Meeting Report
The 5th Meeting of the Rising Generation of Evo-Devo Biologists
“Understanding Evolution Through ‘Epigenetic’ Perspectives”

Reiko Tajiri and Tomonari Kaji

Abstract
The 5th Meeting of the Rising Generation of Evo-Devo Biologists held in Okazaki, Japan, on June 16th, 2012, brought together 62 participants active in evo-devo or in related areas of research. The meeting, entitled “Understanding Evolution Through ‘Epigenetic’ Perspectives” was aimed at introducing (or re-introducing) ‘epigenetics’, originally proposed by Waddington in the 1940s, as a framework in which to tackle a major dilemma that evo-devo researchers face today.

1. Introduction
The overwhelming phenotypic diversity in forms and functions of life on earth has fascinated people from ancient times. The modern evolutionary theory states that phenotypic modifications are underlain by genetic changes. However, researchers have had difficulty providing mechanistic explanation as to how specific genetic changes have caused the changes in phenotypes. We organized this meeting with a prospect of probing for a new way of causal explanation by focusing on the ‘epigenetic’ nature of developmental systems as the direct cause of phenotypes.

Numerous elements of various levels that constitute life (genes, cells, organs, organisms, ...) interact within individual levels, across different levels and with environments. ‘Epigenetics’, by its original definition, is a view that those complex interactions are integrated through development to “cause” phenotypes [1]. According to this view, phenotypes cannot be described as a simple sum of gene functions. Individual genes do not act on phenotypes in a linear fashion. They regulate each other, affect cell behaviours or organ physiologies, in turn get controlled by them, and so on. In the network of interactions spanning all levels, the function of individual elements inevitably involve many other elements of all levels, and project on phenotypes in a highly non-linear fashion.

This non-linearity, derived from the ‘epigenetic’ nature of developmental systems, is crucial in evolutionary biology. The non-linearity allows variations of all levels to accumulate (genotype variations, varied environmental inputs, etc.) without resulting in phenotypic changes, thereby serving as a “capacitor” of evolution. Meanwhile, non-linearity also means that distinct phenotypes are produced in response to a subset of those variations. Müller proposes that the non-linearity of ‘epigenetic’ developmental systems may have served as a major driving force for creating novelties [2]. The initial variation that produced a novelty does not have to be genetic: it can be environmental, cellular, or at any level. Once a novelty is generated and is positively selected subsequently, then genetic changes that stabilize the novel phenotype may follow. This phenomenon is known as genetic
accommodation. Experimental demonstrations of genetic accommodation tell us that even the end products of development ("adult phenotypes") and their interactions with environments can in turn influence basal elements of developmental programs including genes [3].

Evo-devo is a branch of biology that studies how changes in developmental programs drive phenotypic evolution. As suggested by Müller, ‘epigenetic’ aspects of development may have had great impact on evolutionary history. To date, however, the extent of the impact has not been adequately probed by experimental approaches. In this meeting, speakers practicing ‘epigenetic’ approaches in related areas of research were invited, with the aim to foster this approach in evo-devo studies.

2. Emergence of ordered forms and functions from assembled parts

The long history of genetics and the recent advancement of genomics have led to the identification of numerous genes “associated” with individual phenotypes. A challenge for evo-devo now is to account for the mechanism through which a (large) set of genes are integrated during developmental processes to “cause” the phenotype of interest. Studies in other disciplines were presented in the meeting as examples of how we should go about it.

In a study on skull suture morphogenesis, Takashi Miura boldly categorized many genes (signaling molecules and fate determinants) known to be involved in the process into merely two groups, based on their actions on cell behaviors. This approach has enabled him to reproduce by simple mathematical modeling the variety of suture morphologies actually formed under different conditions, and also to describe the morphogenetic mechanism in an intuitive, qualitative manner [4].

Yasuhiro Sugimoto presented a study on passive dynamic walking, whereby a robot composed solely of skeletal parts without any actuator or regulator can walk itself down a slope. This behavior cannot be understood as a simple sum of the movements of individual sticks and joints; the “cause” lies in the way parts interact with each other. Moreover, Sugimoto showed that a complex of tandemly connected robots exhibit distinct patterns of walking depending on the number of robots connected or the mobility of connecting rods. These presentations demonstrated that the mechanistic explanation of a phenotype may be found only by looking at the whole system of interactions. This lesson is well applicable to biology: phenotypes emerge through interactions among multiple organs, as exemplified in section 4.

