Novartis Foundation Symposium 284

TINKERING: THE MICROEVOLUTION OF DEVELOPMENT

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The evolutionary developmental biology of tinkering: an introduction to the challenge

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Abstract. Recent developments in evolutionary biology have conflicting implications for our understanding of the developmental bases of microevolutionary processes. On the one hand, Darwinian theory predicts that evolution occurs mostly gradually and incrementally through selection on small-scale, heritable changes in phenotype within populations. On the other hand, many discoveries in evolutionary developmental biology—quite a few based on comparisons of distantly related model organisms—suggest that relatively simple transformations of developmental pathways can lead to dramatic, rapid change in phenotype. Here I review the history of and bases for gradualist versus punctuationalist views from a developmental perspective, and propose a framework with which to reconcile them. Notably, while tinkering with developmental pathways can underlie large-scale transformations in body plan, the phenotypic effect of these changes is often modulated by the complexity of the genetic and epigenetic contexts in which they develop. Thus the phenotypic effects of mutations of potentially large effect can manifest themselves rapidly, but they are more likely to emerge more incrementally over evolutionary time via transitional forms as natural selection within populations acts on their expression. To test these hypotheses, and to better understand how developmental shifts underlie microevolutionary change, future research needs to be directed at understanding how complex developmental networks, both genetic and epigenetic, structure the phenotypic effects of particular mutations within populations of organisms.

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'Tell you what though, for free, terriers make lovely fish. I mean I could do that for you straight away. Legs off, fins on, stick a little pipe through the back of its neck so it can breathe, bit of gold paint, make good . . .'

Pet Conversion Sketch, Monty Python's Flying Circus, Episode 10

In a favourite Monty Python sketch, an unscrupulous and imaginative pet salesman describes a few simple transformations by which he can convert a dog into a fish. The sketch is preposterous for many reasons, not the least of which

is the feigned biological naïveté of the customer. The pet store's unconventional practices also ridicule scenarios by which non-evolutionary processes might generate novel, adaptive forms. It has traditionally been believed that evolution occurs gradually within populations from the accrual of many incremental, tinkering-like transformations of existing structures. The radical transformation of a terrier into a fish is not only an evolutionary reversal but also absurdly un-Darwinian: 'As natural selection acts solely by accumulating slight, successive, favourable variations, it can produce no great or sudden modification; it can only act by short and slow steps (Darwin 1859, p 471).'

Darwin, of course, didn't know the mechanisms by which evolutionary change occurs, but he clearly believed in gradualism (Mayr 1991). The heart of his theory is that each generation within an interbreeding population inherits a range of phenotypic variations. Because of relentless competition caused by geometric rates of reproduction, limited resources, and/or environmental change, those variations that confer some benefit to an organism's chances of surviving and reproducing will increase in frequency. The accumulation of such variations over time leads not only to organisms that are better adapted to their environments, but is also assumed to be implicated in speciation—the most important type of evolutionary change.

Darwin and his contemporaries were working before modern genetics was born, and they struggled with problems of missing information and temporal scale. Interestingly, many of us continue to struggle with the same challenges even though we now have a much better grasp of the fundamentals of genetics. It requires little imagination to see how selection or other processes such as drift and founder effects can lead to different varieties of finches or tortoises; but it is considerably harder to understand how the same processes can lead from common ancestors to forms as diverse as whales and hippos, or even humans and chimpanzees. A part of this problem of comprehension lies in the nature of the fossil record, whose inadequacies lead inevitably to gaps. Most intermediate forms no longer exist, and (a bit) like Bishop Berkeley's conundrum of the falling tree, how can we understand a given transformation if we can't observe it? We are thus hampered by missing evidence and by a limited understanding of the mechanisms by which one form can transform into another. How does a jaw become an ear, a scale become a tooth, or a swim bladder become a lung? As we begin to understand more about the developmental processes that generate transformations leading to intermediate forms, we can begin to see a more complete and satisfying picture of what really happened in evolution and why.

The burgeoning field of evolutionary developmental biology (EDB) has had a major impact on our thinking about evolution above the level of the species (macroevolution). EDB has become a vital and exciting field by reopening the black box—so long ignored by evolutionary biologists—of the developmental bases for

evolutionary change (see Hall 2003). At the same time, EDB has helped invigorate research on evolution within developmental biology. A key element in the origins of EDB was a set of discoveries in developmental genetics in a few model systems and their extension to non-model system organisms. About 20 years ago we remember the excitement of the first papers on homeobox gene regulation that started to fill the pages of *Nature* and *Science* (e.g. McGinnis et al 1984, Shepherd et al 1984, Dolle et al 1989, Kessel & Gruss 1990, Hunt et al 1991). Other key influences were Gould (1977), Raff & Kaufman (1983) and Atchley & Hall (1991), all of which were eye openers for palaeontologists and other biologists because they outlined more integrative approaches to addressing the evolutionary questions we wanted to study. Suddenly, it seemed possible for palaeontologists, long preoccupied with major trends in macroevolution above the level of the species, to test hypotheses about how evolutionary change occurred within populations (microevolution) and during speciation events. EDB has thus had widespread effects, even in fields remote from developmental biology. For example, in the field of human evolution, researchers have begun to rethink issues of homology, and to ask questions about the developmental bases for the transformations we observe in the fossil record (e.g. Lieberman 1999, Lovejoy et al 1999, Lieberman et al 2004, Pilbeam 2004, Hlusko 2004).

