Neo-Darwinian developmental evolution: can we bridge the gap between pattern and process?

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In the past decade, there has been a surge of renewed interest in the study of developmental evolution. One approach that has been taken is to examine the expression patterns of a candidate gene in divergent taxa and to use these results to infer which aspects of a particular genetic pathway are either conserved or altered. Here we consider this approach from the perspective of the neo-Darwinian paradigm for evolutionary change. If adaptations are typically composed of large numbers of gene substitutions that are of small effect individually, then the candidate gene approach is unlikely to bridge the gap between developmental pattern and evolutionary process: changes in gene expression patterns may identify the steps in developmental pathways that have been altered during evolution but fail to identify the actual genetic changes that have occurred. On the other hand, there is growing support for the view that adaptations often involve large-effect genes; fortunately, the candidate gene approach is well suited to this type of genetic architecture.

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Abbreviations

Abd-A	*Abdominal-A*
Antp	*Antennapedia*
Dll	*Distal-less*
Ubx	*Ultrabithorax*

Introduction

Developmental evolution has finally emerged from the confines of the figurative black box, constructed during the neo-Darwinian synthesis, to which it has been relegated for much of this century [1–5]. The neo-Darwinian synthesis reconciled the Mendelian theory of genetics with evolution in natural populations. To keep the mathematics tractable, it was assumed that evolutionary changes in genotype are translated into phenotypic changes by an undefined set of epigenetic laws; in other words, developmental evolution was ignored in order to focus on the dynamics of allele frequency changes in populations. Thanks to molecular techniques that have proved adaptable for application to a wide variety of organisms, we are now getting our first glimpse of the genetic and molecular detail of the evolution of developmental programs. The most widely heralded result to emerge from such work is that much of the molecular machinery underlying development is conserved — particularly the biochemical functions of homologous proteins — even among phylogenetically distant taxa [6–15]. This triumph of modern biology presents us with an apparent paradox: animals that look nothing like each other develop by using much the same basic 'tool-kit' of molecules and often in much the same ways. How, then, are we to explain the remarkable diversity of life-forms in the world around us?

According to the established neo-Darwinian view of the evolutionary process, large differences in phenotype are based typically on numerous gene substitutions, each having only a relatively small effect on the phenotype individually [16–18]. On the basis of this paradigm, we might expect that numerous, subtle alterations in the actions of similar collections of genes, when compounded over multiple levels of regulation and occurring in increasingly divergent cellular contexts, result in the observed phenotypic diversity. If it is accurate, this depiction does not bode well for the candidate gene approach to studying the evolutionary process at the molecular level; as this is one of the common approaches taken to investigate the evolution of developmental programs [19–21], we consider some of the conceptual issues surrounding, as well as the recent evidence that bears on, the genetic basis of evolutionary change.

The candidate gene approach: examples from arthropods and vertebrates

First, what can we learn from the candidate gene approach as it is used currently to study the evolution of development? For the purposes of discussion, we consider some recent work on the evolution of arthropod limb development [22,23••,24••] and vertebrate axial morphology [25••].

Arthropod limb development

In *Drosophila*, one of the earliest genes known to be activated specifically in the limb primordium is *Distal-less* (*Dll*), which is required for the formation of distal limb structures [26–27]. Homeotic genes function both in positioning the limb primordia and determining their particular adult morphologies [28]. In the abdominal segments, the products of the *Ultrabithorax* (*Ubx*) and *Abdominal-A* (*Abd-A*) genes prevent limbs from developing, apparently by binding directly to *cis*-regulatory elements that would otherwise promote initial *Dll* expression in larval leg primordia [29]. Although *Ubx* and *Dll* are co-expressed in both the second and third thoracic segments later in limb development, early gaps in *Ubx* expression allow limbs to develop in those segments [30••].

The role of *Dll* in the developing limb, in addition to its repression in the abdomen by *Ubx* and *Abd-A*, appears to be conserved between dipterans and lepidopterans [22]. Before larval prolegs begin to develop on the abdomen of a lepidopteran embryo, *Ubx/Abd-A* expression is repressed in ventral patches of cells; soon thereafter, *Dll* is expressed in these patches of cells and the limbs begin distal outgrowth from these locations. These results suggest that, in the time since flies and butterflies last shared a common ancestor, the interaction between the *cis*-regulatory region of the *Dll* gene and the UBX/ABD-A proteins has been conserved but that the expression patterns of the latter have been altered in the abdomen, either to repress larval prolegs in flies or to promote them in butterflies.

