

Embryonic motility: environmental influences and evolutionary innovation

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INTRODUCTION

Embryos do not passively await hatching from their eggs or amniotic containments but begin active movement very early on in their development. The first muscle contractions in the chick embryo start on the third day of incubation and subsequently assume a characteristic pattern of increasing and decreasing motor activity (Fig. 1) (Hamburger 1963; Bekoff 1981, 1992). It is long known that embryonic motility represents an important epigenetic component of development. In vertebrates, active movement of the embryo is required for the correct development of cartilage, bone, and joints; of muscles, tendons and ligaments; and of connectivities in the central nervous system. The intrauterine disturbance of embryonic movements leads to severe malformations and functional disorders, such as pathological motor patterns and neurological deficits that are retained throughout adult life (Bos et al. 2001). It is equally known that embryonic activity depends on environmental conditions, both chemical and physical, and that changes of environmental parameters can strongly affect the motility patterns. However, the effect of such environmental influences on development, via the alteration of embryonic activity, is little explored. Even less is known about the possible evolutionary consequences of this kind of environment–development interaction. This article provides an outline of the relevance of environment dependent embryonic activity for evolutionary developmental biology.

INFLUENCES OF THE ENVIRONMENT ON EMBRYONIC MOTILITY

Motor activity of the embryo is influenced by electrical, mechanical, thermal, and chemical factors (overview in Romanoff 1960). Decrease of temperature, for instance, not only reduces all metabolic activity but as a consequence also

diminishes motor activity (Oppenheim and Levin 1975; Nechaeva and Turpaev 1991). Also, the intensity of ambient light conditions was recently shown to affect embryonic motility (Wu et al. 2001). A 12-fold increase of illumination intensity on the chicken egg results in a 220% average increase of embryo movements (Fig. 2). The relative experimental effect is stronger during periods of low endogenous activity and is less pronounced during periods of high endogenous activity. These effects can be explained in part by the transmission of stimuli via the optic system of the embryo, although reactions can be elicited already before functional optic pathways are established. In addition, an increase of light intensity also affects passive movement of the embryo by amnion contractions. Contraction rates are increased, on average, by 40% during high illumination periods. This effect on the autonomous amnion pacemaker is probably elicited by electrical or chemical stimuli, such as the release of neurotransmitters or prostaglandins, or may reflect a direct sensitivity of the amnion cells (Wu et al. 2001). Together, these results indicate that general changes of ambient light conditions and behavioral activity of the parents affecting the nest and local light intensity can have strong effects on active and passive motility of the embryos.

MOVEMENT-INDUCED CARTILAGE AND BONE FORMATION

In vertebrates, cartilage cell differentiation and cartilage matrix production depend on a mechanosensitive interaction between a number of genes and gene products. The most important of these are *Sox9*, which acts as a transcriptional activator of type II collagen production, and interleukin-1 β , which acts as a transcriptional repressor of type II collagen and negatively regulates the glycosaminoglycan-aggrecan system but positively regulates the matrix metalloproteases (Fig. 3). Under the influence of static compressive force

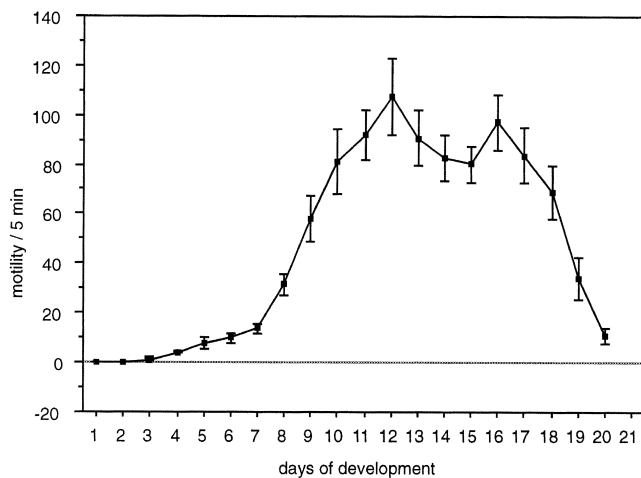


Fig. 1. Frequencies of embryonic movements in the chick (Wu et al. 2001).

