L'enfant terrible at 30: the maturation of evolutionary developmental biology

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Summary

The recent Keystone Symposium on Evolutionary Developmental Biology at Tahoe City in February 2011 provided an opportunity to take stock of where the past three decades have brought this interdisciplinary field. It revealed maturation on several fronts, including increased experimental rigor, the softening of dichotomies that were crucial to its founding and growth, and its growing relevance to both basic and biomedical biology.

Key words: Convergence, Evolution, Model systems, Modularity, Pleiotropy, Population genetics

Introduction

Almost exactly 30 years ago as we write, the Dahlem Workshop on Evolution and Development brought an international group of luminaries from evolutionary genetics, developmental biology and paleontology to the western sector of then-divided Berlin, Germany. Their goal was to define key problems and to set a research agenda to pry open the black box that development had generally occupied in evolutionary theory up to that time. This was, of course, the pre-genomics area, the developmental genetics revolution had only just begun, and the relevant disciplines had been largely separated for many decades. That the language of the report (Bonner, 1981) now seems somewhat archaic is therefore understandable, but it is nevertheless widely recognized as an early milestone in the rebirth of evolutionary developmental biology (EDB). Indeed, a recent conference at the Max Planck Institute for the History of Science, which included several Dahlem alumni, was dedicated to taking stock of the changes that had occurred over the past three decades. In February 2011, the Keystone Symposium on Evolutionary Developmental Biology (Tahoe City, CA, USA) presented another opportunity – one focused on the latest research findings – to see how far we have come since the Dahlem meeting.

Modern EDB was established in the context of far older debates, often framed as dichotomies, such as homology versus adaptation (e.g. Appel, 1987), pattern versus process, macroevolution versus microevolution, fixed species differences versus intraspecific polymorphism, mutationism (the idea that the introduction of new genetic variants biases adaptive trajectories) versus Darwinism (the view that selection alone dictates the course of evolution) (Stoltzfus, 2006) and the evolution of novel forms versus general theories of change (Amundson, 2005). As such, EDB can be seen as a reaction to the abstraction of the Modern Synthesis (which first attempted to unite genetics and evolution into a comprehensive theory), an attempt to get back to the problem of how discrete differences in form evolve (especially between species) and, above all, as a movement to provide empirical specifics that could challenge (or support) the assumptions of neo-Darwinian theory. A major impetus came from advances that revealed development to be a strongly hierarchical process, which in turn suggested an important role for loci (and potentially for mutations) of large effect. This clashed with R. A. Fisher's widely accepted microscope analogy (Fisher, 1930), which asserted that because populations were never far from the optimal phenotype, large phenotypic changes would almost always be deleterious, whereas (as with focusing a microscope) small changes approach a 50-50 probability of improvement.

Despite skepticism from notable evolutionary geneticists [see the compendium of quotes in the supplementary materials of Stoltzfus (Stoltzfus, 2006)], EDB went ahead on its fool's errand. Early studies emphasized class- and phylum-level comparisons but gradually focused on more closely related taxa to allow better insight into the process of change. In parallel, many evolutionary geneticists using quantitative trait locus (QTL) approaches began to discover loci of large effect that underpin various adaptive traits (e.g. Bradshaw et al., 1998; Bradshaw and Schemske, 2003; Peichel et al., 2001). When the mapping of QTL was taken to single-gene resolution, the two approaches converged (Doebley et al., 1997; Shapiro et al., 2004; Steiner et al., 2009). Thirty years later it seems fair to say that the concrete examples thus uncovered greatly expand, and even revolutionize, the way we think about evolution in general, and its temporal dynamics, in particular. The Keystone meeting revealed great enthusiasm for reconciling the insights of EDB with mainstream evolutionary biology and, moreover, for linking it explicitly to biomedical research. Below, we highlight some major themes that emerged during this meeting, suggest areas where consensus now seems to exist and propose areas for future efforts.