In the branchiopod crustacean *Artemia*, a more distantly related arthropod, the *Hox* genes *Antennapedia* (*Antp*), *Ubx*, and *Abd-A* are expressed in largely overlapping domains in all the thoracic segments, consistent with the unvarying morphology of thoracic limbs on different trunk segments in an adult individual [23••]. Thus, it appears that these three genes have acquired non-overlapping thoracic domains since insects and crustaceans last shared a common ancestor. The differences in *Antp/Ubx/Abd-A* overlap between insects and *Artemia* may help explain the evolution of functional specialization of limbs. Furthermore, because all *Artemia* thoracic segments bear limbs, it appears that *Ubx/Abd-A* expression does not repress limb formation in this species. In fact, *Dll* and *Ubx/Abd-A* are co-expressed in the developing thoracic limbs of *Artemia* [24••]; thus, it appears that *Dll* is not repressed by *Ubx/Abd-A* in this species. It is as yet unknown whether *Dll* repression by *Ubx/Abd-A* was gained during the evolution of insects or lost during the evolution of crustaceans.

Vertebrate axial morphology

The expression boundaries of *Hox* genes were examined recently in chicken, mouse, goose, and *Xenopus laevis* embryos [25••]. Interestingly, anterior expression boundaries were found to be shifted in concert with morphological boundaries. For example, the cervical–thoracic transition in each was marked consistently by the anterior expression boundary of *Hoxc-6*. This is despite the fact that the cervical–thoracic transition occurs at different axial positions in these taxa (e.g. the chick has 14 cervical vertebrae, placing the cervical–thoracic transition at somite 19; in contrast, the mouse has 7 cervical vertebrae, placing the cervical–thoracic transition at somite 12). A primary event in the evolution of axial formulae in vertebrates was apparently a change in the expression of genes in the *Hox* cluster.

Interpretations

One strength of the candidate gene approach lies in the convincing case that can usually be made for the mechanistic involvement of the candidate gene(s) examined: for example, mutants in a group 6 paralogue, *Hoxa-6*, in mice show a partial transformation of the seventh cervical vertebra to a more thoracic morphology [31], and the anterior boundary of another group 6 paralogue, *Hoxc-6*, appears to mark consistently the cervical–thoracic transition amongst several vertebrate species. The implication here is that changes in the boundary during evolution played a role in modifying the axial position of this transition. This sort of inference relies upon the soundness of genetic studies in model systems plus the strength of the observed correlations between changes in expression patterns and changes in morphology in divergent taxa; as the correlations are apparently perfect, it would appear that the same genetic pathways are indeed involved in each lineage.

A serious complication arises, however, when we try to go further and fill in the details of an evolutionary interpretation based on the candidate gene approach. The observed changes in expression patterns may identify a step in a developmental pathway that has been altered but fail to identify the actual genetic changes that have occurred. For example, although it might be argued that *Ubx* and *Abd-A* expression was altered in various arthropod lineages by selection upon limb specialization and limb position, nothing has been determined about the specific genetic changes that were responsible. From a comparison of expression patterns, we do not even know whether the changes in gene expression are due to genetic changes in *cis* or *trans*. If these changes are entirely *cis*-regulatory in nature, then we might expect them to be small in number; however, if *trans*-regulatory changes are also involved, then the total number of genetic changes could be extremely large. For example, it is possible that the *Ubx* gene itself (including all *cis*-regulatory sequences) was not altered significantly in any of the lineages leading to extant arthropods but that the *Ubx* expression domain was changed as a result of numerous (unknown) gene substitutions that affect the expression and/or function of upstream regulatory genes (e.g. *hunchback*). Alternatively, changes in the anterior expression boundary could have been caused entirely by a few substitutions in the *cis*-regulatory sequences of *Ubx*. Although the latter scenario would offer the greatest hope for the success of subsequent genetic analysis, it is also the genetic architecture that seems the most unlikely to have been utilized during evolution from the perspective of traditional neo-Darwinism (see below).

Given that we do not know what genetic changes were involved in the evolution of these changes in gene expression, nor even approximately how many allelic substitutions occurred, nor almost anything at all about the actual genetic architecture of the observed alterations of expression, it must remain obscure as to why the genetic changes in question went to fixation in each of the various lineages in the first place. Perhaps they were selected for reasons entirely unrelated to the candidate gene expression patterns (and unrelated to the morphological transition in question) or perhaps they simply drifted to

fixation because they had no significant effects on fitness. Without additional data, we cannot provide these sorts of details.