Not surprisingly, EDB itself is evolving rapidly. Many of EDB's most spectacular and early advances focused on large-scale shifts in body plan. A typical research framework has been to compare the developmental mechanisms that underlie differences in development between two or more standard model organisms (chickens, mice, zebrafish, fruit flies and nematodes) in their phylogenetic context. These comparisons have led to many basic insights about the genetic and developmental bases for major variations in animal body form. However, more and more organisms are now being studied in greater and greater detail and in taxa with closer and closer relationships. One useful consequence of this combination of increased breadth and depth is to permit comparisons of large-scale evolutionary shifts with smaller-scale differences between closely related species. Given that natural selection occurs most fundamentally on individuals within populations, it is especially appropriate that researchers are increasingly studying the developmental bases for the generation of variation within populations (e.g. Stern 2000, Brakefield 2003, Shapiro et al 2004, Frankino et al 2005). As EDB increasingly turns its focus to microevolution and the developmental bases of within-species variation, we can look forward to a new synthesis of genomics, EDB and population genetics.

Ironically, one issue persistently raised by the growing focus on microevolution within EDB and other fields is equivalent to the problem of scale, noted above, that confronted Darwin and his contemporaries: are the evolutionary developmental bases of phenotypic change at the microevolutionary and macroevolutionary

scales comparable in terms of kind or just degree? Does microevolutionary change occur via the same developmental processes that characterize the differences between distantly related model organisms? How useful is it to compare mice and fruitflies (or dogs and fish) if we wish to understand how evolution happens in the sense of the within-population variations of phenotype upon which natural selection acts?

Modelling microevolutionary transformations

In order to test hypotheses about the EDB of microevolutionary versus macroevolutionary events, one needs a framework to compare changes within populations and between species in terms of the transformational processes by which genotype generates phenotype. A good place to start is with three related issues about the relationship between developmental change and evolutionary change: the scale of evolutionary changes within populations, the relationship between genotypic and phenotypic variation, and the hierarchical nature of developmental pathways.

Tinkering

Evolutionary change occurs because phenotypic variation within populations is generated through random alterations to existing pathways or structures. This point has been made many times, including by Darwin (1859), but perhaps was made most clearly by F. Jacob's (1977) useful and brilliant analogy between evolutionary change and tinkering ('bricolage' in French). Unlike engineers who design objects with particular goals in mind based on *a priori* plans and principles, tinkers create and modify objects opportunistically by using whatever happens to be available and convenient. Similarly, heritable novelties upon which selection can act are generated only through the effects of mutations in the genome that lead to alterations in proteins and/or regulatory mechanisms that affect the developmental processes that influence an organism's phenotype. In the case of biological organisms however, tinkering occurs with no goal in mind. With the exception of the purposelessness of biological change, Jacob's analogy of evolution by tinkering is a particularly pithy analogy of how evolution generates novelty at multiple levels of development, and it helps explain several key emergent properties of evolutionary change such as integration, constraint and functionality. Tinkered things tend to work because they make use of pre-existing or easily modifiable functional components. As Jacob (1977) explicitly noted, tinkering explains why novel forms are often capable of developing, reproducing, and avoiding the fate of being hopefull monsters. And because of tinkering, all evolutionary change is constrained by the historical contingency of what happens to be available at given times in given lineages (Gould 2002).

Analogies tend to be dangerous forms of reasoning because, on inspection, they often break down in terms of utility and applicability. Jacob's tinkering analogy, however, has stood the test of time because it is so apt. Interestingly, and as noted by Duboule & Wilkins (1998), Jacob's essay is also one of the first clear expositions of the logic of EDB, but was written at a time when most evolutionary theory came either from systematists and paleontologists or population geneticists (see Mayr 1982). It took at least a decade for developmental biology to catch up with Jacob, but many of EDB's basic theoretical insights recall his analogy, explicitly or implicitly. One obvious example is the reuse of basic toolkit genes during development. As argued by Carroll et al (2001) and others (e.g. Hall 1999, Wilkins 2002), much if not most evolutionary change does not derive from new genes, but from new ways to deploy old genes in new contexts to generate novel forms. Tinkered developmental pathways typically alter phenotype by changing the timing/rate or site of expression of basic processes (leading to heterochrony or heterotopy, respectively). While these new pathways are likely to be successful because they use elements of proven function, they also lead to high 'workloads' for many basic toolkit genes that are expressed in many different contexts, and thus require elaborate *cis*-regulatory control (Duboule & Wilkins 1998). Another, related form of tinkering is the duplication and re-use of entire phenotypic modules that can take on novel functional roles (see Klingenberg 2005).