Key emerging themes

Exploring the micro-macro interface

EDB has generally resisted the idea that a seamless continuum exists between intraspecific variation and fixed differences between species. This resistance reflected, in part, the suspicion that major adaptive transitions require rare mutations that are rate limiting and that are not generally found as detectable polymorphisms. Nevertheless, even such peculiar mutations would have to arise in the context of a population. Speakers at the Keystone Symposium dissected intraspecific variation in development using two approaches. One examined the genetics of polymorphic anatomical traits of known adaptive significance. Examples included the role of standing variation in adaptation to full-time freshwater residency in stickleback fish, which we discuss in more detail below (David Kingsley, Stanford University, CA, USA), and how variant alleles of the gene Agouti mediate adaptive coloration and dorsoventral patterning of fur in beach mice (Hopi Hoekstra, Harvard University, MA, USA) (Manceau et al., 2011). These studies represent the trait-mapping approach mentioned earlier.

The second approach emerges from recent technological advances that allow the characterization of the relationship between standing variation and gene regulation across the entire genome.

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Working in *Caenorhabditis* nematodes, Matthew Rockman (New York University, NY, USA) described the surprisingly strong effect that chromosomal structure and the population genetic phenomenon of background selection have on natural variation in transcript abundance. In this system, the position of a gene on a chromosome is more predictive of population variation in expression than the trait the gene regulates (Rockman et al., 2010). Greg Wray (Duke University, NC, USA) discussed how allelic variation influences – or, in many cases, fails to influence – the expression of components of gene regulatory networks that pattern the sea urchin embryo. The meeting co-organizer Patricia Wittkopp (University of Michigan, MI, USA) explored how transcriptional profiles are altered in interspecies *Drosophila* hybrids.

The relationship between population genetics and macroevolution also came up during an open discussion with panellists Nicole King (meeting co-organizer, University of California, Berkeley, CA, USA), Michael Levine (University of California, Berkeley, CA, USA), David Stern (Princeton University, NY, USA) and one of us (R.E.L.). Stern proposed that elucidating the relationship between population variation and interspecies divergence remained the grand challenge for EDB, and he cautiously suggested that major innovations may indeed often require rare mutations. One of us (E.S.H.) asked whether such mutations might need to be sheltered in the relaxed selective environments of small isolated populations until they are more competitive globally. This view combines ideas of Richard Goldschmidt (who first coined the term 'hopeful monsters') with those of Sewall Wright [the Shifting Balance Theory (Wright, 1969)], Michael Whitlock [Variance-induced Peak Shifts (Whitlock, 1995; Whitlock and Fowler, 1996)] and Michael Lynch (Lynch, 2007). It should be testable in both paleontological and extant field populations, where it predicts that phenotypic variance should be higher in small isolated populations, such as those at the margins of range expansions (Burton and Travis, 2008). Of course, such populations also lose genetic variation by drift, so it is by no means certain that such protection ensures innovation.

Lessons from unicellular organisms

The participants of the meeting were, perhaps surprisingly, very comfortable with the use of unicellular organisms, which do not 'develop' in the usual sense, to inform EDB. Speakers showed how choanoflagellates, algae, yeast and *E. coli* can illuminate the transitions from unicellularity to multicellularity (Nicole King; James Umen, Salk Institute, CA, USA), can reveal the underlying logic of regulatory networks (Alexander Johnson, University of California, San Francisco, CA, USA) and can provide tractable systems for experimental evolution (R.E.L.). This comfort probably reflects the longstanding interest of both population geneticists (Lewontin, 1974) and developmental biologists in the mapping of genotype to phenotype more generally. When viewed in this light, multicellular development is merely one aspect – albeit an especially impressive, complex and interesting one – of a broader effort to extend the Darwinian formalism of variation-selection to include the 'rules' for how genetic differences translate into phenotypic variability.

Parallelism, convergence and the reproducibility of adaptive trajectories

The issue of the repeatability of evolutionary outcomes came up frequently at this meeting. First, in systems where an initially isogenic or freely interbreeding population gives rise to the same adaptation in parallel over short time spans, the precise details of the adaptive walk of each lineage can be investigated. Notable examples of this included Lenski's long-term evolution experiment with *E.*

coli, now beyond 50,000 generations. The replicate populations gain fitness in a new laboratory environment at remarkably similar rates, yet the mutations differ in their details and order, and in some cases their effects are contingent on prior mutations (Woods et al., 2011). The lake-invading sticklebacks described by Kingsley show repeated fixations of the same segregating mutation for lateral plate loss (Colosimo et al., 2005), while the *pitx-1* enhancer region is independently targeted multiple times when pelvic spines are lost (Chan et al., 2010). He suggested that the migratory life history of the stickleback would allow alleles that confer some advantage during their time in freshwater habitats to be maintained at low frequency in the marine population, and thus the same genotypes could later colonize multiple lakes. In a similar vein, Michael Kohn (Rice University, Houston, TX, USA) provided evidence that hybrid introgression was responsible for the repeated evolution of warfarin resistance in some species of house mice, even though those species were not previously known to hybridize in the field.

