Tinkering: a metaphor uniting evolutionary and developmental biology

Thomas J. Sanger

The late 1970s was an exciting time in evolutionary biology. During this time, the long-standing adaptionist program was being critically re-examined and the reintegration of evolutionary and developmental biology was beginning to take hold. In 1977, François Jacob[1] contrasted two competing metaphors for evolutionary change: engineering, determinate, unlimited and approaching perfection, versus tinkering or "bricolage", which is contingent and highly dependent on the resources to hand. The specific assumption, which lies at the foundation of his argument, is that changes in gene regulation underlie phenotypic variation by reusing genes and genetic programs already engrained in an organism's developmental arsenal. While this article has been largely neglected in recent years, it recently served as the motivation for the Novartis symposium *Tinkering: Microevolution of Development* (July 2006), organized by **Dan Lieberman** (Harvard University) and **Brian Hall** (Dalhousie University). Discussions such as those held at this symposium, bringing together evolutionary, developmental, and population biologists, promise to set the groundwork for the conclusive synthesis of these fields.

As demonstrated by **Manfred Laubichler** (Arizona State University) in the opening talk of this symposium, Darwin himself viewed the pattern of natural variation as being "tinkered" with by the process of natural selection.[2] Thus, tinkering at the population level creates the *pattern* of natural phenotypic variation that we observe within and between species. Later 19th century biologists, such as Wiessmann and Kuhn, observed that variation resulted from the "combinatorial rearrangement" of characters, implementing "tinkering" as the *processes* by which variation is generated between individuals. After some discussion, it appeared that deciding whether "tinkering" referred to pattern or process was a moot point in our modern context. What emerged was a consensus that the focus of this meeting would be on minor variation and the developmental processes underlying this variation. While, traditionally, developmental biologists have ignored population or species level variation, understanding the molecular basis for variation at this level will eventually lead to a seamless understanding of the evolutionary processes underlying morphological change. However, such a broad approach needs to be employed at many levels using a wide array of methods. Participants in this meeting described several possible approaches to examining minor, population level variation and these can be grouped loosely into three categories: comparative, quantitative and molecular.

The primary method employed by evolutionary biologists to investigate biological diversity is the comparative method.[3] While broad taxonomic comparisons have been common in evolutionary developmental biology, comparisons among more closely related species are becoming more frequent and proving highly informative. **Rudy Raff** (Indiana State University), for example, presented an update of his work contrasting direct and indirect developing sea urchins within the genus *Heliocidaris*. He demonstrated that this difference in reproductive strategy is the result of several heterochronic shifts in regulatory pathways controlling the development of the left coeloem. In a similar vein, **David Stern** (Princeton University) described his work dissecting the genetic bases of larval trichome pattern in several species of *Drosophila*. He showed that the pattern of trichome loss in these species is the result of several, possibly independent mutations, at the *shavenbaby* locus. Both of these studies demonstrate that the visible morphological differences that we observe between species likely evolved through a series of small regulatory changes rather than through a single step "macromutation" as argued in the past.

Many biologists consider paleontologists to be necessarily restricted to the study of macroevolutionary phenomena or as the suppliers of putative ancestral conditions. **Michael Coates** (University of Chicago) and **Michael Bell** (SUNY at Stoney Brook) each provided evidence that paleontology can provide a much finer-scale picture of evolutionary change. Dr Coates first presented an updated phylogeny of stem tetrapods to demonstrate that the fin-to-limb transition is much more gradual than once thought. He then went on to describe parallels between these transitional species and limb development in extant groups. He demonstrated that a transition, from fins to limbs, once thought to represent a sudden morphological change appears to have been the result of many smaller adjustments rather than one large change.

Department of Biology, Washington university in St Louis, Campus Bod 1229, St Louis, MO 63130. E-mail: tsanger@biology2.wustl.edu
DOI 10.1002/bies.20499
Published online in Wiley InterScience (www.interscience.wiley.com).

Dr Bell used a unique analysis of extant and extinct populations of three-spine stickleback, *Gasteroseus acuteatus*, to examine the morphological history of pelvic structure reduction and loss. Using a 110,000 year time series, Dr Bell was able to show a rapid period of change in pelvic structure following a long period of slow, gradual change. This pattern is consistent with patterns of pelvic change in extant populations of stickleback after the loss of predatory fishes. In addition, the pattern is consistent with the evolution at a major locus. In modern populations, the major locus responsible for pelvic loss is *Pitx1*.[4] Is it possible that genetic variation in *Pitx1* long precedes its phenotypic expression in modern populations? While we cannot examine gene expression in fossils, we can use the comparative method to examine other closely related species for similar patterns of variation to make headway in understanding questions of this sort.[5]

Possibly one of the most important steps in the ratcheting down of evolutionary–developmental studies to the level of the population will be the integration of quantitative methods with developmental data. This is an approach rich in potential. One specific possibility is to use quantitative genetics to elucidate the genetic bases of phenotypic variation. **Jim Cheverud** (Washington University) described his work studying the genetic architecture of several murine phenotypes. While many pure developmental studies search for the single gene underlying a phenotypic change, Dr Cheverud stressed the complex nature of genetic architecture including examples of epistasis, pleiotropy and differential dominance. Dr Cheverud also stressed the fundamental evolutionary concept that evolution proceeds through selection on *heritable* genetic variation, hence the statistical relationships between genotype and phenotype in populations.