Transformations of genotype-to-phenotype

A second issue to consider is the complex relationship between genotypic and phenotypic variation. Natural selection acts within populations on phenotypically different individuals who vary in fitness. Since most phenotypic variation is complexly structured by many genes and by many developmental interactions, it follows that any theory of evolutionary change must be able to account for the relationship between genetic variation and the variation of complex phenotypes within populations.

This problem was highlighted succinctly by Lewontin's (1974) classic model of evolutionary change, shown in Fig. 1, which lays out the four sets of transformational 'rules' that generate evolutionary change in the relationship between genotype and phenotype. These four transformational processes are: (1) developmental transformations by which genotype becomes phenotype; (2) population-level transformations such as natural selection, founder effects and so on that lead to changes in gene frequencies within an interbreeding population; (3) transformations of the genotype during gamete formation such as mutation, segregation and recombination; and (4) transformations of the genotype caused by reproduction, such as fertilization biases, assortative mating and so on.

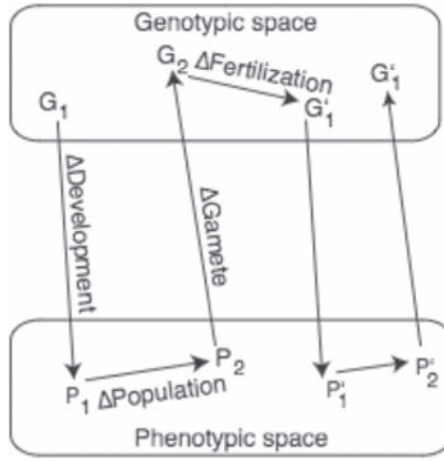

FIG. 1. Lewontin's model of evolutionary transformations. See text for details.

Although Lewontin's model clarifies the relationship between developmental biology and population genetics, most population geneticists have focused on the last three of his transformations, and most developmental biologists have focused on his first transformation. A major and important exception to this division of labour was Atchley & Hall's (1991) model for the development and evolution of complex morphological structures, summarized in Fig. 2, which explicitly integrated developmental biology and population genetics. In particular, Atchley & Hall (1991) examined Lewontin's (1974) first transformation in the context of a particular empirical model, the mouse mandible. Atchley & Hall reasoned that complex structures such as the mandible initially comprise a finite number of semi-independent units (modules), each of which can be described by five parameters (the number of stem cells in the precondensation, the time of condensation initiation, the rate of cell division, the percentage of mitotically active cells, and the rate of cell death). Once initiated, these units then interact with each other through various epigenetic processes (e.g. induction) and through the pleiotropic effects of particular genes. In some cases, these units also interact with the environment (e.g. responses to mechanical loading or nutrition).

A number of key features made the Atchley & Hall (1991) model important in the origins of EDB. First, the model outlined how just a limited number of transformational processes by which genotype becomes phenotype can be used as parameters to study the evolution of complex structures. Second, by drawing on the work of Lande (1979), Cheverud (1984) and others, they explicitly formalized

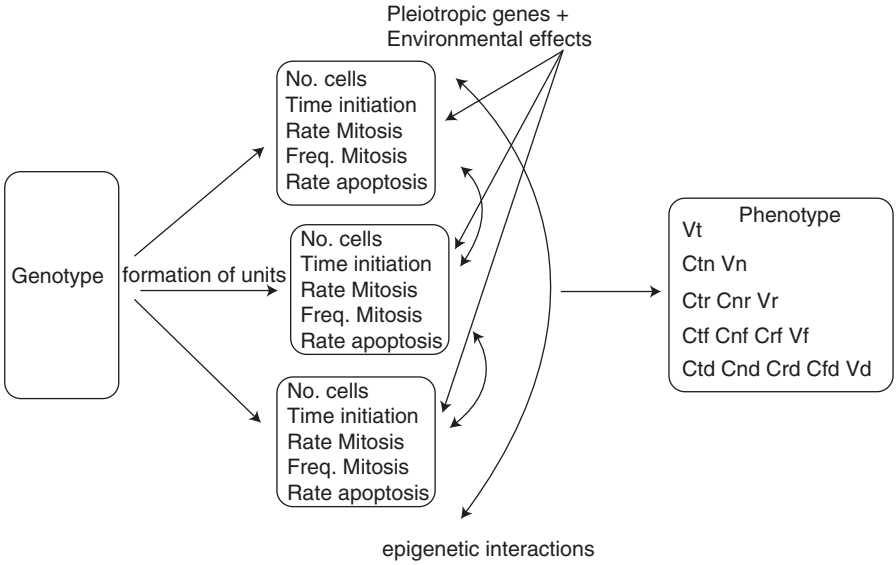

FIG. 2. A simplified view of the Atchley & Hall (1991) model whereby genotype generates units (here modelled in terms of skeletal condensations) whose size, shape and integration are influenced by a wide range of interactions. The end result of these interactions and processes is a particular phenotype characterized by a unique variance/covariance matrix. Changes to any of these generative steps will lead to predictable changes in phenotype as expressed not only in terms of heterochrony but also integration.