Sox9 is up-regulated in chondrogenic cells and interleukin-1 β is down-regulated, leading to an increase of glycosaminoglycan synthesis by up to 59% and of type II collagen mRNA by about 15%, whereas metalloprotease production is down-regulated. This results in an overall 2- to 3-fold increase of type II collagen and aggrecan in compressed three-dimensional culture systems containing chondrocytes (Takahashi et al. 1998). Tension forces act on the BMP system (Sato et al. 1999; Aspenberg et al. 2000; Ikegame et al. 2001) and influence the arrangement and proliferation of chondrogenic cells (Harris et al. 1980, 1984; Bard 1990). Recent results suggest that *indian hedgehog* is a key signaling molecule upstream of BMP in the mechanotransduction pathway leading to chondrocyte proliferation (Wu et al. 2001a).

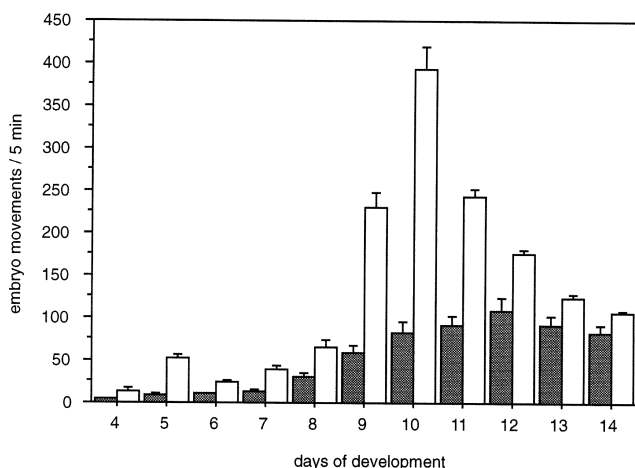


Fig. 2. Effects of a 12-fold increase of illumination intensity on movement frequencies in the chick embryo. Open bars, experimental; shaded bars, controls. (Wu et al. 2001.)

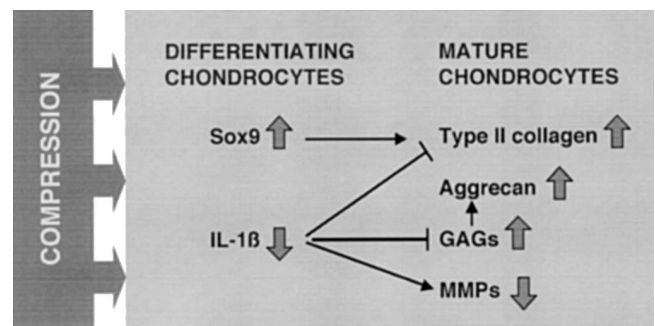


Fig. 3. Molecular mechanisms involved in compressive force regulation of vertebrate chondrogenesis. IL, interleukin; GAG, glycosaminoglycan; MMP, matrix metalloproteases. (After Takahashi et al. 1998.)

Based on these and other molecular mechanisms (e.g., Klein-Nulend et al. 1995; Nomura and Takano-Yamamoto 2000), movement-dependent biomechanical stimulation is responsible for cartilage and bone remodeling during embryogenesis and postembryonic life (Carter et al. 1987). Motor activity thus has a direct influence on the size and shape of skeletal elements and, in particular, on the formation of joints (Drachman and Sokoloff 1966). The formation of secondary cartilage on membrane bone and of constant sesamoids in tendons also depends on mechanical stimulation (Hall 1979, 1986; Sarin et al. 1999). Equally it is known that *de novo* formation of cartilage elements can be elicited in fetal and postfetal vertebrate mesenchymal and connective tissues (Fig. 4) when certain pressure regimes are applied either experimentally or under pathological conditions (Tagil and Aspenberg 1999; Vogel and Koob 1989). A reduction of movement reduces pressure stimulation and results in the reverse of the compression effect, namely a drop of type II collagen, aggrecan, and glycosaminoglycan expression and a consequential reduction of cartilage matrix synthesis. Hence it can be postulated that pressure-sensitive skeletal elements, such as sesamoid cartilages, will not form in the absence of movement.

