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Recommendations and Goals for Evo-Devo Research

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RECOMMENDATIONS AND GOALS FOR EVO-DEVO RESEARCH: SCENARIOS, GENETIC CONSTRAINT, AND DEVELOPMENTAL HOMEOSTASIS

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ABSTRACT

The rapidly growing field of evolutionary-developmental biology (evo-devo) arises from the fusion of formerly disjunct scientific disciplines that traditionally generate very different scientific products. What should the scientific product of evo-devo be? I propose it should be a testable evolutionary scenario. Evolutionary scenarios have suffered eclipse and even opprobrium in recent years, but analysis of genes that control development may make evo-devo scenarios testable, hence scientifically valid. Hypothesis-based studies are more likely than descriptive studies to generate testable evolutionary scenarios. Candidate-gene studies are risky if only one or a few genes are known in the pathway that is putatively responsible for the evolutionary innovation. General questions that may be addressed by evo-devo include the nature of genetic and evolutionary constraint and, conversely, why some clades show "tendencies to evolve." High levels of developmental homeostasis may result in genetic constraint on evolution. Although genetic constraint is largely hypothetical, developmental homeostasis can be measured, so the possibility of its impact on evolutionary potential can be tested. I introduce the "rock band" model to provide a metaphor for the genetic control of development that may allow evolution to occur. The rock band model also illustrates conditions that may lead to greatly increased stability (resembling developmental homeostasis) rendering change unlikely. This paper is Floral Genome Project Contribution number 24.

Key words: candidate gene approach, developmental homeostasis, epihomology, evo-devo, evolution of development, evolutionary-developmental biology, fundamental homology, genetic constraint, rock band model, tendency to evolve, testable evolutionary scenario.

INTRODUCTION

The new field of evolutionary-developmental biology (evo-devo) is growing rapidly by attracting scientists from varied backgrounds (Dalton 2000; Cronk et al. 2002). The excitement it generates (Raff 2000; Goodman and Coughlin 2000) is easy to understand: studies in evo-devo offer the promise of completing the broad-scale understanding of evolution by bridging the chasm between whole-organism evolutionary studies and mechanistic analyses at the genetic and molecular levels.

A deep understanding of evolutionary history should encompass not only the *pattern* of organismal relationships, but should also account for the *process* of evolutionary change, including genetic changes that created novel morphological and physiological attributes, and the selective forces or chance events that allowed novel features to become characteristic of particular lineages. In the past, study of evolutionary process has been problematic because of the lack of mechanistic framework to limit the imagination of those trying to explain the origin of novel structures or of major taxonomic groups, that is, of macroevolutionary events. Ignorance of how genes determine organisms' attributes prevented evaluation of evolutionary hypotheses using the tools and data from other parts of biology, such as genetics, population genetics, or ecology, and precluded any resulting inferences that could feed back into taxonomy, systematics, or paleontology.

The admittedly euphonious pairing in the term "evo-devo" fails to identify the crucial new field that now makes evo-devo possible. Already in the nineteenth century the study of animal development was a core component of evolutionary biology (Hall 2000; Hossfeld and Olsson 2003; Wagner and Larsson 2003), but it is the new developmental genetics (not simply development) that provides the crucial bridge from evolutionary biology to fields that previously could not contribute significantly to evolutionary understanding.

Population biology was fundamental to the understanding of speciation in the *Evolutionary Synthesis* 60 years ago, but population biology first grew as a theoretical science without knowledge of the genes that control evolutionarily significant phenotypic differences. As a result, this field has had remarkably little practical impact on the understanding of macroevolution, that is, evolution above the species level.

Interactions of organisms with their biotic and abiotic environments result in differential selection on varying phenotypes, which is the rudder of evolution. Such studies lie within ecology, but without knowledge of the genetic determinants of phenotypes, such data are disconnected from the mechanisms underlying evolutionary change.

Molecular systematics only superficially bridges the gap, because its inferences fall squarely to one side or the other of the divide: they either involve relationships of taxa or instances of gene evolution, but the genes are usually experiencing purifying selection, which indicates that the function of the gene product is not changing (Kellogg 2004), whereas intergenic regions and introns typically exhibit neutral evolution.

Traditional studies in physiology, genetics, molecular biology and biochemistry have commonly ignored evolution completely.

Evo-devo interconnects these many fields, so that knowledge from each will illuminate the others, making biology into a contiguous intellectual enterprise, which it has not been for a century. Ultimately, all attributes of organisms may become subsumed under an evolutionary understanding, even including biogeography, ecology, population biology, physiology, anatomy, and macromolecular structural biology (David 2001).

The most important fields of study in this burgeoning fusion are ones that hardly interacted in the past, most notably developmental genetics (including aspects of molecular biology) and systematics (including taxonomy, molecular taxonomy, morphology, and anatomy) (Roush and Pennisi 1997). The traditional scientific products of these fields differ so dramatically that practitioners seldom understood each other, and there was even animosity between them. For example, it was a commonplace that some molecular biologists attacked taxonomists as doing purely "descriptive" work that should not be considered science. Such ill feeling perpetuated this "great divide" in biology, variously termed "skin out" vs. "skin in" or whole organism vs. biochemistry/molecular biology studies.

Because the scientific products of the component fields are so different, the question arises: what should be the ultimate scientific product from the field of evo-devo? I propose that the ideal product is a Testable Evolutionary Scenario, preferably one that gives a full account of the evolutionary events that generated the novel feature(s) or the group of organisms. The complexity of such a full description makes it a "scenario," defined by the Oxford English Dictionary as (definition 1) "a sketch or outline of the plot of a play, ballet, novel, opera, story, etc., giving particulars of the scenes, situations, etc.," or (definition 2) "A sketch, outline, or description of an imagined situation or sequence of events; esp. . . . (c) A scientific model or description intended to account for observable facts." Such an account is far too complex to be considered a unitary hypothesis or theory, but typically would not rise to the level of a paradigm, that is, a new way of thinking about the subject or a new conceptual structure for its understanding. A scenario might be separated into numerous individual hypotheses, some of which might be relatively independent from other hypotheses subsumed within the scenario. If component hypotheses within the scenario do interact, such interactions are themselves elements of the scenario. The scenario as a whole should generate a full, consistent picture of the evolutionary event. Testing may force a scenario to be modified, re-cast, pruned, or discarded, generating a new or improved scenario that (we hope) is closer to historical truth. A full scenario that convincingly passes all available tests would offer a deep understanding of the evolutionary event it portrays.

Evolutionary scenarios have had bad press since the 1970s (Gould 1978; Gould and Lewontin 1979). Even the Oxford English Dictionary takes note, commenting, "the over-use of this word [scenario] in various loose senses has attracted frequent hostile comment." Such hostility has seriously inhibited attempts to create useful scenarios. That is why scenarios need defense now.

In the following discussion I first discuss the nature of evolutionary scenarios, and then the criticism leveled at their use. Then I offer specific suggestions for effective evo-devo studies, and consider major questions that evo-devo may address.

DISCUSSION

Evolutionary Scenarios

Evolutionary scenarios have a very long history, extending back even before Darwin's *Origin of Species* (Darwin 1859). Lamark's explanation for the giraffe's long neck (Lamark 1809: 122; Mayr 1982: 344, 352–358), is probably the most familiar early scenario. Evolutionary scenarios were central in the *Origin of Species* and in early Darwinian studies. Such scenarios served the important function of showing evolution to be a reasonable explanation for the diversity of living things. Scenarios connected distinct taxonomic groups to each other, making explicit the claim of historical relationships among them.