3. Proximate cause of phenotypes

As illustrated by Miura’s work mentioned above, functions of a large number of genes are often integrated at the level of cells to “cause” organ-level phenotypes. A visual example of cell behaviors serving as a proximate cause of morphogenesis was presented by Takefumi Kondo. At the initial phase of tracheal development in the fly, a group of epithelial cells per hemisegment invaginate into the body cavity to start forming a tube. Kondo found that mitotic
cell rounding, a requisite behavior preceding division in most cell types, serves as a physical trigger for rapid invagination. Direct visualization of cell behaviors by live imaging was crucial to this new finding, hitherto overlooked by exhaustive genetic screenings. His work illustrates the importance of choosing the right level to look for the proximate cause of a phenotype.

A mixed approach of paleontology and fluid mechanics has enabled Yuta Shiino to unravel the perfect link between morphologies of the bivalve shells of extinct brachiopods and their interactions with the environment. The animals captured food particles solely by exploiting sea water flows to generate passive flows within their bodies. The efficiency of the passive flows depended on their shell shapes. Shiino showed that their shell morphologies fell within the small range of the optimum predicted in fluid dynamics simulation to generate robust passive flow under ambient conditions [5]. The stringent fit inspires an exciting possibility that the interaction between the “end product” of development (adult morphology) and the environment could have had significant influence back on development itself.

4. Interactions across all levels
Organs regulate organismal physiology, which in turn influences all levels of life. Nutrition and hormones are among the well-known examples. Kota Miyasaka showed a surprising mode of interaction across genes, organs, and the organism. It had been known that heartbeat is required for development of the heart itself in vertebrates. Miyasaka found that blood circulation generated by heartbeat acts as a physical cue on heart cells themselves to induce expression of a specific set of microRNAs, which in turn regulate other genes. This regulation is essential for heart cells to form a perfectly functional pump [6]. His study signifies that phenotypes (forms and functions) emerge as a net result of ramified interactions across multiple levels: organs, organismal physiology, cells and genes.

Yoko Matsumura presented a case where a behavior of the adult brings an imperfectly formed organ into a completely functional one. She focuses on the extremely elongated morphology of genitalia (as long as twice the body length) in some leaf beetles [7]. The long distal tube (flagellum) is normally stored in the internal sac, a membranous invagination whose folds envelop the flagellum, protecting it from entanglement and preparing it for efficient ejection. Matsumura found that, when initially formed in the pupa, the flagellum is coiled up by itself. After eclosion, males engage in a solitary “training” behavior: repeated ejection and withdrawal of the genitalia. The repeated movement mechanically comforms the flagellum to the sac. Thus, even phenotypes, the end products of development, may interact with each other to “cause” yet another phenotype: a developed organ (= a phenotype) requires a behavior (= a phenotype) to achieve functionality (= a phenotype).

5. Lessons from outside: guide to the future of evo-devo
There have been some cases in which the molecular function of a single gene (or a few) sufficed to directly account for phenotypic evolution. Technical advances have availed us with huge data on genetic changes associated with
evolution. We are inclined to delude ourselves into thinking that all phenotypic changes will eventually be reduced to individual gene functions: some day, if only made technically possible, we can resurrect dinosaurs by putting their DNA into frog eggs. It may be unfortunate but also quite fascinating that life is not so simple. Lessons from outside of evo-devo presented in this meeting may be summarized as below:

(A) Focus on the integrated level rather than reductionistic decomposition into basal elements is often key to understanding the mechanism that causes the phenotype.
(B) Mechanisms that best explain the phenotypes do not necessarily lie in the genes: you may find ones at the cellular level, at the level of environment, or at any level.
(C) Mechanistic explanation of a phenotype may not be contained in a single level: it may be best explained as an ‘epigenetic’ result of interactions across different levels, sometimes including the end phenotypes themselves.

We believe that introduction of ‘epigenetic’ perspectives into our research practices will help us open up a new horizon of evo-devo.

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References