This postulate can be tested by experiments that arrest embryonic movement. One method is pharmacological paralysis of the embryo by a single injection of the postsynaptic blocking agent decamethonium iodide into chicken eggs (Sullivan 1966; Hall 1975). The resulting paralysis strongly affects the development of four constant cartilages that form in the hindlimb of the chick: the patella, a sesamoid in the common tendon of the femorotibial muscles; the crest cartilage, a sesamoid in the tendinous precursor of the syndesmosis tibiofibularis; the cartilage inside the supratendineal ligament on the distal tarsometatarsus, termed pons cartilage; and the tibial cartilage, a conduit for tendons near the intertarsal joint. The absence of embryonic movement leads to size reduction or complete aplasia of these cartilages (Fig. 5)

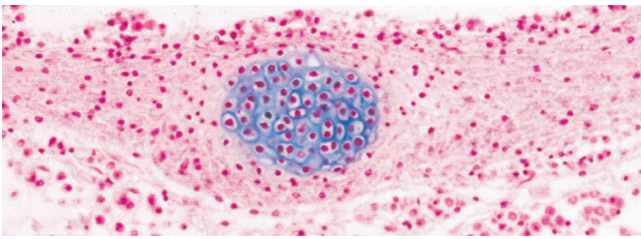


Fig. 4. Cartilaginous sesamoid in a tendon anlage of a chick embryo.

(Wu 1994). Long bone growth and craniofacial development are also severely affected (Herring and Lakars 1982; Hall and Herring 1990; Bertram et al. 1997). These experimental results highlight the important epigenetic role of embryonic movement in skeletogenesis and indicate that environmental factors that influence embryonic motility via physical and/or chemical stimuli can have subtle effects on skeletal development.

EVOLUTIONARY ROLES OF MECHANOSENSITIVE SKELETOGENESIS

Given the sensitivity of skeletogenesis to mechanical stimulation and its dependence on environmental conditions, it is legitimate to consider potential evolutionary roles of these interdependencies. One straightforward inference is that this kind of reactivity of the skeletogenic system provides a source of phenotypic plasticity. Natural selection would be able to operate on variations of size and shape of skeletal elements induced by environmental change. Such a role of an

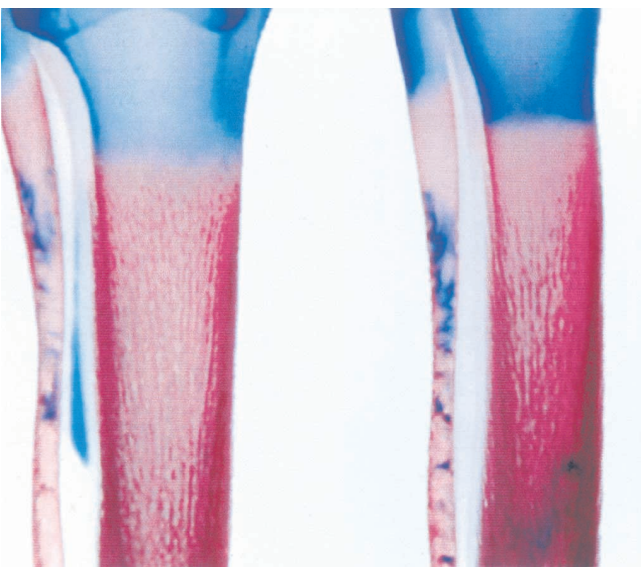


Fig. 5. Complete aplasia of the crest cartilage in a paralyzed chick embryo. Left, control.

environment-dependent developmental mechanism is in line with the standard evolutionary theory and its emphasis on variation and selection.

However, there is a second and possibly distinct evolutionary role of mechanosensitive skeletogenesis: the *de novo* formation of skeletal elements from responsive tissues. In this case, the starting point would be selection acting not on the new element itself (because it would not yet exist) but on other characteristics, such as size, shape, or proportions of body parts. Continuous selection on these parameters, for instance the relative size of limbs and their skeletal support, will alter the biomechanical conditions in the affected region and result in new pressure and tension loads in the involved tissues. If these tissues have a chondrogenic capacity, they will respond by producing cartilage matrix and begin cartilage cell differentiation at certain threshold levels of mechanical load. A new skeletal element will result. This new element could remain selectively neutral for long periods of time, its continuous existence merely depending on the maintenance of the same biomechanical conditions in every subsequent generation. But eventually it can become itself subject to selection and will thus be stabilized and integrated in the developmental-genetic machinery.