More recently, practitioners of the *Modern Synthesis* (or the *Evolutionary Synthesis* [Mayr 1982: 566–570]) endeavored to use knowledge from all branches of biology to understand evolution as a process, and from this understanding to generate the best possible account of organismal relationships—but without a firm connection between whole organism and sub-organismal studies, the only way this could be attempted was by creation of evolutionary scenarios. Direct tests of such scenarios were not possible. Instead, one argued relative merits of competing scenarios, to determine which appeared to be the most reasonable. To an unfortunate degree this approach resembled the algebra problem of solving a single equation containing two unknowns: one unknown can assume practically any value, depending on the value assumed for the other unknown. In the *Modern Synthesis,* the analogs of the two (actually three) unknowns were phylogenetic history and the mechanisms of gene action and evolutionary change.

Competing evolutionary scenarios were judged, I believe, using the criterion of internal self-consistency of the data, of explanations of the data, and (very crucially) of which data were thought important and thus required explanation (Frohlich 1999, 2003). Such analyses were especially difficult at the macroevolutionary level, which led to a focus on species-level questions. In spite of the difficulties, much progress was made in the understanding of speciation and in species-level taxonomy as organismal diversity became better known and ideas regarding evolutionary processes were gradually honed.

Insurmountable problems at the macroevolutionary level are confirmed by the significant differences within the angiosperm classifications proposed in the *Modern Synthesis* era by Hutchinson (1973), Takhtajan (1980), Cronquist (1981), Thorne (1983), Dahlgren (1983) and Dahlgren et al. (1985). The incompatibilities between their systems highlight one attribute of internal self-consistency tests: small changes in the data, or in which data are considered important, sometimes cause gross changes in the preferred scenario, to the extent that scenarios worthy of consideration appear to be intellectually discontinuous. The criterion of internal self-consistency can sometimes result in apparently convincing support for grossly erroneous conclusions.

An example from biogeography.—An example from biogeography illustrates the problem of double unknowns and also shows the effects of removing one unknown. Here, one of the unknowns consisted of past continental positions, while the other involved evolutionary patterns and dispersal abilities of organisms. In his superb study of animal distributions, Darlington (1957) concluded that continental movements were NOT required to explain the distribution of living animals on the Earth. He believed that major groups commonly arose on the "world continent" (Eurasia), and then dispersed to other, more distant continents. Later the group would become extinct in Eurasia as new, superior groups arose there and replaced it. This repeating pattern could account for organisms restricted to the Southern Continents as remnant groups that had originated and spread from Eurasia, but had subsequently been out-competed in Eurasia. Darlington also believed that long-distance dispersal by wind (Darlington 1938) could explain some peculiar distributions, such as the presence of a (primitive) leptodactylid frog on New Zealand. To demonstrate the reasonableness of frogs blowing to New Zealand he would tell the story of throwing a frog off the roof of the Museum of Comparative Zoology at Harvard, while the director, Thomas Barbour, stood below to determine the outcome. The frog hit, and the director yelled up to the roof "It's quite dead!"—At which point the frog hopped away. The seminar audience would laugh, and the possibility of frogs blowing to New Zealand would then be treated as reasonable. The criterion of internal self-consistency was satisfied. (In the 1960s Darlington re-evaluated Southern Hemisphere distributions, concluding that continental movements probably had occurred [Darlington 1965], but he still accepted the World Continent as the source of major groups sequentially replacing earlier groups across the Earth.)

The discovery of plate tectonics removed one of the unknowns from biogeographic work, by providing detailed historical data on continental movements. It rendered moot the requirement that the World Continent be the font of successive waves of organisms invading distant landmasses and serially replacing earlier invaders.

Knowledge of plate tectonics, coupled with vastly more robust phylogenies, has dramatically shrunk the hypothetical space in which one might erect competing self-consistent theories. Vicariance biogeography (Humphries and Parenti 1999) offers explanations that are more detailed and more explicit than the accounts of traditional biogeography, allowing hypotheses of vicariance to be tested. Some distributions do result from vicariance (Haddrath and Baker 2001; Swenson et al. 2001). In other cases, phylogeographic analyses suggest that long-distance dispersal generated modern distributions (Dick et al. 2003; Nagy et al. 2003)—even for frogs (Vences et al. 2004). Biogeographic spread through Laurasia explains yet other Southern Hemisphere distributions (Davis et al. 2001). Recent advances in estimating divergence times within phylogenies greatly augment the power of biogeographic analyses (Sanderson 2002). Together, new theories and new analytical methods promise a renaissance of biogeography (Donoghue and Moore 2003).

One must emphasize that error in one part of a scenario may not invalidate its other components. For example, Darlington's *Zoogeography* (1957) provides a profound discussion of biogeography, in spite of his rejection of continental drift. One must be careful not to reject a whole opus or an entire scenario because a (separable) component is wrong. A famous example of the latter was the dismissal for many decades of Wegener's (and others') continental drift theory, largely because proposed forces to move the continents were thought too weak and the mantle too rigid (Frankel 1988; MacDonald 2003; but see Oreskes 2003). Physics provides an even more remarkable example, although Sadi Carnot was luckier than Wegener. Although Carnot used the caloric theory of heat in his publication on heat engines, and then died young, it was recognized that the same reasoning would apply to the modern (mechanical) theory of heat, so Carnot has long been recognized as the father of the second law of thermodynamics (Erlichson 1999).

In a sense, evo-devo is the culmination of the *Modern Synthesis.* That endeavor attempted to use knowledge from all fields of biology to understand evolution. Of the crippling unknowns mentioned above, phylogenetic history is being resolved by molecular systematics, and the mechanisms of gene action are being elucidated by developmental genetics, allowing a more focused question on evolutionary mechanisms to be the central issue addressed in evo-devo.

Just so stories.—If a scenario is sufficiently self-contained or involves multiple unknowns that can all be adjusted to account for any possible observation, then the theory cannot be refuted by any data, nor can internal self-consistency ever fail. Gould attacked such evolutionary explanations as "just so stories" (Gould 1978), in reference to Kipling's whimsical explanations for the origins of animals' attributes. Gould rejected theories that cannot be refuted as being unworthy of science. In their "spandrels" paper, Gould and Lewontin (1979) attacked the uncritical use of adaptationist explanations, noting that if one adaptationist explanation failed, any number of others could be proposed. They suggested that attributes of organisms could have arisen through a variety of mechanisms that did not require selective advantage for the particular feature under discussion. Among their alternatives were genetic mechanisms such as drift, genetic constraint, allometry (presumably due to genetic constraint), and pleiotropy, which grades into evolutionary mechanisms such as preadaptation, that is, the origin of a feature for a function no longer of importance (Bock 1959; Mayr 1960), (or exaptation [Gould and Vrba 1982]). These alternative explanations may well become testable if the underlying genetics of particular attributes are known.

For example, genetic drift, directional selection, and stabilizing selection of amino acid coding regions may be distinguished by the ratio of non-synonymous to synonymous substitutions (dN/dS), especially when estimated on individual internodes in a phylogeny (Yang 2002). In noncoding regions (including promoters), both stasis and rapid sequence change may be detected by phylogenetic foot printing. Regions showing stasis may be interpreted as conserved regulatory regions (Ayre et al. 2003; Hong et al. 2003). Regions showing especially rapid change, i.e., significantly faster than expected from neutral substitution (recognized by comparison with other regions of the same genome or by comparison to the same region on a sister-taxon lineage) have been termed "evolutionary hotspots" (Yap and Pachter

2004), where such rapid change might be adaptive. In population studies the presence of genetic sweeps identifies regions experiencing strong positive selection. Genetic sweeps might suggest regions that are of particular importance in evolution at and around the species level (Diller et al. 2002). These methods can be combined synergistically with the increasingly powerful ab initio bioinformatics analyses of genomic sequences (e.g., analyses that detect primer binding sites, the spacing of binding sites and, by inference, potential transcription factor interactions, and maybe even protein function). This will allow increasingly powerful inferences of mechanisms that control gene expression, and result in expression differences between different alleles and loci in the same or different organisms (Qiu 2003; Whisstock and Lesk 2003). Even the ab initio prediction of protein folding shows good progress (Wolynes 2004), perhaps eventually allowing direct calculation of the effects of amino acid substitutions and indels on protein structure and function. Perhaps, some day, possible functions of uncharacterized domains or whole proteins might be inferred directly from their amino acid sequences.