the effects of these changes in terms of patterns of variance and covariance. Finally, they also clarified how changes to developmental processes can be studied using heterochrony and/or allometry. Not surprisingly, the most profound impact of Atchley & Hall (1991) was on vertebrate morphologists, helping to spawn a rich literature on morphological integration (see Cheverud 2007, this volume). Unfortunately, Atchley and Hall's model was not applied as widely by geneticists and developmental biologists who work on plants, invertebrates and/or non-skeletal tissues. Part of this problem was that Atchley & Hall (1991) focused primarily on cellular processes relevant to skeletogenesis. It is much easier to study morphological integration in bones than in other tissues. In addition, Atchley and Hall mostly considered those cellular processes that regulate the size and origins of particular units (skeletal condensations), but did not discuss explicitly the genetic and developmental regulation of these processes. Thus, it has been a challenge for many developmental biologists to extrapolate Atchley and Hall's model to the particular tissues and/or developmental processes they study.

Regulatory hierarchies in development

A final issue to consider is how recent advances in our understanding of developmental pathways and their regulatory hierarchies help us clarify where and how selection can tinker with development to generate evolutionary change. This is a dauntingly large topic that has been the subject of many recent and diverse reviews (e.g. Gerhardt & Kirschner 1998, Hall 1999, Carroll et al 2001, Davidson 2001, Wilkins 2002, West-Eberhard 2003, Carroll 2005), but stepping back from the many details, one can make a few useful generalizations, illustrated in Fig. 3. Along the many steps by which genotype transforms into phenotype, there is a constant interaction between two interrelated sets of pathways: genetic and epigenetic (the latter defined, *sensu* Waddington, as interactions between a given gene and its environment, including the actions of other genes). Both genetic and epigenetic pathways are generally hierarchical, but in very different ways.

In terms of genetic pathways, there is a general hierarchical unfolding of connected gene activities, a network, that is characteristic of each developmental

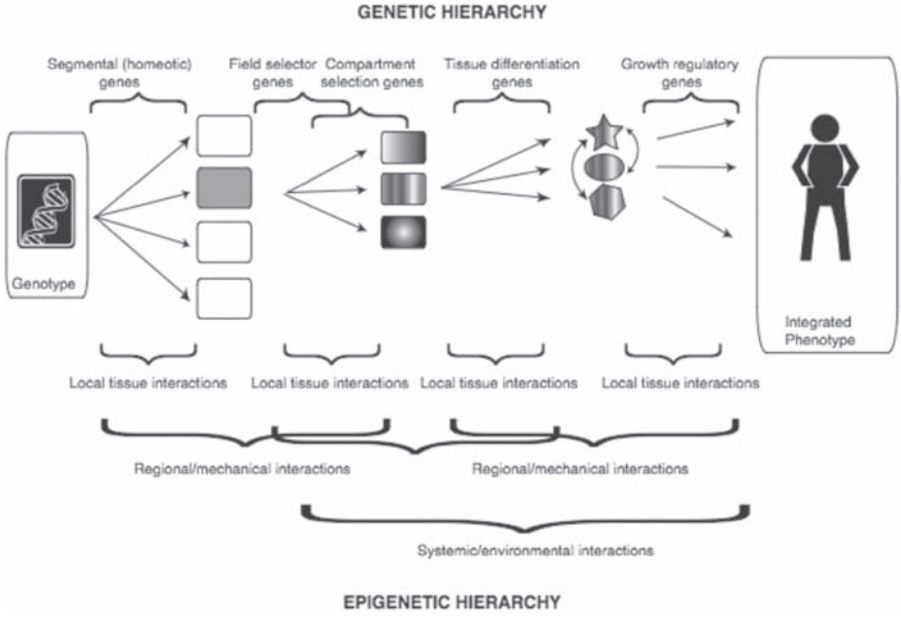

FIG. 3. A simplified model (based on *Drosophila*) of the interaction between genetic networks and epigenetic networks. These hierarchies occur concurrently and interactively. Note that the hierarchies depicted are arbitrary and not universally applicable; rather they are meant to illustrate the point that genetic networks combined with epigenetic interactions lead to integration at multiple levels of structure. See text for further details.