Artificial selection experiments, such as those performed by **Paul Brakefield** (Institut Biologie Leiden), capitalize on the heritable genetic variation underlying a trait and allow one to examine constraints on evolutionary change. Dr Brakefield's experiments aim at genetically decoupling the size and color of serially repeated *Bicyclus* butterfly wing eye spots to examine the individual basis of these characters and their developmental linkages. Through these experiments, Dr Brakefield showed that, while the size of different eyespots can be decoupled and evolve independently, the color composition of different eye spots cannot be separated and will thus almost certainly evolve in concert when individual elements are selected. Studies such as those by Drs. Cheverud and Brakefield bring a note of caution to studies of morphological evolution because complex genetic architectures and integration of characters are likely much more common than most researchers currently assume.

One of the most highly integrated structures of the vertebrate body is the skull. Composed of many different bones from different developmental origins and serving many functions, the skull has a complex history. Due to its complex development, however, the skull has been difficult for evolutionary developmental biologists to dissect in detail. Nevertheless, this is an area where the use of quantitative techniques has made great progress. By studying the patterns of covariance in the skull, **Benedikt Hallgrimsson** (University of Calgary) and **Rebecca Ackermann** (University of Cape Town) have been able to make specific predictions about the developmental integration of the skull and its evolution. Dr Hallgrimsson described a unique approach of comparing variance and covariance patterns in the skull between standard and mutant strains of mice to understand the genetic basis of the skull's integration. Interestingly, he found that an increase in variation is localized to the point of the mutation while other structures only change according to the wild-type covariation pattern. Dr Ackermann presented data on the divergence of craniofacial ontogeny between human and non-human primates to understand when in development the differences between these species arise. Surprisingly, the differences between these species are largely present early in ontogeny meaning that later growth plays little role in the divergence of these forms. The two sets of studies have implications for long-held beliefs about primate cranial evolution and will certainly lead to new insights into the evolution of modern human form.

Reconciling molecular data with quantitative variation among species remains difficult. However, this is precisely what the collaboration of **Irma Thesleff** (University of Helsinki) and *Jukka Jernvall* (University of Helsinki) are working towards with their experimental models of tooth development. Dr Thesleff presented data regarding the molecular bases of cell and tissue interactions in the developing tooth bud. Her work demonstrates that variation in tooth morphology and tooth number is likely to be the result of fine-scale changes in the modulation of conserved signaling molecules. Dr Jernvall then described how patterns of mammalian dentition, generally a decrease in the number of teeth corresponding with an increase in specialization, relate to what is known about the development of teeth and cusps. His analysis suggests that, through small changes in the process of enamel knot formation, new cusps can be added easily without disrupting formation of existing knots. During the open meeting that followed the symposium, Dr Jernvall elaborated on this work to describe the use of GIS software to map the topology of the developing tooth bud with respect to the gene expression patterns underlying enamel knot formation.

A central aim of evolutionary developmental biology is to relate variation at the molecular level with morphological variation within and between species. While practically this may only be possible for a few well-studied genes at this time, progress is beginning to be make this more tractable. As this area of research develops, **Adam Wilkins** (*BioEssays* Editorial Office) stressed the importance of using a hierarch-

ical network approach to examine variation in gene expression since development is largely the unfolding of loosely nested developmental modules. In particular, he showed how the formal structure of genetic networks enables them to serve as devices for both transmitting and amplifying genetic change. These properties, he argued, relate to the well-known but still poorly understood phenomenon of rapid morphological change that can occur in evolution.

Changes in the spatial and temporal regulation of gene expression are widely accepted as principal agents of evolutionary change. Illustrating this, **Denis Duboule** (University of Geneva) examined the regulation of posterior Hox genes associated with anterior–posterior polarity in the tetrapod limb. He described a complex pattern of spatial and temporal co-linearity based on the distance between an enhancer and the protein-coding region of the gene. On the basis of these data, he suggested that the asymmetry of a tetrapod limb is merely a byproduct of Hox gene function rather than a trait selected for during the transition onto land.

One of the current dogmas of evolutionary developmental biology is that *cis* regulation of gene expression is responsible for the generation of most biological diversity. To examine whether genetic variation in *trans*-acting factors is also important, **Gunter Wagner** (Yale University) dissected transcriptional regulation down to its component parts. Dr Wagner presented a model in which he compared the efficiency of selection on a novel binding site, *cis*, versus selection on a novel protein interaction, *trans*. This model suggests, contrary to popular belief, that evolving new regulatory links is probably more easily accomplished through selection on protein–protein interactions. Examining this conclusion using a broad collection of data sets across a wide variety of gene families should help determine whether the standard beliefs of the field should be revised.

To my knowledge, this is only the second meeting specifically devoted to the microevolution of development.[6] It is my belief that meetings such as this will change the direction of research in evolutionary developmental biology and possibly lead to its decisive synthesis with the field of evolutionary biology. While the first stages of evolutionary developmental biology have largely progressed on the backs of technical advances, allowing detailed comparisons beyond the scope of model organisms, subsequent research should focus on answering long-standing evolutionary debates. The topics discussed at this meeting represent a handful of research programs, each of which could each fill a personal research career. What is the relationship between interspecific phenotypic variation and standing genetic variation in related species? Of this variation, what percentage of it is heritable and how does the genetic variance covariance structure evolve itself? At the molecular level how do novel regulatory interactions evolve and how much hidden genetic variation lies in the genome? The list could go on and on; the future of the field beckons with its possibilities.

Acknowledgments

This meeting was hosted by the Novartis Foundation, London, England. Special thanks to the Novartis organizers **Greg Bock** and his assistant **Allyson Brown**. Many of the ideas discussed in this review are the result of discussions with the meeting participants.

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