Genetic changes that seem likely to result in evolutionary constraint have been found in a clade of *Ipomoea* L. in which red, rather than blue pigment is produced in the petals. At least two genetic changes inactivate the blue-pigment producing pathway, suggesting that a reversion from red to blue pigment production is unlikely (Zufall and Rausher 2004).

Crystallins provide a remarkable example of evolutionary origin for an attribute no longer of significance. These proteins are highly expressed in eye lenses, and give eye lenses their high indices of refraction. Yet many crystallins are identical to (or derived from) enzymes used in intermediary metabolism; surely these enzymatic functions are not of significance in the lens (though chaperonin-derived crystallins may still have chaperonin function) (Piatigorskya 2003).

Pleiotropic genes are clearly important in evolution. Iltis' (1983) "catastrophic sexual transmutation" scenario for the origin of maize from teosinte was tested by the identification of quantitative trait loci (QTLs) that account for the major differences between maize and its ancestor teosinte. This implied that the origin of maize was both simpler and more complex than Iltis had hypothesized: it was simpler in that a few genes could account for the major differences between the two plants, but more complex in that the pleiotropic action of the genes involved accounted for multiple dramatic changes in the evolution of maize (Doebley 1995; Doebley et al. 1997; Martienssen 1997; Wang et al. 1999; Hubbard et al. 2002; Jaenicke-Després et al. 2003). This work has now led to newer scenarios (Iltis 2000; Lauter and Doebley 2002; Smalley and Blake 2003).

These examples show that the alternative mechanisms for evolutionary change offered by Gould and Lewontin (1979) may be discernable, at least in favorable circumstances, thus allowing tests of evolutionary scenarios. In his magnum opus on evolutionary theory, Gould (2002) discusses evo-devo at length, joyously acknowledging the importance of the new knowledge it is generating. Gould still downplays the evolutionary importance of adaptation relative to genetic constraint, but these very claims constitute implicit models (or vague scenarios) of evolutionary mechanism that can now be tested. Evolutionary scenarios that are testable are not "just so stories" (Gibson 1999). Testable evolutionary scenarios lie within science, as they are fundamentally different from Kipling's marvelously inventive explanations that were never intended to be believed, and so could not benefit from support nor suffer from refutation.

The Diversity of Evo-Devo

What constitutes an evo-devo study? The critical element of evo-devo is the creation of a bridge over the great divide between organismal evolutionary studies (broadly defined) and developmental genetics (including related elements of molecular biology, biochemistry, and physiology). I believe that any study that establishes such a bridge should be considered evo-devo.

This is a broad definition, which includes at the extremes studies that do not directly use genetic data and others that only use genomic sequence data. For example, the study by Boyce and Knoll (2002) on developmental potential and evolution of leaves employs data from fossil leaves (or leaflets), in particular, from their venation patterns and lamina structure. Boyce and Knoll's approach is to use venation patterns to infer the developmental mechanism that generated the leaf lamina. Their analysis is informed by studies of living plants with comparable venation patterns in which the leaf meristems have been studied. They suggest that the limited range of possible meristem organizations, with their characteristic effects on venation, does allow such inferences to be made. Their analysis is facilitated by the multiple instances of parallel evolution that gave rise to leaves and independently generated diverse venation types in many lineages. They carefully documented the wide extent of this parallelism, and note the similar sequence of venation pattern changes in each lineage. It is the multiple instances of parallel evolution that allows such strong claims to be made. They point out that developmental genetic studies of laminar structures in modern plants, including leaves, petals, winged fruits, etc. can provide tests of their inferences. This makes their scenario testable and places the study firmly within evo-devo.

The calcichordate theory of Jefferies (Jefferies 1986; Jefferies et al. 1996; Jefferies 1997; Dominguez et al. 2002) grows from the *Modern Synthesis* tradition and also uses cladistic analysis on paleontological data. It presents a scenario for the evolution of chordates, suggesting most famously that the earliest chordates possessed a calcite skeleton. It identifies, as stem-group chordates, fossil animals that lay down on their right sides on the ocean bottom, and so exhibited extreme left-right asymmetry. This implies that left-right asymmetry is likely homologous throughout the living chordates, and that modern chordates show less asymmetry than their deep ancestors. Living amphioxus shows remarkable asymmetries in early development, even for such canonical structures as the gill slits, which are essentially symmetrically placed in the adult, but whose development involves stunning asymmetries (Boorman and Shimeld 2002). In particular, some of the fossils show gill slits on the left side of the head only, and this recalls the strange fact that larval amphioxus has left gill slits only. Genes specifying left-right asymmetry in vertebrates are expressed in

similar patterns in lower chordates (Cooke 2004*a, b*) suggesting that such asymmetry is homologous throughout the chordates, and derives from stem-group ancestors. The calcichordate theory also has implications for possible homologies, in different major groups, among genes involved in skeletal deposition. This subject clearly falls within evo-devo, especially when considering both the paleontological and the developmental genetic approaches of different workers.

The work by Bateman and Rudall (2006) in this volume, and their previous work (Bateman and DiMichelle 2002; Rudall and Bateman 2003) clearly fall within evo-devo, although their observations of *natural* terata and the absence of crossing experiments or DNA sequences would likely prevent geneticists from claiming these studies as lying within their field.

At the other extreme there are many studies that focus on analyzing DNA sequence information derived from genomic sequencing projects or from studies of expressed genes. When such studies have a comparative focus, either on related genes of different organisms, or on members of a gene family in one organism, then they approach evo-devo. If such studies go beyond the creation of a gene phylogeny, to consider functional changes of genes, then they extend into evo-devo. Firmly within evo-devo are studies that focus on developmentally important gene families to elucidate roles that the gene family members have in various organisms. Even without expression data such work can have major implications for the evolution of gene function and even for morphology. A famous example is the early work by Kramer et al. (1998) on B gene phylogeny in basal angiosperms.

Work by Finnegan (2002) also falls within evo-devo. She notes that epialleles (genetic differences due to DNA or histone methylation, rather than to DNA sequence change) can affect various attributes of plants, including, for example, flowering time through the *Arabidopsis* Heynh. genes *FWA* and *FLC*. Epialleles such as *fwa-1* are heritable in plants, although loss of methylation of epialleles (epimutation) is generally far more frequent than DNA sequence change. Furthermore, the frequency of epimutations increases in plants under stress. If epialleles exist that affect phenotypic attributes that are under strong differential selection in different habitats, then epialleles might allow plants to have very high mutation rates, especially when stressed, for these few genes, without suffering the high genetic load that would result if all genes experienced high mutation rates. This work falls within evo-devo because it suggests a hypothetical mechanism to achieve evolutionary plasticity for attributes that may experience especially strong selection, such as flowering time, although no epialleles have yet been found to operate in this way. Epialleles are also involved in gene silencing and in increased phenotypic diversity in polyploids. (Note that "epigenetic" and related terms have a different meaning in population genetics—gene interaction—compared to usage in genetics and molecular genetics. Here I am using it in the genetics sense. Unrecognized conflicts in semantics can cause difficulty when separate scientific fields merge.)