process. Carroll et al (2001) recently tried to summarize this sort of hierarchy for invertebrate development. While their scheme is overly simplified and not completely relevant to vertebrate development, it is nonetheless heuristically useful as a kind of pathway that may exist in certain cases. According to the Carroll et al scheme, *Drosophila* pathways typically begin with 'big gun' *patterning genes*, many of them homeobox genes, that define the basic units of the body such as the embryonic germ layers, neural crest, axial segments, and so on. Within these units, *field specific selector* genes then operate to trigger or constrain cascades of developmental events that help form major units such as organs or appendages. A now classic example is the eyeless gene, *ey*, which starts the development of eyes in *Drosophila*. Within these units, *compartment selector* genes then act to specify particular groups of cells that form axes (such as the way that the apical ectodermal ridge patterns the anteroposterior, dorsoventral and proximodistal axes of the limb). On top of these axes, *cell-type selector genes* then induce the differentiation of appropriate cell types to form the essential tissues of the body such as muscle, bone, or kidney. Finally, each of these cell groups can be subdivided into further units with differential rates and timing of growth, all of which are subject to regulation by specific *growth-related genes*. As noted above, other kinds of networks exist for different tissues and organisms (see Salazar-Ciudad & Jernvall 2004, for another example). The point is that genetic networks, by their very nature, tend to be hierarchical.

Genetic pathways—more appropriately considered networks because of their non-linear complexity—are not autonomous. Once started, they do not necessarily end up in the same place because a fundamental property of developmental pathways is that many, if not most, of the changes that occur during ontogeny are the result of interactions among different cells. These epigenetic interactions also have their own sort of hierarchy that runs interactively (and thus concurrently) with the genetic hierarchy described above (for a comprehensive review, see West-Eberhard 2003). At the local, most regionally specific level they include inductive interactions—sometimes reciprocal—between neighbouring cells that regulate the differentiation, proliferation and growth of particular cell lines, as well as influence their rate of migration and apoptosis. Classic and well-understood examples include the interactions between mesenchymal and epithelial cells in the face that regulate the formation of tooth germs (see Peters & Balling 1999, Jernvall & Thesleff 2000), or the interactions between mesodermal and ectodermal cells in the limb bud that initiate limb formation and regulate its patterning (see Tickle 2002). At a higher level, cells in a particular region are also influenced by signals such as diffusion gradients of morphogens (e.g. *Shh* in the limb bud). Cells in a given region can also be affected by mechanical and other environmental stimuli that, in turn, induce regional genetic and developmental responses. A good example is the way that brain growth up-regulates FGF2 expression in its surrounding membrane (the dura mater), which then up-regulates growth in sutures by turning on various

transcription factors (e.g. MSX2 and TWIST) that stimulate osteogenesis (see Opperman 2000, Wilkie & Morriss-Kay 2001). Finally, cells within a given region or even the whole body are regulated generally by systemic endocrine factors, which, themselves, are regulated by a wide range of environmental and intrinsic stimuli.

Although the above model of developmental hierarchies summarized in Fig. 3 is necessarily rather general, and arguably too simplistic, it illustrates an important point about how tinkering can operate to generate potentially useful phenotypic change. Notably, genetic networks combined with epigenetic interactions at increasing levels of specification from the local to regional to organism levels, lead to integration at multiple levels of structure. Put differently, a key emergent property of increasing specification combined with regional and organism-level organization is a highly integrated phenotype. Change at any level, from mutations that affect the *cis*-regulation of how, when and where a particular gene is transcribed in a given cell line, to how much oestrogen is pumped throughout an organism's body, rarely lead to large-scale independent effects. Instead, their actions are modulated by numerous interactions at different levels. For example, mutations that lead to extra fingers or toes, typically lead to digits that have (more or less) an appropriate set of muscles, nerves and vessels to permit some degree of function.

Modelling the degree and rate of tinkering events

Let us now consider how the above insights can be used to model and test hypotheses about the degree and rate at which major transformations occur at the microevolutionary level. Recall that our basic question is whether the developmental differences we observe between distantly related organisms are comparable in type (their developmental and genetic bases) or merely in their degree of effect. In addition, do mechanisms of developmental change at the microevolutionary level provide us with any insights about whether speciations occur through gradual versus saltational transformations? In other words, can major transformations evolve rapidly with few or no intermediate states, and if so, are they similar to the differences we observe among distantly related organisms? EDB has rekindled interest in these questions by providing potential developmental support for the hypothesis that evolution can occur rapidly without intermediate transitional stages (e.g. Lovejoy et al 1999, Gould 2002, Raff et al 2003, Byrne & Voltzow 2004). The most extreme statement of this view, the hypothesis of punctuated equilibrium, posits that evolutionary patterns typically show long periods of stasis punctuated by rapid periods of saltational evolutionary change (Eldredge & Gould 1972, Gould & Eldredge 1993). Although the hypothesis was initially rooted in palaeontological observations, it potentially fits comfortably with EDB findings