Even in the case of more ancient occurrences of interspecies divergence, which cannot be mapped genetically, repeated evolution can be instructive. Presenters described studies of convergently evolved multicellularity (King and Umen), loss of pigmentation in unrelated cave animals (Meredith Protas, University of California, Berkeley, CA, USA), and self-fertile hermaphroditism in *Caenorhabditis* nematodes (E.S.H.). Such parallel transformations in separate lineages reveal both general principles and the scope for different solutions to similar selective forces. For example, Protas discussed how cave isopods have followed two distinct genetic pathways that lead to albinism (Protas et al., 2011), whereas cave fish seem to have used only one (Protas et al., 2006). Similarly, a conserved RNA-binding protein plays opposite roles in sexual patterning of the hermaphrodite germ line in different nematode species (E.S.H.).

Evolution as a tool for deciphering structure-function relationships

Several speakers effectively showed how an evolutionary perspective allows rules to be inferred that govern structure-function relationships. One approach is to use closely related phenotypic variants to reveal causal links between structure and function. This approach was elegantly explored at the level of protein structure and ligand specificity by Joseph Thornton (University of Oregon, OR, USA), who used phylogenetic inferences to guide the reconstruction of ancestral protein sequences, and then assayed the changing functionalities of the ancestral and derived protein variants (e.g. Bridgham et al., 2009; Bridgham et al., 2010). The frequently observed specification of the same phenotypes by divergent mechanisms (True and Haag, 2001; Weiss and Fullerton, 2000) also provides opportunities to elucidate rules that govern structure-function relationships (Li and Johnson, 2010). Johnson discussed how variant forms of regulatory circuits in yeast clarify the overall logic of such circuits. Similarly, Scott Barolo (University of Michigan, MI, USA) described how the poor conservation of a complex transcriptional regulatory region that drives a conserved expression pattern in the *Drosophila* eye allowed comprehension of its overall regulatory 'grammar' (Swanson et al., 2010). In both cases, relationships emerge that cannot be observed and understood by focusing on a single taxon.

New models, increased rigor

Just a decade ago, only a few multicellular organisms were studied intensively enough to merit their designation as developmental 'models', and, for some of them, only recently have genome
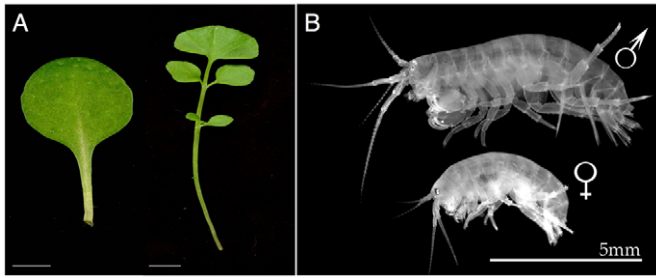
Fig. 1. Emerging model organisms in evolutionary developmental biology. (**A**) *Cardamine hirsuta* is related to the model crucifer *Arabidopsis thaliana*, and is readily transformable and amenable to forward genetics. Unlike *A. thaliana* leaves that are simple (left), *C. hirsuta* leaves are subdivided into distinct units called leaflets (right). Image courtesy of M. Tsiantis. Scale bars: 1 cm. (**B**) The beach hopper *Parhyale hawaiensis* is a lab-friendly model for crustacean development. Image courtesy of N. Patel.

sequences, transgenesis, and forward and reverse genetic methods become available. This meeting highlighted that many researchers in the EDB community are actively developing new model organisms (Fig. 1), often chosen to allow precise functional comparisons with existing models (Abzhanov et al., 2008). Examples of such emerging models that now sport an impressive array of tools include the cruciferous angiosperm *Cardamine hirsuta* (Miltos Tsiantis, University of Oxford, UK), the marine annelid *Platynereis dumerilii* (Detlev Arendt, EMBL, Heidelberg, Germany), the crustacean *Parhyale hawaiensis* (Nipam Patel, University of California, Berkeley, CA, USA), the beetle *Tribolium castaneum* (Dominik Stappert, University of Cologne, Germany), and non-*elegans* species of *Caenorhabditis* nematodes (E.S.H. and Ronald Ellis, University of Medicine and Dentistry of New Jersey, NJ, USA).