Most evo-devo studies use data from two or more sources: genetics and/or gene sequence and/or gene expression and/or development and/or morphology. Even if the broad goal is to evaluate evolutionary mechanisms, this may be approached indirectly. The proximal goal may be to evaluate homology of morphological structures, or to determine whether a gene sequence was under selection, or some other narrow (or even peripheral) question. In the strongest, most direct use of such data, one searches for the actual genes responsible for an evolutionary innovation, to understand how and maybe even why they changed.

Microevolution.—In microevolutionary studies very powerful tools may be available. If an interesting evolutionary innovation separates organisms that can be hybridized, and if the F_1 hybrids show high fertility and normal crossing-over, then a QTL approach can be used to directly detect the chromosomal regions responsible for the phenotypic differences. Finding the actual genetic difference within the QTL is much more difficult, but is increasingly being achieved (Remington and Purugganan 2003; Borevitz and Chory 2004). Maize QTL work by the Doebley lab is now classic (Doebley 1995; Doebley et al. 1997; Wang et al. 1999; Hubbard et al. 2002; Jaenicke-Després et al. 2003). Typically, to find the actual gene, one selects a candidate gene that maps within the QTL. This is typically possible only for organisms with extensive genetic resources, for which potential candidate genes are known, e.g., model organisms and crop plants (or their close relatives). Alternatively, ultra fine mapping with a huge population can directly identify the responsible gene, as in the 7000-plant population that mapped a tomato fruit quality QTL to an exon and adjacent intron of an invertase gene (Fridman et al. 2000). Technological improvements are likely to make both types of methods increasingly feasible for non-model organisms.

In rare favored cases it is even possible to recreate the evolutionary innovation. Rieseberg and colleagues resynthesized diploid hybrid species in *Helianthus* L. Their careful mapping of the parental chromosomal segments that are retained as different lineages regain fertility demonstrates that this process is surprisingly deterministic. Both natural and resynthesized hybrids retain nearly the same segments, and some natural diploid hybrid species appear to have arisen independently multiple times (Schwarzbach and Rieseberg 2002; Gross et al. 2003). Their work demonstrates genetic constraint for the return of fertility beyond any that might have been expected, placing this work within evo-devo. Their studies invite further analyses to identify the genes responsible for selectively important phenotypes of the hybrids (Lexer et al. 2004).

Resynthesis of polyploid species of hybrid origin was often attempted in the *Modern Synthesis* era, by crossing the putative parents to evaluate the morphology of the F_1s, usually without attempts to double their chromosome numbers. These studies could provide strong evidence for the parentage of the hybrid species (Stebbins 1971), but encountered a road block at that point, preventing further analysis of mechanisms of morphological change; such classical studies approached—but did not lie within—evo-devo.

Some more recent studies of natural and artificial polyploids have focused on the fates of the newly duplicated genes, including silencing, conversion to pseudogenes, subfunctionalization, etc. (Adams et al. 2003; Soltis et al. 2003).

Polyploidization provides a wonderful tool for such studies, which clearly lie within evo-devo.

Macroevolution.—The most impressive evolutionary innovations are macroevolutionary changes that separate genera, families, or larger groups of organisms. Typically the candidate gene approach is used in attempts to elucidate evolutionary change between organisms that cannot be hybridized. Candidate genes are selected based on genes known from model organisms. Homologs of these genes are cloned from the organisms of interest and expression patterns determined. Gene phylogenies may be constructed to evaluate gene orthology and to search for gene duplications. This can be very informative, especially if the morphological innovation and the expression pattern are consistent with the known function of the homologous gene in model organisms. For example, in tulip, B class flower homeotic genes are expressed in both the outer and inner tepals (Kanno et al. 2003). Expression of B class genes (with A genes but not C genes) specifies petals in *Arabidopsis,* and ectopic expression of B genes in the first whorl changes the sepals into petals (Krizek and Meyerowitz 1996). Hence, expression of B genes in the first whorl of tulip "explains" the petaloidy of those organs.

Geneticists who are used to analysis of null mutants (i.e., with fully inactivated genes) for defining gene function may object that expression patterns can never provide proof of function, but expression data are the most commonly available evidence of function in evo-devo. Certainly when a genetic control pathway is reasonably well understood in one or more model organism(s), and a similar function of the homologous gene in the study organisms would account for the evolutionary novelty, then it is reasonable to use expression pattern to infer that that gene does "account" for the novelty. Each scientific specialty develops a tradition that regulates acceptable evidence and allowable inferences that may be drawn from it. This is based in part on what is practical. The tradition from genetics is not necessarily appropriate for evo-devo. In genetics, null mutant phenotypes are traditionally treated as proof of gene function, even though gene redundancy may mask a gene's full function, and early effects of the gene may preclude observation of its later effects if the relevant structures become too malformed in the mutant for full analysis at late stages. These potential problems are well known, but tolerated in the analysis of gene function in genetics.

Note the quotes surrounding "account" in the previous paragraph and "explain" in the paragraph before that. Expression of a gene (such as the B genes in tulip outer tepals) may constitute a *step* in the genetic control system that generates the evolutionary novelty (i.e., petaloid outer tepals) but this may not be the primary *cause* of the new feature (Baum 2002). The primary cause could reside in a gene that is upstream of the studied gene, with the changed expression of the studied gene merely reflecting these upstream events. The primary cause must be a DNA sequence change (or epigenetic change) that alters promoter function or protein function for one or more genes. This primary change may operate through a cascade of other genes to generate the altered phenotype.

If a gene expression change, such as the B gene expression in tulip, is part of a well understood genetic control network that seems to be widely conserved, then one can study homologs of the putative upstream genes to find the highest gene in the hierarchy with altered expression, and then examine its promoter (and perhaps the amino acid sequences of the proteins that bind to it) to find the primary cause of the new feature. We have studied the evolution of petaloid bracts in *Cornus* L., and have found that homologs of at least three of the genes required to specify petaloidy in *Arabidopsis* are expressed in bracts of *Cornus florida* L. (J. M. Hu, N. M. Maturen, and M. W. Frohlich unpubl. data). It seems unlikely that regulatory mutations would occur independently in three or four genes, so we are now studying expression of homologs of the upstream genes *LEAFY* and *UFO,* because overexpression of these genes in *Arabidopsis* leaves is sufficient to cause expression of the B genes and to convert the leaves into petaloid structures (Pelaz et al. 2001).

Gene loss (or conversion to a pseudogene) is a stark indicator of expression change, and may provide particularly useful evidence, especially if the lost gene has broadly conserved function and is in a gene family that seldom shows duplications or losses. Gene loss could be the primary cause of an evolutionary novelty, but not necessarily so. For example, even the loss of an enzyme gene might not be the primary cause for evolutionary loss of the enzyme's product. The primary cause could have been an upstream regulatory change that greatly reduced the enzyme's expression. With little expression, there would no longer be stabilizing selection to prevent loss of the enzyme gene or its conversion to a pseudogene. The loss, on the lineage leading to angiosperms, of one of the two gymnosperm paralogs of *FLORICAULA/LEAFY* was pivotal in suggesting the Mostly Male theory for the evolutionary origin of the flower (Frohlich and Parker 2000; Frohlich 2001, 2002, 2003). This gene loss probably *resulted* from the innovation of the flower, but is unlikely to have caused the innovation (Frohlich 2001, 2002).