that distantly related organisms often use many of the same developmental genes and mechanisms, thereby potentially permitting a wide range of useful novelty to be generated (or lost) rapidly and without transitional forms via minor shifts in their regulation (Carroll 2005). As noted above, new segments can be generated by simple homeotic duplications; new appendages can be formed by heterotopic expression of existing field-specific selector genes (e.g. the much publicized *ey* mutants); and new tissues can be grown in new places by altered inductive interactions between neighbouring cell lines, many of which are changes in *cis*-regulation. Moreover, because developmental pathways necessarily take advantage of pre-existing mechanisms that generate integration, such shifts can lead to fully operational integrated organisms with new body plans rather than hopeless monsters. If major evolutionary transformations can and did occur via such simple shifts, then it follows that these shifts might have been rapid and saltational (that is, without many intermediate transitional forms). Although comparisons of distantly related organisms indicate that relatively simple developmental shifts can and do underlie major phenotypic differences, it does not necessarily follow that those changes occurred all at once without many intermediate transitional forms. As Darwin (1859, p 481) himself noted, one of the great challenges to thinking about evolution is to imagine the many transitional steps that can lead to large-scale changes: 'We are always slow in admitting great changes of which we do not see the steps'. Indeed, a number of arguments and observations support a more gradualist, transitionalist perspective whereby tinkering events typically generate small-scale changes with intermediate transitional forms. First, as noted above (e.g. Lewontin 1974), evolution occurs at its most basic level through the action of natural selection on individuals within populations. Thus, for organisms to remain part of interbreeding populations they cannot change so radically that their differences lead to reproductive barriers and/or isolation. In addition, since mutants must remain part of the gene pool in an evolving population (even one experiencing strong directional selection), any degree of reduced fitness in F1 backcrosses to the rest of the population and will select against change. Studies of hybridization generally support this point. Although distantly related species with distinct phenotypes (e.g. camels and llamas [Skidmore et al 2001]) can sometimes interbreed and produce fertile offspring, they rarely do. Even closely related and very similar species tend to have lower rates of reproductive success when they hybridize, leading to minimal introgression (Harrison 1993, Barton & Hewitt 1989). For example, geladas (*Theropithecus gelada*) and olive baboons (*Papio anubis*) regularly interbreed, but their F1 hybrids have low fitness (Jolly et al 1997).

A second, more developmentally based reason to argue that evolutionary change is often if not usually gradual and transitional derives from the overlapping, interactive, and mutually-dependent genetic and epigenetic hierarchies illustrated in

Fig. 3. As noted above, the hierarchical nature of developmental pathways enables mutations of ultimately large effect to 'work', but the expression of such mutations is also modulated and constrained by processes of integration that are a fundamental property of developmental pathways. To use Waddington's (1957) terminology, the expression of a given mutation depends largely on its epigenetic landscape. The intrinsic effect of most mutations is neither big nor small, rather it is the genetic or environmental context of the mutation that determines its effect (see Wilkins 2007, this volume). One can therefore hypothesize, following Schmalhausen (1949) and Stern (1949), that mutations of large effect are more likely to be successful if their phenotypic consequences are initially constrained by their developmental milieu. Over time, via the effects of natural selection on other parts of the genetic network, the expression of such initially cryptic mutations is predicted to be manifested or even become enhanced as their regulatory control becomes modified through changes in integration (Futuyma 1987, Lauter & Doebley 2002). This kind of change presumably underlies the phenomenon of genetic assimilation whereby the expression of mutations occurs many generations after the mutation itself because of tinkering driven by environmental factors (see Palmer 2004).

Given these different scenarios, it is useful to compare alternative models for how evolutionary transformations at the subspecies or species level might generate evolutionary changes that vary in terms of their rate of transformation (saltational or gradual) and effect (small-scale or large-scale). Figure 4 attempts to summarize, in a highly simplified manner, several different kinds of pathways by which phenotypic units (squares) transform during ontogeny through various developmental processes (arrows). Because the number of units and their potential interactions with each other and the environment increase during ontogeny, the units in each pathway become increasingly integrated over time. However, the pathways differ in the degree of change that a given mutation can cause. At one of end of the continuum of possibilities are simple changes in development that have small-scale effects on a few aspects of the organism's phenotype (Fig. 4A). Such transformations, which can occur at different times during ontogeny (Fig. 4A illustrates one that is rather late), may be the most common type of evolutionary change. There are many examples, but one type that is especially well studied is the mutations to *Hox* expression in axial somites that generate variation in vertebral numbers and in the boundaries between vertebral types (e.g. Kmita & Duboule 2003). As shown by Pilbeam (2004), such mutations can account for the substantial variation in thoracic and lumbar vertebral numbers within hominoids, and may have played a role in the origins of bipedalism when modal number of lumbar vertebrae apparently changed from three in the last common ancestor of apes and humans to six in australopithecines. Other examples of this kind of small-scale tinkering include tandem repeat sequences in the *cis*-regulatory region of *Runx2* (an up-regulator of