What types of future experiments might allow us to address these issues of *cis* versus *trans* and many versus few genetic changes? In the case of *Ubx*, for example, it would be interesting to determine the promoter elements of *Artemia Ubx* that drive the proper anterior border of expression in *Artemia* and then see how this element behaves in *Drosophila* and do the reverse with the well-characterized promoter elements of *Drosophila Ubx*. If the *Artemia Ubx* promoter drives expression in an *Artemia*-like pattern in *Drosophila* (with an anterior boundary at the front of the thorax) and the *Drosophila Ubx* promoter drives expression in a *Drosophila*-like pattern in *Artemia* (with an anterior boundary around the third thoracic segment), then it would be reasonable to argue that differences in expression are caused by *cis*-regulatory evolution. Subsequent dissection of the promoter elements could then be used to address how many nucleotide changes are sufficient to alter the anterior expression domains. Alternatively, if the *Artemia Ubx* promoter gives a *Drosophila*-like expression domain when placed into *Drosophila* then it can be argued that evolutionary changes occurred primarily at the level of *trans*-regulatory factors. A comparison of expression domains of *Artemia* and *Drosophila* gap genes — such as the known negative regulator of *Ubx*, *hunchback* — might provide some information about the nature of these *trans*-regulatory changes. Upstream changes themselves might be caused by only a few genetic changes (e.g. specific changes in the *hunchback* promoter) or a large constellation of changes (e.g. many mutations in various proteins that ultimately affect the stability or binding of a whole array of regulators of *Ubx*). Clearly, these are not experiments that can be tried in the near future as the *Artemia Ubx* promoter has yet to be analyzed and no transformation technique has yet been determined for crustaceans; furthermore, complicating issues such as auto-regulatory feedback must be addressed. Nevertheless, these are the types of approaches that might resolve issues of specific genetic causes for changing patterns of candidate regulatory genes and hence bridge the gap between developmental pattern and evolutionary process.

Focusing Fisher's microscope: what exactly is a saltation?

Why are population geneticists particularly skeptical about the evolutionary importance of mutations of large effect? To address this question, it would be helpful to bear in mind what is meant exactly by a genetic change that has a large effect on an organism's phenotype. Unfortunately, there is no universally accepted definition for such a change; in our experience, however, most population geneticists would include in this category most allelic differences that would be readily apparent to a human observer, thereby allowing for the easy recognition of discrete phenotypic classes (this would include generally the entire spectrum of mutations that are studied by developmental geneticists). It is thought that individual mutations with phenotypic effects that are large enough to be easily noticed are unlikely to contribute much to evolution because they rarely go to fixation within an evolving population; instead, large numbers of mutations, with much smaller phenotypic effects individually, are believed to account for most adaptive evolution. The basis for this view, which permeates much of the thinking in modern evolutionary biology, lies at the very heart of the neo-Darwinian synthesis [16–18,32,33].

The neo-Darwinian view of the dynamics of diverging gene pools is essentially a Mendelian translation of evolution by natural selection as envisaged by Darwin. When discussing complex adaptations (e.g. the vertebrate eye), Darwin reasoned that a complex adaptation must arise from the successive incorporation of small changes to its component parts, each of which is advantageous to the current state of the system and is therefore favored by natural selection. The architects of the modern synthesis (e.g. [16,34,35]) formalized Darwin's arguments by showing that natural selection operating on Mendelian variation is expected to overcome the effects of mutation and random genetic drift.

Of the architects of the neo-Darwinian synthesis, it was Fisher [16] who argued most strongly that evolution must proceed gradually by a series of extremely small steps. He reasoned that if fitness is a function of a multidimensional character set (i.e. the whole phenotype), then an infinitesimally small change in a character related to fitness has a reasonable chance of carrying the system closer to a nearby fitness optimum; in contrast, a large change in phenotype has a far greater chance of carrying the system away from the optimum rather than towards it. To make his point, he compared the effect of a random mutation on fitness to the effect of a random change in the focusing of a microscope that is, at present, only slightly out of focus: minute adjustments to the focusing knob have a reasonable chance of improving things; larger adjustments, however, will almost certainly worsen the situation. On the basis of this type of logic — along with calculations showing that even allelic differences conferring extremely small fitness advantages should be readily seized upon by natural selection — Fisher proposed and adhered to an extreme form of what has now been termed 'micromutationism' [33]; he apparently believed that most adaptations are based on loci that are essentially innumerable, each having a minuscule effect [36].

One fundamental objection to this extreme interpretation was noted by Kimura [37]: favorable mutations of large effect are not only less likely to occur but are also more likely to be fixed once they do occur; hence, Kimura argues, mutations of intermediate phenotypic effect will often end up the winners during evolution. In other

words, because the overall frequency of a given class of substitution must take into account not only the mutation rate to more favorable alleles (which may well favor mutations of smaller effect) but also the probability of fixation once a mutation has arisen (which should favor mutations of larger effect), it is the mutations of intermediate value for both parameters that will end up as the most frequent components of adaptations. Furthermore, Hill [38] argued that each mutation of large effect that went to fixation will contribute more to the trait under selection than will each mutation of small effect so that even if large mutations are fixed less frequently than smaller ones, they may still contribute most of the response to selection. Finally, another theoretical objection to Fisher's position is that we really have no idea how many different possible 'adaptive peaks' may be near a population's current position in the imaginary 'adaptive landscape' [33]. For example, if a large mutation actually moves the population onto any of several nearby peaks, then this may more than outweigh the fact that it is not likely to improve the population's position on the slope of whatever adaptive peak it is currently climbing.