Changes in promoters (cis-regulatory regions) have been considered for some years to be the most likely sources of evolutionary change (Carroll 2000; Durbin et al. 2003; Levine and Tjian 2003). At present, promoter analysis is difficult, but bioinformatics efforts to understand promoters are progressing (Qiu 2003). Recognizing primary changes in promoters may be simplified if such changes commonly arise from transposon insertion (Bennetzen 2000; Walbot 2002; Casacuberta and Santiago 2003; Jordan et al. 2003; van de Lagemaat et al. 2003).

In some cases strong evidence of altered promoter function may be obtainable by in vitro binding assays (Kanno et al. 2003) or by yeast two-hybrid assays (Elomaa et al. 2003), or in favored cases by transforming the genomic region containing the promoter into a heterologous test organism. Shu et al. (2000) studied the evolutionary origin of rosette flowering in *Jonopsidium* Rchb. using a candidate gene approach focusing on the *LEAFY* homolog. They suggested, based on expression data, that altered regulation of the *LEAFY* homolog may have generated this morphological novelty. Yoon and Baum (2004) transformed genomic sequences containing *LEAFY* homologs with the promoter regions from three rosette flowering crucifers into *Arabidopsis,* and for two of

them found moderate morphological changes reminiscent of rosette flowering. This suggests that in those cases the evolutionary novelty is likely due to changes in the LEAFY promoter, at least in part. There could also be changes in other genes involved in the transition to flowering, and/or *Arabidopsis* may not have been able to respond fully to the heterologous promoters.

Changes in protein function may also be studied by in vitro or in vivo analyses. One can even determine functionality of an inferred ancestral protein using a synthetic DNA sequence that codes for the ancestral amino acid sequence (Jermann et al. 1995; Benner 2002; Chang et al. 2002; Chang 2003; Thornton 2004), and one may be able to show what amino acid sequence changes are important for altered function (Opitz et al. 1998; Zhang and Rosenberg 2002).

The variety of approaches that fall within evo-devo is striking. This is due to the broad fusion of such diverse fields that are merging to create evo-devo, and from the individual dynamism of those fields.

Suggestions for the Design of Evo-Devo Studies

If the goal of evo-devo is to erect a testable evolutionary scenario that bridges the great divide, how should evo-devo projects be designed? That science seeks to test hypotheses has been its orthodox benchmark, derived from studies of physics, yet some of the largest current scientific projects in biology are almost purely descriptive. The Human Genome Project is not testing any hypothesis, and hypotheses are typically absent from standard Expressed Sequence Tag (EST) projects. In a curious reversal, molecular biologists involved in such work are now engaged in descriptive studies, whereas practitioners of the new taxonomy, based on modern phylogenetic theory, focus on evaluating competing hypotheses of relationship. In spite of past name-calling, descriptive studies have always been an important component of science. Data gathering is often scientifically important, especially in the early stages of a new scientific endeavor. However, unless one carefully justifies the inherent value of the data, relative to the effort required to obtain it, such studies may well fail to produce significant results.

Any new field is likely to suffer growing pains, and evo-devo is no exception. Investigators moving into evo-devo not uncommonly wish to pursue projects closely related to their previous work; for example, they may continue to study their favorite gene, but they now look for its homologs in phylogenetically interesting non-model organisms. Those who enter the field from organismal biology may continue to focus on their favorite organisms, but now study interesting genes from these organisms. In each case, the goal is to describe the genes' sequence and expression patterns, and in favored cases the genes' function(s), but there is no real hypothesis under consideration. A number of early evo-devo studies followed this pattern. Even when the technical objectives were met, commonly nothing could be inferred from the results.

The major genomic sequencing projects of important organisms (e.g., human, *Caenorhabditis, Arabidopsis, Drosophila,* chimpanzee) are examples of valuable data gathering projects. Genomic sequencing of *Populus trichocarpa* Torr. & Gray is justified by the economic importance of the genus as well as by scientific interest, and by the declining cost of such projects. Although most EST projects are not testing hypotheses, others are, such as the Floral Genome Project (Soltis et al. 2002). However, evo-devo projects that examine a few genes (selected only because of a researcher's previous interests) in only one or a few organisms—but without a scientific goal in mind—have an unfavorable ratio of cost to benefit.

Table 1. Some plant features controlled by known genes operating at more than one level in the genetic control hierarchy.

Flower organ specification (i.e., sepals, petals, stamens, carpels)
Flowering time
Carbon fixation system (C_3, C_4, CAM)
Flower color and fruit color
Apical meristem homeostasis
Control of dorsiventrality in lateral organs

One should be wedded neither to a favorite gene, nor to a favorite organism, when planning an evo-devo project. Instead, one should find a scientific question that is both interesting and tractable. The hypothesis need not be especially elaborate. It could be an assertion of homology (or lack of homology), or that a particular evolutionary process did or did not occur in the origin of a novel character or a new taxonomic group. Let the question dictate what organisms and what genes will be studied. The older evolutionary literature is full of interesting hypotheses, including very many embedded within evolutionary scenarios (Burian 2000). As ever-more developmentally important genes are discovered, a growing number of such hypotheses and scenarios will become amenable to serious investigation in evo-devo. Hypothesis-based inquiry is the way to generate interesting results.

Practicality and the circumstances of the investigator are important considerations in choosing projects. Graduate students and others under time constraints need projects with minimal risks. Unlike molecular taxonomy, which virtually always gives significant results regardless of the phylogeny of the organisms under study, some evo-devo projects do not generate significant results.

In an evo-devo project it can be extremely important that the candidate genes turn out to act at an appropriate level in the genetic control hierarchy, either at the level of the primary cause of the innovation, or at least at a level that can reveal something of the mechanism of evolutionary change. It is tempting, if an evolutionary innovation is (more or less) mimicked by a mutation or by gene overexpression in a model organism, to assume that that gene must surely be responsible for the innovation. Alas, nature has many tricks! One must consider whether the study will still give interesting results if some other gene is actually responsible for the innovation. If not, then the study is risky. Risk is reduced if one studies genes at several levels of the control hierarchy, at least in the initial stages of the study, before settling on particular genes for intensive analysis. Table 1 lists some of the better-understood developmental systems in plants, for which genes are known at multiple levels. Innovations related to these processes are good candidates for evo-devo projects.

Note that one favorite subject for plant evo-devo—dorsi-

ventrality of the flower—is not on this list. The superb work on *Antirrhinum majus* L. has identified only three genes that control this attribute. *CYCLOIDEA* and *DICHOTOMA* are closely related *TCP* genes that specify dorsal identity, while *DIVARICATA* is a *Myb* gene that specifies ventral identity (Luo et al. 1999; Galego and Almeida 2002). Mutant screens have as yet not revealed additional genes controlling zygomorphy in *Antirrhinum.*

These may be reasonable candidate genes for plants related to *Antirrhinum,* in which floral zygomorphy is homologous to that of *Antirrhinum* (Donoghue et al. 1998), and such studies have generated very interesting results (Cubas et al. 1999; Hileman et al. 2003). However, studies of more distantly related plants, in which floral zygomorphy evolved independently are risky, as other genes might have been recruited to establish zygomorphy (though recruitment of the same genes would be extremely interesting). Furthermore, the discovery that the *CYCLOIDEA* homolog is expressed in a zygomorphic pattern, even in the radially symmetric flower of *Arabidopsis,* undermines the use of expression patterns of *CYCLOIDEA* homologs for causal explanations of zygomorphy (Cubas et al. 2001).