In previous EDB meetings, the comparison of expression patterns for a few key genes might have been the endpoint of most talks, but these new models are allowing such comparisons on a genome-wide basis. More impressive still, these new model organisms have been developed to the point where routine comparisons of phenotypes resulting from loss or gain of expression of orthologous patterning genes are possible. An especially provocative use of the new models was for 'synthetic evolution', i.e. the induction by genetic manipulation of phenotypes normally seen only in relatives of the model organism. Two striking examples include the production of an apparently vertebrate-like two-chambered heart in the ascidian *Ciona intestinalis* (Levine) (Stolfi et al., 2010) and the homeotic transformation of legs from one type to another by the knockdown (Liubicich et al., 2009) or transgenic overexpression of a Hox gene (Patel). This latter experiment is a wonderful confirmation that the relationship between Hox expression boundaries and appendage identity in crustaceans is indeed causal.

Emerging principles: optimal pleiotropy and the Stern-Carroll Rule

EDB was reborn in part because of suspicion that the dominant neo-Darwinian theory had been formed in the absence of sufficient empirical information. But after three decades of work, the field has produced data that are leading to the crystallization of some general principles. Two such principles were explicitly proposed in talks at the meeting; both dealt with pleiotropy, whereby a single gene affects multiple phenotypic traits in the same organism. Specifically,

these principles address how the reuse of conserved developmental signaling pathways (such as Hedgehog and Wnt) and transcription factors (such as *engrailed* and *ovo/shavenbaby*) at multiple times and places during development can bias the attributes of adaptively important mutations. One principle, recently dubbed 'optimal pleiotropy' (Kopp, 2009), was explored by Stern in the context of his work on the evolution of the *Drosophila* larval cuticle. Stern argued that when a discrete trait is gained or lost, neither global patterning factors (which impact the entire body) nor the battery of structural genes that build the organ in question are the targets for selection. Instead, evolution typically works through local regulators that respond to global cues and coordinate many downstream factors. These regulators thus provide mutationally accessible paths to produce coherent phenotypic changes.

Another, related general principle was put forward by meeting co-organizer Sean Carroll (University of Wisconsin, WI, USA). In the wake of a much-debated review that questions whether the emphasis of EDB on cis-regulatory evolution might be premature (Hoekstra and Coyne, 2007), Carroll sought to define precisely when cis-regulatory regions are expected to be the locus of evolution; under these conditions, this proposition met with no opposition at the meeting. Specifically, if an anatomical novelty is spatially or temporally restricted, and if development of the novel trait is controlled by pleiotropic regulators of the sort described above, then the relevant mutations will be in the cis-regulatory elements of those regulators, and not in protein-coding sequences (Carroll, 2008). This idea was also put forth some time ago by Stern (Stern, 2000), and we therefore suggest it henceforth be called the 'Stern-Carroll Rule'. We also note that others have stressed the general importance of regulatory evolution (e.g. King and Wilson, 1975; Wray, 2007).

The extent of pleiotropy and its relation to adaptive evolution has long been a central issue in evolutionary genetics. Fisher (Fisher, 1930) realized that pleiotropy was an important factor, and that if it were widespread it would constrain a sequence of adaptive steps to progressively smaller and smaller effect sizes. More recently, Allen Orr's theoretical work has suggested that as organisms become more complex, their ability to adapt would be increasingly constrained by pleiotropy (Orr, 2000). The Stern-Carroll Rule appears to offer one way around this 'cost of complexity'. Genetic models also suggest that mechanisms that restrict pleiotropy may allow at least modest gains in adaptive response (Welch and Waxman, 2003) and more predictable evolution (Chevin et al., 2010). Recent empirical studies of pleiotropy further indicate that strong modularity of the genotype-phenotype relationship is the norm in eukaryotes (Wagner et al., 2008; Wang et al., 2010). Such modularity may help to minimize pleiotropic constraints and thereby allow organisms to adapt more quickly (Wagner and Altenberg, 1996; Wagner et al., 2007). However, this suggestion should not be construed to mean that regulatory changes are a silver bullet for the adaptive evolution of all traits in all organisms. For example, unicellular organisms cannot localize gene expression in space (at least not at the level of distinct tissues), although they can and do localize expression in time, turning genes on and off in response to local environmental cues. In addition, adaptations that affect the entire body of a multicellular organism (e.g. metabolism, body-wide pigmentation) might be expected to use a wider variety of genetic mechanisms, including coding changes (e.g. Carroll, 2008).