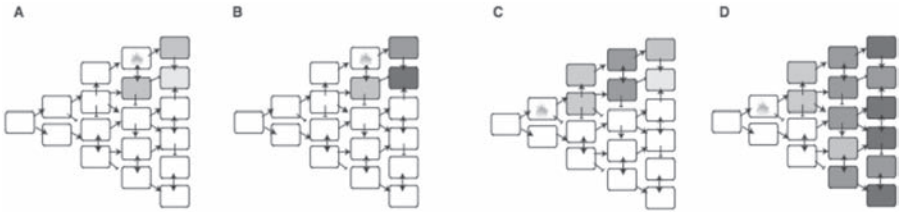

FIG. 4. Four models of evolutionary change. Boxes represent units of phenotype; arrows indicate developmental processes; fires represent mutations; ontogenetic time/stage progresses from left to right. In A, a mutation late in ontogeny leads to small-scale phenotypic change. In B, a mutation late in ontogeny leads to a larger phenotypic change, but with restricted effects on overall phenotype. In C, a mutation early in ontogeny leads to small-scale phenotypic change because of constraints imposed by other aspects of the genetic and epigenetic network of development. In D, a mutation early in ontogeny leads to wide-scale phenotypic change because of a lack of constraint by changes to the network.

osteoblasts) that apparently influence the length of the rostrum in dogs (Fondon & Garner 2004); and regulatory changes to *Pitx1* which leads to pelvic reduction in stickleback fish (Shapiro et al 2004).

A second possible type of transformation, illustrated in Fig. 4B, is a simple change in development that has large-scale effects on a few aspects of the organism's phenotype. Because such transformations have extensive effects with strong selective consequences, they might become rapidly fixed within a population by natural selection or other processes such as founder effects or drift, possibly causing rapid microevolutionary change. However such transformations, if they exist, are probably much more rare, and thus are hard to document. One possible example, which remains mostly untested, is the transformation from fins to limbs. Comparisons of distantly-related vertebrates such as mice and zebrafish suggested that the initial transition from fins to limb-like appendages most likely involved the recruitment of segments 9–13 in both *HoxA* and *HoxD* that, when expressed in the limb bud, generate an autopod with mobile wrists, ankles and digits (e.g. Shubin et al 1997, Coates et al 2002). The recent discovery of a fossil fish with a manus-like fin (*Tiktaalik roseae*) appears to confirm the existence of such a predicted transitional organism (Daeschler et al 2006, Shubin et al 2006), although it is not known to what extent the tetrapod manus is under extensive regulatory control, and how gradual the transition was in evolutionary time.

It is important to note, however, that neither of the hypothetical pathways described above in Figs 4A and B is highly integrated, and both model evolutionary changes in a few genes that have restricted effects. As is commonly appreciated, this sort of change is rare or unlikely because of extensive levels of pleiotropy, linkage and epistasis that constrain and modulate the expression of many

mutations (Cheverud 1996). As a result, most organismal phenotypes exhibit substantial degrees of integration, typically manifested by high levels of correlation and covariation, as well as by various scaling relationships among their different components (see Chernoff & Magwene 1999, Dworkin 2005, Klingenberg 2005, Hallgrimsson et al 2005). Put differently, the widespread presence of extensive integration suggests that hierarchies of genetic and epigenetic pathways act as a sort of funnel to limit and structure the vast reservoir of genetic variation present in a population into a more restricted range of phenotypic variation. Evidence that developmental pathways restrict the generation of variation was recently demonstrated by an experiment by Hallgrimsson and colleagues, which compared the phenotypic effects of various mutations that influence the growth of different components of the skull in mice. Although the mutations themselves were completely different in terms of their primary effects (e.g. one acted on brain size, another on cranial base length), the different mutations led to similar patterns of integration, probably because their effects were structured by the many epigenetic interactions among the components of the skull that occur during development.

With these concerns in mind, a third more likely model, illustrated in Fig. 4C, is that most mutations of potentially large effect actually tend to have muted effects on phenotypic outcomes because of processes of canalization that are a fundamental property of most developmental pathways (Dworkin 2005, Klingenberg 2005). These processes, which include various stabilizing interactions and genetic redundancy, are adaptive (i.e. have been subject to selection) because they buffer organisms from the effects of major mutations. Consequently, they also lead to gradual rather than punctuated phenotypic change. Over time, however, selection may act on these pathways either to release the constraints they impose on development and/or to enhance the effect of the primary mutation (as shown in Fig. 4D). In such cases, we expect to see, eventually, widespread changes of large effect, but with intermediate, transitional forms.