Major Questions for Evo-Devo

Tendencies to evolve and developmental homeostasis.—Evo-devo has the promise to answer some of the largest questions regarding evolution. One such question is why do particular evolutionary innovations happen in some groups of organisms but not in others? To be more specific, why is it that some features are very stable in some large clades, yet in other clades the same feature evolves highly diverse forms? For example, orchid flowers show an amazing diversity of form, yet in the grasses and in Marantaceae the flowers are by comparison highly uniform. Grasses show great variability in the organization of the inflorescence, yet in orchids and Marantaceae inflorescences show little variation. Marantaceae show great variation in its colorful leaf markings, yet in grasses and nearly all orchids such variations are absent. Claiming that selection is responsible does NOT answer the question! The critical issue is why these plants are able to respond to selection with such evolutionary plasticity of form.

In the *Modern Synthesis* era a group of organisms was sometimes said to have a "tendency to evolve" certain types of features. Indeed, in my undergraduate plant taxonomy course (from W. H. Wagner), such "tendencies to evolve" were cited as notable characteristics of plant families, helping to define those families. Tendency to evolve implicitly included both a tendency to undergo parallel evolution (though without explicit phylogenies it was not clear which similar characters represented homology and which parallelism) and also a tendency to create diverse attributes of particular features, as in the cases of the three families mentioned above.

Such "tendencies to evolve" (especially in parallel) would be anathema in cladistics, with its goal of minimizing evolutionary change on a cladogram, and with a basic assumption of parsimony being the equal cost of character state change on any internode, anywhere in the cladogram. Yet many features have repeatedly evolved in parallel in many groups, for example, many of the features used to delimit tribes, families, and orders in traditional angiosperm classifications. This is a large part of the reason why traditional taxonomy in the plants has been in such dramatic conflict with (the presumably more historically accurate) phylogenies derived from molecular studies. The examples of the orchids, grasses, and Marantaceae indicate that tendencies to evolve morphological diversity of particular structures also exist. Genetic constraint (or the lack of it) can be used as a catchall explanation for such phenomena, but genetic constraint is mostly hypothetical.

This tendency to evolve (or not) and genetic constraint may both be related to developmental homeostasis. Developmental homeostasis is the tendency of an organism to make uniform, standard structures in spite of assaults from the environment or from mutations. Failure of developmental homeostasis may be detected as fluctuating asymmetry, that is, slight asymmetries between the left and right sides of the body in individual animals, or comparable aberrancies observed in individual plants. Plants can show not only left-right asymmetries, but also variation among structures that are made repeatedly, such as flowers along an inflorescence (termed "translational asymmetry," Alados et al. 2001). Fluctuating asymmetry has been most studied by ecologists as a measure of fitness (Freeman et al. 1999; Møller and Shykoff 1999; Alados et al. 2001), although the evolutionary significance of fluctuating asymmetry and developmental homeostasis were considered in detail in an important paper by Fenster and Galloway (1997).

Developmental homeostasis might be increased by duplication of genes in a genetic control network (Wilkins 1997), but simply making extra copies of genes may not allow the system to respond appropriately under different environmental conditions. Hence, there may be multiple but rather different genetic control systems operating to achieve developmental homeostasis for each developmental pathway. Evidence in support of this comes from the great diversity of genes found in enhancer-suppressor screens, which are now routine in developmental genetics studies of model organisms. In such screens, one mutates an organism that is already mutant for a weak allele that causes only moderate defects. After mutagenesis, one searches among the selfed offspring for individuals with more severe or less severe defects. New null mutants that show more severe defects identify genes that, by definition, contribute to developmental homeostasis, because the normal form of that gene had helped minimize defects in the parent plant that were caused by the original weak mutant allele. Typically such newly discovered genes show little phenotypic effect as single mutants. Most often they are not paralogs of the weak allele gene used in the screen, and they may operate through very different mechanisms, for example, in chromatin remodeling, whereas the original weak mutant may have been a transcription factor. Furthermore, most of the classic flower homeotic mutants in *Arabidopsis* also show translational asymmetry, resulting in the mutant phenotype becoming progressively weaker or stronger along the length of the inflorescence (M. P. Running pers. comm.; S. E. Jacobsen pers. comm.). This confirms that very many genes are involved in generating developmental homeostasis.

If multiple, different genetic control systems redundantly

specify correct development of a structure then this should result in increased developmental homeostasis. However, this should reduce the chance of evolutionary modification of that structure. With much developmental homeostasis, multiple mutations in the different genetic systems might be required for such novelty. Although multiple mutations might arise, and may rarely come together in the same individual, they would likely be separated by sexual recombination, reducing the chance that positive selection on the novel phenotype would lead them to near fixation. Hence, developmental homeostasis may reduce the chance of morphological evolution (Fenster and Galloway 1997). Developmental homeostasis may be a source of genetic constraint that prevents evolutionary novelty.

Lack of developmental homeostasis for a feature may predispose it for evolutionary diversification (Fenster and Galloway 1997). Rudall and Bateman (2002, 2003) and Bateman and Rudall (2006) have already noted the frequent occurrence of certain types of teratologies (terata) in the structures of orchid flowers. They suggest that these terata can result in the formation of new species and give numerous examples where such mechanisms appear to have operated. Furthermore, they note that other conceivable terata are very uncommon, which they attribute to developmental constraint. Developmental constraint is related to developmental homeostasis. Those terata that are common indicate lack of developmental homeostasis for the attributes that become teratological, which apparently has resulted in morphological evolution of orchid flowers.

Understanding the mechanisms of developmental homeostasis and genetic constraint (and developmental constraint) on evolution are lofty goals for developmental genetics and for evo-devo, but they are goals that may eventually be achieved. Unlike genetic constraint, developmental homeostasis can be measured, and compared to evolutionary diversification.

Parallelism and convergence.—Parallel and convergent evolution are especially common in plants. Parallelisms and convergences may be treated as replicate experiments in evolution, affording avenues to study evolutionary potential and evolutionary constraint.

Gould (2002: 1068) cites his "older view" that convergences effectively constitute replicate evolutionary experiments, in order to highlight what he calls the "magnitude of the reversal" of these views. On the surface this is only a dispute whether the evolution of elaborate eyes in many phyla represents convergence or represents parallelism. The implicit larger issue follows from Gould's (2002) suggestion that these parallelisms are due to genetic constraint operating on similar developmental-genetic systems, implying that such parallelism is pre-ordained by the similar genetic developmental systems of even the most disparate animal phyla (Gould 2002: 1068–1069). However, even he points out that in different phyla different tissues form analogous portions of eyes (Gould 2002: 1123–1132), suggesting that evolutionary constraint has limits, and there are indeed major examples of convergence in eye evolution, though perhaps not quite as large as previously thought. Comparison of different instances of parallel and convergent evolution will test his assertions. How often do parallel or convergent evolution involve comparable changes in the same developmental genetic control systems? If the same pathways are modified, are orthologous genes changed? If homologous genes are involved, how similar are the specific changes that generate the innovation, or that elaborate it, or that contribute to developmental homeostasis for the innovation?

Table 2. Plant attributes and life styles that have frequently changed or arisen through parallel or convergent evolution. Life style changes commonly involve parallelism or convergence in both morphology and physiology. Asterisks (*) mark items also appearing in Table 1.

