **57**, 69–78.

Weber, K.E.: 1992. 'How Small are the Smallest Selectable Domains of Form?', *Genetics* **130**, 345–353.

Weiss, K.M and Fullerton, S.M.: 2000. 'Phenogenetic Drift and the Evolution of Genotype-Phenotype Relationships', *Theoretical Population Biology* **57**, 187–195.

Wilkins, A.S.: 2002, *The Evolution of Developmental Pathways*, Sinauer Associates, Sunderland, MA.

Willmer, E.N.: 1960, *Cytology and Evolution*, Academic Press, New York.

Wilson, E.B.: 1896, *The Cell in Development and Inheritance*, Macmillan, New York.

Wilson, E.B.: 1925, *The Cell in Development and Heredity* (3rd edn.), Macmillan, New York.

Wolf, J.B., Brodie, E.D. III., Cheverud, J.M., Moore, A.J. and Wade, M.J.: 1998, 'Evolutionary Consequences of Indirect Genetic Effects', *TREE* **13**, 64–69.

Woodger, J.H.: 1945, 'On Biological Transformations', in W.L. le Gros Clark and P.B. Medawar (eds), *Essays on Growth and Form Presented to D'Arcy Wentworth Thompson*, Cambridge University Press, Cambridge, pp. 95–120.

Wray, G.A. and Raff, R.A.: 1989. 'Evolutionary Modification of Cell Lineage in the Direct-Developing Sea Urchin', *Heliocidaris erythrogramma, Developmental Biology* **132**, 458–470.

Wray, G.A. and Raff, R.A.: 1990. 'Novel Origins of Lineage Founder Cells in the Direct-Developing Sea Urchin', *Heliocidaris erythrogramma, Developmental Biology* **141**, 41–54.

Zimmermann, B.: 1984, 'Assembly and disassembly of Gap Junctions During Mesenchymal Cell Condensation and Early Chondrogenesis in Limb Buds of Mouse Embryos', *Journal of Anatomy* **138**, 351–364.