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What Comes First: the Structure or the Egg? Ross Granville Harrison on the Origin of Embryonic Polarity

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Ross Granville Harrison's paper " *On relations of symmetry in transplanted limbs,*" published in JEZ in 1921 investigated the mechanism governing the development of embryonic axes through systematic transplantation experiments on the forelimb of the salamander, *Amblystoma punctatum*. The questions these experiments addressed remain as fundamental, and largely unanswered, today as they were over 80 years ago: how does an embryo establish its three axes, and how is this positional information conveyed to developing organs? The experiments themselves exemplified the transition from embryology as a purely descriptive field, to modern experimental embryology.

Harrison's interest in embryology began while he was working towards his Ph.D., when he visited Germany and studied the development of fins in teleost fishes with Nussbaum. At that time in Germany, Wihelm Roux established a new journal "Archiv fur Entwicklungsmechanik," designed for the study of mechanisms of development, as opposed to mere description of developmental processes. It is highly likely that this shift in approach to problems in developmental biology had a significant impact on Harrison also. Harrison was clearly an outstanding observer, and the care which he applied to the descriptive aspects of embryology becomes apparent in the JEZ paper. The experiments are systematic, and the documentation of the experimental results is detailed and totally objective. The beautiful line drawings along with the detailed experimental histories convey the actual experimental results more accurately than many multicolor photoshop images in the current scientific literature. The data is presented exactly as Harrison saw it, not as he predicted it to be. For example, in figure 37, the transplanted limb is incidentally covered by the gills, and Harrison does not alter the drawing to "move" the gills and make his observation more obvious. It would be possible for a reader to do an independent analysis of Harrison's experiments just based on the raw data in the manuscript, and for that reader to form independent conclusions not influenced by those Harrison himself presented later in the paper.

The conclusions that Harrison did draw from his experiments far outlasted the experiments themselves, however. The experiments were sparked by a discrepancy in observations between two notable embryologists of that time, Streeter and Spemann, regarding the equipotentiality of the ear placode. Although the discrepancy turned out to be spurious, the questions raised by those observations lead Harrison to search for other equipotential developmental systems. The limb bud of *Amblystoma* presented a manipulable and observable model for the development of embryonic axes. The limb bud could be transplanted either in its entirety, or in parts, both to the normal location, and to a location which does not normally give rise to a limb. The degree to which symmetry was pre-patterned in the limb bud could then be investigated by transplanting it to the same or to the opposite side of the embryo, and by positioning the transplanted limb with its dorsal-ventral axis preserved or inverted. The outcome of these experiments showed that limb buds can regulate their development in some respects: halved or doubled buds gave rise to a normal limb. Thus, with regard to size regulation the limb bud behaves as an equipotential system, which can respond to information from the rest of the embryo and re-set its developmental program. Development of asymmetry, however, presented a more complex picture.

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Harrison asks two predominating questions here: when is the adult form established in the primordium, and what is the nature of the signal that shapes the undifferentiated limb bud into the adult limb? His experiments provided a partial answer to the first question, leading to the conclusion that "the posture and asymmetry of the limb is determined neither by the limb itself, nor by its surroundings exclusively." At the stage of the transplants described in this manuscript, the anteroposterior axial differentiation is already determined, while the dorsoventral specification is imposed on the limb bud by the surrounding tissue. To this day, the precise timing during development when a limb bud is specified remains an area of active investigation (Tickle, 2003). For example, there are currently two differing models describing the timing of specification of the proximo-distal axis of the limb, the "progress zone model" and the "early specification model" (Dudley et al., 2002). In the progress zone model, the different parts of the limb are progressively specified as the limb extends. In contrast, in the early specification model, the fate of limb segments along the proximo-distal axis is already encoded in the very early limb bud. Of note is that recent experiments designed to address these questions employed some of the same basic experimental methods used by Harrison, the transplantation of chick limb buds to other sites of the embryo. The analysis today is guided by the use of molecular markers, but the question Harrison at least partially addressed using only careful observation has still not been definitively answered.

The second question, namely the nature of the embryonic signal specifying adult form, is the principal subject of the discussion in his 1921 JEZ paper, and was one that Harrison spent considerable time on in his subsequent scientific endeavors. At the time of Harrison's experiments, a vigorous debate was ongoing between Harrison and Childs regarding the nature of this signal. Child and others espoused the idea that the phenomena of axial differentiation are the result of gradients, which were thought to be metabolic in nature. Harrison, on the other hand, questioned the existence of such gradients as the origin of embryonic axes, believing that there is a molecular basis underlying the development of asymmetry. In the 1921 JEZ paper, the argument for a molecular determination of form arises from the observation that the limb bud is, at least partially, an equipotential system. Harrison concludes that the "Existence of an equipotential system necessitates, in fact, the assumption of some sort of molecular hypothesis for the representation of adult form in the germ." Since each elementary unit comprising the limb bud is able to assume a range of adult fates, depending on the environment in which it develops, determination of form must reside in the elementary unit itself, rather than in the arrangement of elementary units at the time of transplantation. He then goes on to describe a hypothetical carbon molecule, showing how progressive modification of the four side chains leads to an asymmetric, chiral structure. This structure could provide the internal reference found in each cell of the limb bud that would permit alignment along the anteroposterior axis, and thus determine the correct dorsoventral and left-right axes. Childs raised some major objections to the "molecular" approach. In particular, he suggested that " protoplasms in general have not yet been shown to posses any structure that might serve as a basis for developmental pattern," and advocated instead that the site of attachment of the egg established a metabolic gradient that formed the basis for the development of future asymmetry. Harrison and the crystallographer W.T. Astbury went on to search for the molecular structures underlying developmental asymmetry through the use of X-ray crystallography of air-dried specimens of *A. punctatum*. These experiments were unsuccessful, largely not because the idea would turn out to be wrong, but because of the technical limitations of the experiments. Harrison does not deny that gradients may have a role in developing embryonic symmetry. For example, he wrote that "It seems to the present writer that such gradients may well be an expression of polarity rather than its cause." In other words, something, such as inherent molecular asymmetry must initiate organismal asymmetry. The initial asymmetry can then be converted to a gradient.

What has been learned about development of embryonic symmetry since Harrison's publication? Strikingly, both gradients and underlying molecular asymmetry have been shown to govern the formation of the 3 embryonic axes. In *Drosophila*, a gradient of the *bicoid* molecule is one of the first steps in the creation of anteroposterior axis. Notably, the *bicoid* gradient is regulated by cytoskeletal elements, which Harrison alluded to when he stated that "in the egg, such an arrangement of protein molecules would form a lattice or framework in which substances could be differentially distributed.'' The recent observations that provide the greatest support for Harrison's hypothesis of an underlying molecular asymmetry are those concerning the development of the left-right axis. In this instance, the asymmetric macromolecular structure has been identified. It is the axoneme of the cilium, which is a large, highly asymmetric macromolecular structure (McGrath and Brueckner, 2003). The cilium is aligned relative to the anteroposterior and dorsoventral axes in the embryo, and then acts on the surrounding tissue to generate gradients of ions and effector molecules.

Over 80 years ago, the discussion in Harrison's JEZ paper accurately predicted both the existence of molecular asymmetry and gradients as the mechanisms underlying the development of embryonic asymmetry. It is remarkable that this insight was generated without any molecular biology, mouse knockouts, or genomics, but through careful, thoughtful experimentation and observation alone.

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