Flower color*
Flowering time*
Carbon fixation system (C_4 and CAM)*
Acquisition of petaloid attributes by other organs*
Leaf shape (including leaf lobes, teeth, and compound leaves)
Size of leaf, flower, etc.
Growth habit (tree, shrub, herb)
Aquatic plants
Desert plants (e.g., succulents, quick-cyclers, phreatophytes, and plants tolerant of extreme water potentials)
Epiphytes
Plants tolerant of cold climates
Plants tolerant of deep shade
Plants tolerant of unusual soils (e.g., high or low pH, low nutrient availability, heavy metal contamination, etc.)
Plants that synthesize pyrrolizidine alkaloids
Indument forms, especially trichome types

Table 2 lists a few of the features that exhibit much parallel or convergent evolution in plants. Note that many of these are ecologically important. Also note that flower color, carbon fixation system, and flowering time also appear in Table 1 as features amenable for evo-devo because many of the genes involved have been studied in model organisms. Some evo-devo work has begun on these subjects, but far more could be done. Some examples include, for flower color, Bradshaw et al. (1998), Farzad et al. (2002), Hodges et al. (2002), Bradshaw and Schemske (2003), Durbin et al. (2003) and Zufall and Rausher (2004); for carbon fixation system, Hibberd and Quick (2002), Keeley and Rundel (2003), and Sage (2004); and for flowering time, Le Corre et al. 2002, Österberg et al. (2002), and Michaels et al. (2004).

Homology.—The concept of homology is already changing dramatically (Gould 2002). It is now clear that the same genetic system can be recruited from one organ (in the ancestor) to function in another non-homologous organ in the descendent. For example, genes active in the shoot apex can function in leaves to generate the separate leaflets of compound leaves (Kessler and Sinha 2004). We have shown that petal-specifying genes are active in *Cornus* petaloid bracts (J. M. Hu, N. M. Maturen, and M. W. Frohlich unpubl. data). If genes and gene cascades characteristic of different structures operate together to form a third structure, why is the third structure not homologous to both of the others? Perhaps homology as a concept is too simplistic to reflect the burgeoning knowledge from evo-devo, and has outlived its usefulness. At a minimum, partial homology seems to be real (Sattler and Rutishauser 1997; Rutishauser and Isler 2001;

Vergara-Silva 2003). Perhaps one might say the structure has double homology, with one homology overlain upon the other, if one can infer the order in which the different gene cascades came to be expressed in that structure. Perhaps "fundamental homology" would refer to structures (or specific attributes of structures) that existed down through the organisms' lineages back to the common ancestor, generated by gene cascades descended from those of the fundamentally homologous structure in the common ancestor, with only incremental changes along the subsequent lineages. Perhaps "epihomology" would refer to attributes, and the gene cascades that generate them, acquired *after* divergence from the common ancestor, through ectopic expression of whole gene cascades that had been functional in (and had evolved for) some other structure elsewhere on the organism. With these definitions, heterotopy could still reflect fundamental homology, if the structure involved moved to a new location with all its gene cascades, and did not merge with pre-existing cascades. Epihomology would arise when gene cascades move to a new location and are overlain on pre-existing gene cascades, so the resulting structure combines attributes of its ancestral form and features specified by the ectopic gene cascade.

Evo-devo will require a deeper and more detailed understanding of morphology and development than is available at present (Wagner and Larsson 2003; Kellogg 2004). Comparative morphology and anatomy as descriptive sciences unrelated to other biological endeavors have long been in decline. Such studies will experience a renaissance in the course of merging with the other fields creating evo-devo.

A crucial issue is the generally hidden question of which data are considered important and in need of explanation in a theory or a scenario. Observations considered unimportant are typically not reported, so no attention is called to them, so the tradition of ignoring them is strengthened. For example, the presence of vascular strands in the outer integument of ovules and seeds has been reported in more than 30 plant families (Eames 1961). The orientation of these strands—whether the xylem faces the inside or the outside of the ovule—is important for determining dorsiventrality of the outer integument to compare it with possible gymnosperm antecedents (Frohlich 2001, 2002), but this orientation is almost never reported. I have found only three papers that provide this information (that the xylem faces the inside), and in two of the papers this was not noted in the text; but was only apparent from illustrations (Chamberlin et al. 1993; Svoma 1997).

Sometimes people even ignore the obvious. The familiar greenhouse weed *Kalanchoe daigremontiana* Raym.-Hamet & Perrier forms tiny plantlets at the edges of its leaves, providing a clear example of heterotopy (with stem apex forming on a leaf). Asexual reproduction through plantlets produced by heterotopy on leaves or inflorescences is well known in many plants. Yet heterotopy (the movement of a structure from one place to another on an organism) has been considered an unlikely event in evolution since the time of Haeckel. Evo-devo will encourage re-evaluation of which observations are important. For example, the highly variable number and placement of ovules in the carpel, compared to the uniformity of anther placement on stamen supports the suggestion in the Mostly Male theory that ovules (but not anthers) could have been ectopic on the carpel antecedent (Frohlich and Parker 2000; Frohlich 2001, 2002, 2003). Ovule position has long been an important character for angiosperm classification. Yet I am not aware of anyone who has questioned why it should be that ovule number and position are so variable in flowering plants, especially as compared to anther number and position.

Macroevolution versus microevolution.—A question of longstanding importance is how similar are macroevolution and microevolution (Mayr 1982: 607–620; Carroll 2000; Bateman and DiMichelle 2002; Gould 2002: 21, 1296ff; Kellogg 2002; Simons 2002; Vergara-Silva 2003). Microevolution can be studied directly at the population level in many species, but the origin of dramatically distinct new features in macroevolution happens rarely, so we are unlikely to directly observe its occurrence. Does it occur through saltation, and if so, does a dramatic novelty arise in a single step in one or more individuals (Bateman and DiMichelle 2002; Vergara-Silva 2003; Bateman and Rudall 2006), or by very rapid but sequential changes within a population (Mayr 1982: 618)? How important is heterotopy, which would seem to require dramatic novelty in an individual? To what degree are such changes controlled by genetic constraint? How important is chance in evolution? Can extensive knowledge of the genetic systems that control development directly suggest the evolutionary origins and trajectory of such systems? For that matter, how is it possible for evolution to occur at all?

A Model of Development and of Evolutionary Change

Understanding how and why evolution occurs depends on one's model of how genes determine the attributes of organisms. A model may be described through metaphor; that is the method I use here. Work over the last century has revealed the incredibly intricate systems that provide the mechanisms for physiology, genetics and development. They are a wonder to behold. Such intricacies elicit the metaphor of the well-oiled machine, that is, an intricately designed piece of human-manufactured equipment, in which each part is crucial and perfectly designed to fulfill its function. This metaphor lacks any equivalent of the genes, though, so instead I prefer the metaphor of the symphony orchestra.

The orchestra has many musicians (who symbolize genes). They all work together in a precise pattern to generate the wonderful music that symbolizes the phenotype. A large orchestra can indeed make spectacular, intricate music. In my orchestra no single musician is vastly more important than the others (as my orchestra doesn't play concertos).

In an orchestra the musicians always try to play exactly the notes written by the composer. Even the shading of their play is dictated by the conductor. In such a complex interacting system anything unplanned is bad. All mutations (wrong notes) are deleterious. There is no opportunity in a symphony orchestra for improvisation, and in practice improvisation is not done. This metaphor, like the well-oiled machine, could represent the intricacies of organisms, but it makes evolution effectively impossible, because all change is bad. Only Creationists could favor such a model.

Instead, I propose the "rock band" model. In a rock band there are a few important musicians (genes), with a number

of back up musicians of lesser importance, and still lesser people who drive the bus, arrange hotels, etc. There are also lots of groupies, but the groupies are so unimportant that some of them may get dumped, that is, they become "pseudogroupies."

Note that in a rock band it IS possible to have improvisation. While the majority of wrong notes may be deleterious, quite a few attempts at improvisation are good. Occasionally a back-up musician may become a lead. Rarely, even a groupie may acquire power through unexpected interactions, which may have a profound effect on the band. It is the relatively haphazard organization of such a band that allows improvisation and permits occasional, even more drastic changes in the way the band produces music. If these changes are successful then the band prospers and is likely to retain these novelties. This is a metaphor for evolution.