More generally, it must be remembered that evolution will often find an initial solution that works well enough, even though it is not the best solution. Later changes may then compensate for maladaptive pleiotropic effects produced by the initial solution (e.g. Lenski, 1988). We suspect that some of the curious and convoluted

developmental programs that EDB seeks to unravel acquired their complexity through a historically contingent series of adaptations and compensations that relieved some problems but caused others. Perhaps this process even generated some of the regulatory modules that were used more elegantly in later stages of adaptive radiations, as selection could better refine and exploit those interacting elements that were tightly linked on a chromosome.

Conclusions

As was in evidence at this meeting, the bar continues to be raised for the standards of evidence in EDB, allowing more definitive conclusions to be attained and blurring the line between model organisms and everything else. It should therefore become clear in the years ahead which principles of EDB are general and which may apply only in specific taxa or circumstances. Moreover, there are tremendous opportunities for integrating across scales, from genome organization through mutational and molecular mechanisms to phenotypic and ecological effects.

Keystone participants heard several talks describing major changes in form mediated by alteration of the activity of single genes – some experimentally induced transformations and others natural transformations. Yet there were also other presentations that demonstrated a surprising conservation of developmental output, despite major changes to demonstrably important factors. What rules might distinguish these disparate outcomes? Answering this question requires a deeper understanding of structure-function relationships over different biological scales. In our view, comparisons of natural variants within and between taxa will probably elucidate the diversity of mechanisms and rules more effectively than will the focused dissection of any single organism. Moreover, this resolution is fundamental to biomedical research, because it will help to reveal those mechanisms that make individuals more or less susceptible to everything from genetic mutations and developmental perturbations to pharmaceutical manipulations and aging. As was discussed at this meeting, EDB therefore deserves sustained support from biomedical funding agencies.

EDB is also being pursued by 'multi-lingual' scientists, who are fluent in multiple biological sub-disciplines. At this Keystone meeting, Neil Shubin (University of Chicago, IL, USA) exemplified this fluency by combining expeditionary paleontology and molecular developmental biology to understand major transitions in vertebrate limb development (Shubin et al., 2006; Dahn et al., 2007). Too often, however, these relevant sub-disciplines are balkanized by our universities, funding agencies and journals. These barriers are not insurmountable, but we need to work to ensure that such integration continues and even grows. In particular, it will be essential to the future success of EDB that such cross-training flourishes at the level of graduate and postdoctoral education and research. In these respects, books for the general reader such as *Endless Forms Most Beautiful* (Carroll, 2005) and *Your Inner Fish* (Shubin, 2008) are extraordinarily helpful in attracting bright students and engaging the public that funds most research. The same joy of discovery and interdisciplinary synthesis that makes these books so engaging suffused the meeting, and challenged the participants to both think and read more broadly.

The program of this Keystone meeting emphasized research that uses empirical genetic approaches. Although such approaches have been highly productive, other areas of EDB research focus on morphogenesis as a cell- and tissue-level phenomenon, and on the use of sophisticated theory to infer general principles. As the vast majority of organisms are still not amenable to genetic analysis,

these approaches are crucial. In this respect, it is encouraging that the meeting showed that sustained work on marine invertebrates (e.g. sea urchins, marine annelids and ascidians) allowed the development of surprisingly rich and powerful sets of tools. However, the growing theoretical branch of EDB was essentially absent from this meeting. Although EDB will be rooted in empirical data for the foreseeable future, some groups now combine data with models to reveal general principles (e.g. Kavanagh et al., 2007), and we expect these efforts to continue their development. With any luck, we will see the fruits of this labor when l'enfant terrible reaches its full stature.

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