Numerous cases of new skeletal traits that may have arisen by this mode exist in vertebrates, such as the famous additional “digits” in giant pandas or the falciforme in moles. One example studied in more detail is the origin of the fibular crest on the tibia of theropod dinosaurs and birds. Müller and Streicher (1989) suggested that progressive reduction of the fibula, a characteristic trend in the evolution of bipedal locomotion in reptiles and birds (Streicher and Müller 1992), led to an increasing rise of mechanical load on the connective tissue between the tibia and the fibula during embryonic movement. At a certain threshold point a stress-dependent sesamoid cartilage arose—and can still be observed as a transient structure in the embryos of recent birds. The sesamoid may have existed long before its ossification and integration into the tibia occurred, giving rise to the sudden appearance of the prominent fibular crest on the theropod tibia. The other embryonic hindlimb cartilages described above also each gave rise to osseous innovations in various avian lineages.

These examples support the proposed relationship between embryonic motility and stress-dependent skeletogenesis in evolution. Paralysis experiments confirm that the formation of the same elements that represent the kernels of skeletal innovations in the theropod–avian lineage still depend on biomechanical stimulation during the development of recent birds. By extension, this argument can be taken one step further: Because environmental factors affect embryonic motility, it is conceivable that changes in environmental conditions have similar consequences on skeletogenic tissues via the alteration of embryonic movement regimes and bio-

mechanical stimulation. Experimental studies are required to confirm this conclusion. A prediction would be that strong effects can be elicited during sensitive periods when biomechanical stimuli are particularly essential for normal development.

ORIGIN OF NOVELTY IN EVOLUTION

The de novo formation of skeletal elements addresses an important but largely neglected issue in evolutionary theory: the origination of morphological novelty. This generative problem of organismal evolution is sidestepped in traditional accounts that focus on the gradual variation and adaptation of characters and calculate their population genetic underpinnings. The studied characters are usually taken as given, and their origination is tacitly assumed to be based on the same mechanisms as their variation and adaptation. There is growing awareness that this does not need to be the case and that innovation should be treated as a distinct problem of evolution (Nitecki 1990; Müller and Newman 2003). The key to this issue is an operational definition of novelty that permits to distinguish the phenomena that require explanation (Müller and Wagner 1991).

The potential mechanisms for the origin of phenotypic novelty are many and include point mutations, cumulative mutations, and other forms of genetic rearrangement, but each of these possibilities has shortcomings in explaining the origin of complex characters (Müller and Wagner 2003). This article argues that an alternative mode for innovations to arise is through thresholds in development. If selection acts on body proportions and skeletal proportions, the ensuing biomechanical changes and the reactive potential of skeletal tissues will automatically generate new elements. This effect is neither the direct result of a mutation or “new genes” for that specific character nor the result of selection “for” that character but rather a developmental by-product of general selection regimes, affecting growth rates for instance, that trigger a specific developmental response at certain threshold points of the affected developmental system. This response is “unforeseen” and the ensuing character could be called “neutral,” because it may exist for prolonged periods of time purely for developmental reasons without selective advantage.

Clearly such thresholds do not reside in biomechanical sensitivity alone but in numerous other properties of developmental systems, such as any rate, size, topology, or concentration dependent process. The theoretical framework for this mode of origination of phenotypic novelty has been called the “side effect hypothesis” (Müller 1990). The biomechanical aspects of character origination are part of a more general approach that assigns a key role in the evolution of multicellularity and primitive body organization to generic physical properties of cells and tissue masses (New-

man and Müller 2000). This position does not deny that mutation and selection are involved in innovation but argues that their effects are indirect and that the major role of these processes is ensuring inheritance and variation of the phenotypic characters that originated primarily from epigenetic properties of developmental systems undergoing evolutionary modification.