A few rock bands never do improvisation. These are typically older bands that play concerts for their single-cohort of aging fans, fans that idolized the band years earlier. Such fans typically want to hear exactly the same sounds as are on their old LP albums. Bands at this stage of their careers seldom produce highly successful new music, however, such bands do commonly provide highly polished shows, with accurately rendered music that satisfies even fanatic fans.

By analogy, an organism with a relatively haphazard developmental genetic system—in which some genes are much more important than others in determining phenotype—CAN have the ability to evolve. An overly tight organization, in which nothing can go wrong, prevents change. A tight organization is comparable to having very much developmental homeostasis.

As in the case of the geriatric rock band, that for decades has tried to please the same audience, a long experience of stabilizing selection should favor the appearance of strong developmental homeostasis, which would, in turn, limit the potential for evolutionary novelty. This may be a source of evolutionary stasis. Conversely, recent morphological evolutionary change should reduce developmental homeostasis for the newly arisen attributes, allowing further evolutionary change. This might contribute to apparent saltation, through a rapid sequence of smaller changes. It might also result in a "tendency to evolve," characteristic of a clade, as hypothesized in the *Modern Synthesis* era.

The discovery of homeotic mutants with profound morphological effects shows that genes with major effects on development do exist. The suggestion that very many genes are of little importance, not unlike groupies following a rock band, is supported by a comparison of the *Arabidopsis* ecotypes Columbia and Landsberg erecta, showing that as many as 1 to 2% of genes present in the one ecotype may be missing in the other (Borevitz et al. 2003). Comparison among many ecotypes suggests the proportion of genes missing in at least one ecotype could be as high as 4.3% (T. Mitchell-Olds pers. comm.). Such gene losses are not limited to *Arabidopsis*. Fu and Dooner (2002) found that four expressed genes of one maize BAC are missing from the homologous BAC of another inbred line.

What is the Future of Evo-Devo?

The future of evo-devo could not be brighter. Evo-devo benefits from the rapid increase in knowledge in the diverse fields that are merging to form evo-devo. Due to rapid technological advance (e.g., Shendure et al. 2004), data and experiments formerly possible only with the best-established model organisms are becoming practical with semi-model organisms (that have about five labs studying them) and even with non-model organisms. High-throughput systems, such as microarrays, allow study of full gene regulatory networks (Davidson et al. 2003), an approach which is already underway in plant evolutionary studies (Soltis et al. 2002).

Effective methods to inactivate genes, applicable to any plant, would allow gene function to be determined as done with mutant analysis in standard genetics.

Such "reverse genetics" methods are now used for transformable model plants, but have typically required generation of large libraries of random transformants and elaborate screening systems (Sussman et al. 2000; Sessions et al. 2002). Homologous transformation, in which an inserted DNA molecule replaces an endogenous gene with closely similar sequence is practical in the moss *Physcomitrella,* but not in other higher plants (Egener et al. 2002). TILLING [Targeting Induced Local Lesions IN Genomes] does not depend on transformability, and promises recovery of mutants for nearly any gene, as long as the mutagenized plants are homozygous for the gene(s) of interest and have life cycles that permit several generations to be grown and studied (McCallum et al. 2000). Extensive screening for desired mutants is still required, but can be done so efficiently that screens for natural alleles of specific genes are practical (Comai et al. 2004).

Methods based on RNA silencing of genes are potentially far more powerful (Pe'ery et al. 2003; Matthew 2004; Pennisi 2004). Plants have at least three distinct systems for inactivating genes similar to a particular RNA sequence (Baulcombe 2004).

Plants and animal share endogenous RNA interference (RNAi) systems that will degrade mRNA if its sequence matches short (ca. 22 base pairs) double-stranded RNAs (dsRNA). RNAi has already been used for high-throughput analyses of gene function in *Caenorhabditis elegans* (Kamath and Ahringer 2003). In worms and some other animals, feeding with dsRNA can inactivate genes (Pennisi 2004), but such simple methods do not work with plants.

In plants, virus induced gene-silencing (VIGS) shows the greatest promise. In VIGS, the plant is inoculated with a modified virus construct containing sequence of the plant gene to be inactivated. A VIGS vector has been developed for *Nicotiana benthamiana* that is easily inoculated and spreads throughout the plant, even into the apical meristem, and effectively inactivates the target gene (Ratcliff et al. 2001). This vector is not effective in *N. tabacum.* The recent discovery of a genetic difference that renders *N. benthamiana* especially susceptible to viruses hints that a vector that inactivates the comparable endogenous gene as well as the experimental target gene might function well in other plants (Yang et al. 2004). If this, or some similar system, allowed VIGS to be used generally, it may be effective even in long-lived perennials that would be totally unsuitable for standard genetics methods. In animals, the feeding method for RNAi is already generating spectacular evo-devo results (Pennisi 2004). A broadly applicable VIGS system could be as important for plant evo-devo as PCR is in molecular taxonomy.

Evo-devo may become of major practical and economic importance. If one thinks back to the world of 40 years ago, most modern people would regard that era as "before computers." However, if a time traveler said that to a person of the year 1964, the 1964 person might respond indignantly that, on the contrary, there IS a computer at this University—it occupies a whole floor in the Computer Center. The time traveler from 2004 would not be impressed. Likewise, a future person visiting 2004 would probably say we live before genetic engineering. So far, genetically engineered plants typically have, in addition to the selective marker, only a single inserted gene, typically transcribed from a universal promoter. No one has successfully engineered a novel multi-step biosynthetic pathway into a transgenic organism. No one has substantially modified the morphology of a transgenic organism (except, inadvertently, to make it deformed or sick). At present such projects would be far too ambitious to attempt, because no one knows how to modify genes or gene systems to achieve a desired morphological or biochemical novelty. Evo-devo may suggest answers. Evo-devo will show how evolution has accomplished changes in morphology, physiology, and biochemistry. We can learn from these examples how we humans might modify organisms in complex, useful ways (Miyao 2003). The earliest genetically complicated modifications will most likely be precise imitations of natural evolutionary innovations. Among these may be the insertion of entire biosynthetic pathways for secondary compounds, to make the transgenic plant resistant to insect pests. For example, the mustard-oil defensive system might be inserted into maize or cotton. It is unlikely that existing pests of these crops, which can deal with the endogenous defensive systems of these plants, would easily become resistant to natural defensive chemicals that have stood the test of evolutionary time in the plants from which they were transposed.

Many scientists are entering evo-devo from a molecular biology or developmental genetics background. Soon, all the obvious evo-devo projects will be under study. Evo-devo is an inherently collaborative endeavor. Researchers with molecular or genetics backgrounds would be wise to establish collaborations with evolutionary biologists, especially with taxonomists and systematists. Scientists with molecular expertise will need taxonomists to find organisms and systems for which hypotheses can be erected and effectively tested. In many cases these hypotheses may involve evolutionary change that occurred within a group of rather closely related organisms (i.e., within a genus or a family). There are examples where evolutionary innovation between related organisms shows a major adaptive shift or morphological changes reminiscent of differences between major groups (Carroll 2000). Such examples may be especially informative for evo-devo because the evolutionary innovation of interest is accompanied by only modest changes in unrelated systems or genes. The living world is incredibly diverse. No one person is intimately acquainted with large parts of this diversity, but taxonomists are likely to know more than other people, and are likely to know whom to ask for more information and how to select and to obtain the most appropriate organisms for study.

There are a great many molecular biologists. Today there are many fewer taxonomists and systematists. I urge molecular biologists interested in evo-devo to strengthen taxonomic/systematic studies in their own institutions. The growth of evo-devo will be most successful in institutions where all the requisite parent fields are strong.

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