Testing the latter two models is much more challenging because it requires knowing more about developmental pathways that is currently often the case. Knockout and knock-in experiments, however, provide useful evidence which support the constrained models illustrated in Figs 4C and 4D. As is well known, many are genes are identified and characterized because their knockouts have dramatic and widespread effects on phenotype. Typically, knockouts that affect coding regions of widely used transcription factors have especially pronounced and often lethal effects, while mutations that affect the transcriptional regulation of key genes can have large phenotypic effects in one genetic background but often produce much less of an effect when expressed in a different background (Pearson 2002). There are many examples of this phenomenon. To highlight one: knocking out the masterblind gene (*mbl*) in Zebrafish leads to an expanded jaw and reduced neural components in a TL background, but to much less expression in an AB

background which partially rescues the phenotype (Sanders & Whitlock 2003). These and other similar cases (see Flatt 2005) indicate that mutations to complex pathways can be expected to lead to gradual or rapid evolutionary change depending on several factors, including the intensity of the selection pressure involved, and the degree of redundancy and constraint within the developmental pathway.

Indeed such effects are a predicted evolutionary outcome of tinkering itself. Over time, as tinkering events increasingly co-opt the same toolkit genes into more pathways, these genes take on new functions, and their combinatorial control becomes more complex (Kaufman 1987, Duboule & Wilkins 1998). Such complexity inevitably leads to more developmental stability and canalization, which means that many mutations that influence complex pathways may initially be unexposed to selection because they have minimal phenotypic effects. In other words, complex developmental pathways may be constrained to undergo more gradual change than simple systems, a phenomenon that Duboule & Wilkins (1998) term 'transitionism'.

Testing the models

Unfortunately, models of how tinkering events generate evolutionary change in populations produce more questions than answers. In order to resolve these questions, we especially need more information about how differences in developmental pathways generate variation within populations and between closely-related species (e.g. through QTL analyses on inbred lines and closely related species). For example, do mutations of large effect play a significant role in microevolutionary change, or are such changes simply the observed by-products of broad-scale comparisons? Moreover, to what extent and when are mutations of big effect (e.g. shifts in patterning) modulated and constrained by existing developmental pathways, both genetic and epigenetic? In addition, do phenotypic changes that lead to microevolutionary events typically occur from shifts at particular levels of development (von Baer's Law predicts they occur at later ontogenetic stages)? Finally, how do organisms in the same population cope with novel variations of integration and/or shifts in modularity (i.e., how developmentally different can a reproductively successful mutant be?).

Answering these and other questions will be an enjoyable challenge, one that requires a new synthesis of population genetics and developmental biology along the lines of Atchley & Hall (1991). Given the diversity and complexity of developmental pathways it is unclear if we will ever derive widely generalizable models applicable to a broad range of tissues and organisms. Our hunch, however, is that the developmental bases for most microevolutionary changes will follow Darwin's prediction of gradualism. As noted above, mutations with simple, direct effects on phenotype tend to "work" only when they result in minor phenotypic changes,

whereas mutations of big effect are much more likely to lead to maladaptive organisms with low fitness that will be quickly removed from the gene pool. In reality, most developmental pathways tend to be complex and integrated, with many redundant steps, and to rely heavily on a small set of genes with heavy workload. Such complexity has, itself, evolved because it has enhanced fitness, and has permitted evolvability via tinkering. In such circumstances, natural selection favours mutations whose effects are buffered by complex, integrated pathways. One predicts these sorts of pathways to favour selection on aspects of pathways that release constraints and/or enhance the effects of chance mutations, thereby leading to gradual evolutionary change with intermediate phenotypes. When viewed over long time scales (as is typically the case when we compare distantly related organisms) we are seeing the cumulative effects of changes resulting in major shifts, but we are missing many of the complex changes in regulatory machinery that influence their expression.

Finally, although much of the work needed to better understand the developmental biology of microevolution will occur in the lab with model organisms such as mice and butterflies, it is useful to remember that such research has much broader implications. A particularly interesting challenge will be to test hypotheses about our own species' origins. In addition to satisfying our intrinsic interest in our own evolutionary history, several reasons make humans an exciting test case for a synthesis of EDB, population genetics and genomics. First, we have the complete human and chimpanzee genomes, along with extensive data on human genetic variation. In addition, we know from several lines of evidence that humans and chimpanzees shared a last common ancestor 5–8 million years ago that, phenotypically, must have been very much like a chimpanzee (Ruvolo 1997, Pilbeam 1996, Patterson et al 2006). In addition, we have a superb, well-studied and well-dated fossil record that extends back close to the estimated divergence time of apes and humans. And, finally, we have a rich knowledge of human developmental genetics from 'natural' knockout experiments in the form of various syndromes and diseases. Human and chimpanzee development will never be studied experimentally in the lab, but the above sources of data, combined with emerging new technologies, may help us figure out what genes changed in human evolution and how they were deployed. It may be funnier to imagine transforming a terrier into a fish, but it is far more interesting to decipher what processes actually transformed a chimpanzee-like last common ancestor into the earliest bipedal hominids, and thence through a series of transitions into modern humans.

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