The particular environment–development interaction sketched out in this essay highlights that activity of the embryo is an important epigenetic factor in evolution. It represents one of many ways in which the environment interacts with development and by which changes in environmental conditions can translate into morphological change. These properties have never been systematically studied and provide a rich field for experimental analysis. Many of the sophisticated methods and tools of functional morphology could be applied with great profit to embryonic problems to form a starting point of what could become a *functional embryology*. This would contribute a new kind of data to evolutionary developmental biology.

REFERENCES

- Aspenberg, P., Basic, N., Tagil, M., and Vukicevic, S. 2000. Reduced expression of BMP-3 due to mechanical loading: a link between mechanical stimuli and tissue differentiation. *Acta Orthop. Scand.* 71: 558–562.
- Bard, J. B. L. 1990. Traction and the formation of mesenchymal condensations in vivo. *BioEssays* 12: 389–395.
- Bekoff, A. 1981. Embryonic development of chick motor behaviour. *Trends Neurosci.* 4: 181–183.
- Bekoff, A. 1992. Neuroethological approaches to the study of motor development in chicks: achievements and challenges. *J. Neurobiol.* 23: 1486–1505.
- Bertram, J. E., Greenberg, L. S., Miyake, T., and Hall, B. K. 1997. Paralysis and long bone growth in the chick: growth shape trajectories of the pelvic limb. *Growth. Dev. Aging* 61: 51–60.
- Bos, A. F., Einspieler, C., and Prechtel, H. F. 2001. Intrauterine growth retardation, general movements, and neurodevelopmental outcome: a review. *Dev. Med. Child. Neurol.* 43: 61–68.
- Carter, D. R., Orr, T. E., Fyhrie, D. P., and Schurman, D. J. 1987. Influences of mechanical stress on prenatal and postnatal skeletal development. *Clin. Orthop.* 219: 237–250.
- Drachman, D. B., and Sokoloff, L. 1966. The role of movement in embryonic joint development. *Dev. Biol.* 14: 401–420.
- Hall, B. K. 1975. A simple, single-injection method for inducing long-term paralysis in embryonic chicks, and preliminary observations on growth of the tibia. *Anat. Rec.* 181: 767–778.
- Hall, B. K. 1979. Selective proliferation and accumulation of chondroprogenitor cells as the mode of action of biomechanical factors during secondary chondrogenesis. *Teratology* 20: 81–92.
- Hall, B. K. 1986. The role of movement and tissue interactions in the development and growth of bone and secondary cartilage in the clavicle of the embryonic chick. *J. Embryol. Exp. Morph.* 93: 133–152.
- Hall, B. K., and Herring, S. W. 1990. Paralysis and growth of the musculoskeletal system in the embryonic chick. *J. Morph.* 206: 45–56.
- Hamburger, V. 1963. Some aspects of the embryology of behaviour. *Q. Rev. Biol.* 38: 342–365.
- Harris, A. K., Stopak, D., and Warner, P. 1984. Generation of spatially periodic patterns by a mechanical instability: a mechanical alternative to the Turing model. *J. Embryol. Exp. Morphol.* 80: 1–20.
- Harris, A. K., Stopak, D., and Wild, P. 1980. Fibroblast traction as a mechanism for collagen morphogenesis. *Nature* 290: 249–251.
- Herring, S. W., and Lakars, T. C. 1982. Craniofacial development in the

- absence of muscle contraction. *J. Craniofac. Genet. Dev. Biol.* 1: 341–357.
- Ikegame, M., Ishibashi, O., Yoshizawa, T., Shimomura, J., Komori, T., Ozawa, H., and Kawashima, H. 2001. Tensile stress induces bone morphogenetic protein 4 in preosteoblastic and fibroblastic cells, which later differentiate into osteoblasts leading to osteogenesis in the mouse calvariae in organ culture. *J. Bone Miner. Res.* 16: 24–32.
- Klein-Nulend, J., Roelofsens, J., Sterck, J. G., Semeins, C. M., and Burger, E. H. 1995. Mechanical loading stimulates the release of transforming growth factor-beta activity by cultured mouse calvariae and periosteal cells. *J. Cell. Physiol.* 163: 115–119.
- Müller, G. B. 1990. Developmental mechanisms at the origin of morphological novelty: a side-effect hypothesis. In M. H. Nitecki (ed). *Evolutionary Innovations*. The University of Chicago Press, Chicago, pp. 99–130.
- Müller, G. B., and Newman, S. A. (eds.) 2003. *Origination of Organismal Form*. MIT Press, Cambridge, MA, in press.
- Müller, G. B., and Streicher, J. 1989. Ontogeny of the syndesmosis tibiofibularis and the evolution of the bird hindlimb: a caenogenetic feature triggers phenotypic novelty. *Anat. Embryol.* 179: 327–339.
- Müller, G. B., and Wagner, G. P. 1991. Novelty in evolution: restructuring the concept. *Annu. Rev. Ecol. Syst.* 22: 229–256.
- Müller, G. B., and Wagner, G. P. 2003. Innovation. In B. K. Hall and W. M. Olson (eds.). *Key Concepts and Approaches in Evolutionary Developmental Biology*. Harvard University Press, Cambridge, in press.
- Nechaeva, M. V., and Turpaev, T. M. 1991. The effect of temperature on the motor activity of the chick embryo and amnion at 5–14 days of development. *Zh. Evol. Biokhim. Fiziol.* 27: 743–748.
- Newman, S. A., and Müller, G. B. 2000. Epigenetic mechanisms of character origination. *J. Exp. Zool.* 288: 304–317.
- Nitecki, M. H. (ed.). 1990. *Evolutionary Innovations*. University of Chicago Press, Chicago.
- Nomura, S., and Takano-Yamamoto, T. 2000. Molecular events caused by mechanical stress in bone. *Matrix Biol.* 19: 91–96.
- Oppenheim, R. W., and Levin, H. L. 1975. Short-term changes in incubation temperature: behavioral and physiological effects in the chick embryo from 6 to 20 days. *Dev. Psychobiol.* 8: 103–115.
- Romanoff, A. L. 1960. *The Avian Embryo*. Macmillan, New York.
- Sarin, V. K., Erickson, G. M., Giori, N. J., Bergman, A. G., and Carter, D. R. 1999. Coincident development of sesamoid bones and clues to their evolution. *Anat. Rec.* 257: 174–180.
- Sato, M., Ochi, T., Nakase, T., Hirota, S., Kitamura, Y., Nomura, S., and Yasui, N. 1999. Mechanical tension-stress induces expression of bone morphogenetic protein (BMP)-2 and BMP-4, but not BMP-6, BMP-7, and GDF-5 mRNA, during distraction osteogenesis. *J. Bone Miner. Res.* 14: 1084–1095.
- Streicher, J., and Müller, G. B. 1992. Natural and experimental reduction of the avian fibula: developmental thresholds and evolutionary constraint. *J. Morphol.* 214: 269–285.
- Sullivan, G. E. 1966. Prolonged paralysis of the chick embryo, with special reference to effects on the vertebrate column. *Aust. J. Zool.* 14: 1–17.
- Tagil, M., and Aspenberg, P. 1999. Cartilage induction by controlled mechanical stimulation in vivo. *J. Orthop. Res.* 17: 200–204.
- Takahashi, I., Nuckolls, G. H., Takahashi, K., Tanaka, O., Semba, I., Dashner, R., Shum, L., and Slavkin, H. C. 1998. Compressive force promotes sox9, type II collagen and aggrecan and inhibits IL-1beta expression resulting in chondrogenesis in mouse embryonic limb bud mesenchymal cells. *J. Cell. Sci.* 111: 2067–2076.
- Vogel, K. G., and Koob, T. J. 1989. Structural specialization in tendons under compression. *Int. Rev. Cytol.* 115: 267–293.
- Wu, K. C. 1994. *Entwicklung, Stimulation und Paralyse der embryonalen Motorik*. Dissertation, University of Vienna.
- Wu, K. C., Streicher, J., Lee, M. L., Hall, B. K., and Müller, G. B. 2001. Role of motility in embryonic development. I. Embryo movements and amnion contractions in the chick and the influence of illumination. *J. Exp. Zool.* 291: 186–194.
- Wu, Q., Zhang, Y., and Chen, Q. 2001a. *Indian hedgehog* is an essential component of mechanotransduction complex to stimulate chondrocyte proliferation. *J. Biol. Chem.* 276: 35290–35296.