Although much discussion and theoretical consideration has been devoted to these issues, the results have been inconclusive [39–41]. It would seem that this debate, as with so many others in biology, will be decided empirically. This raises the question: what is known about the genetic basis of evolutionary change in natural populations?

Genetics of evolutionary change in natural populations

Although numerous selection experiments have shown that there is abundant genetic variation present within most species for most quantitative traits [42], these findings have contributed little to the debate about which types of genetic architecture are usually involved in an evolutionary change. Attempts have been made recently to characterize the genetic architectures underlying phenotypic differences within and between species directly; we will consider a few of the most informative examples.

Bristle number in *Drosophila*: variation in candidate genes

Exciting progress has been made recently in the study of a set of classic quantitative traits: numbers of abdominal and sternopleural bristles of adult *Drosophila melanogaster* [43,44••,45,46]. In flies, bristles are sensory organs of the peripheral nervous system [47]. Beginning with an outbred laboratory stock of wild-type flies, artificial selection has been used to produce inbred lines that differ with respect to their bristle numbers [44••]; the authors then measured the association between bristle numbers and marker locus genotypes in backcross, F_2, or recombinant lines derived from the parental strains. From these data, quantitative trait loci affecting bristle number have been mapped with high resolution. The following results were obtained: first, although many of the segregating loci have small effects on bristle number, a few have large effects and are responsible for most of the phenotypic variation; second, alleles at bristle-number quantitative trait loci exhibit variable degrees of dominance, strong epistatic interactions, and large pleiotropic effects on fitness; and third, candidate genes, which are known to be involved in bristle development based on previous genetic studies, are often found to have polymorphisms with large quantitative effects on bristle number (e.g. *scabrous*).

According to these results, if we are interested in studying natural variation in bristle number, we would do well to examine polymorphisms associated with a small number of candidate genes (e.g. loci necessary for normal sensory organ development, including the neurogenic genes [47]). By doing so, we would apparently account for most of the observed phenotypic variability. This astonishing result was not predicted by the neo-Darwinian paradigm of numerous genes each having only a minuscule effect individually.

Ultrabithorax polymorphisms: variation in a candidate regulatory gene

Gibson and Hogness [48••] have demonstrated recently that natural populations of *Drosophila* harbor polymorphisms at the *Ubx* gene that appear to have significant phenotypic consequences; these polymorphisms seem to affect the developmental stability of the regulatory identity of the third thoracic segment. To be specific, certain *Ubx* alleles — which can be identified by using DNA polymorphisms as markers — increase the frequency with which an environmental perturbation (exposure to ether vapor in this case) effects a partial transformation of the third thoracic segment towards the identity of the second thoracic segment. For our purposes, the important point here is that there appears to be functionally relevant extant genetic variation for the determination of thoracic segment identity and that this variation maps to the obvious candidate gene (identified initially as a mutation of large effect during genetic screens).

Insecticide resistance: single-gene responses to strong selection

In recent years, great strides have been made in our understanding of the molecular genetics of insecticide resistance [49–51]. In particular, it has been shown that the resistance of *D. melanogaster* to cyclodiene insecticides is caused by a single amino acid substitution in a GABA receptor gene. Even more astonishing is the fact that exactly the same point mutation confers resistance to cyclodienes in the natural populations of a wide range of insect species, including the house fly, the yellow fever mosquito, the red flour beetle, and the American cockroach. Although it can be argued that insecticides provide an unusually strong selective agent, hence favoring genes of major effect despite any pleiotropic consequences, these results should at least give pause to any who doubt that major-effect genes are ever selected in evolution. When judging the relevance of these results for evolution

as it occurs normally in natural populations, one critical issue is the strength of selection that is usually operating in the wild. Interestingly, Endler [52] concludes that strong selection could indeed be common in nature; on the other hand, recent analyses of synonymous codon usage [53•] suggest that even extremely minute differences of fitness are 'seen' by natural selection.

Hybrid fitness: coadapted genomes in rapid flux

In general, it appears that interspecific hybrids suffer from reduced fitness because of substitutions at an extremely large number of genes [54,55•,56]. This is true even for species that produce fertile hybrids and have not diverged much from each other morphologically. Although each genetic change often has only a small effect on the hybrids individually, they may interact to have a joint effect which is far larger than that of individual components. Post-mating reproductive barriers are the negative, pleiotropic consequences of the underlying allele substitutions that have occurred in each lineage. Such results show clearly that many of the substitutions that have occurred during evolution exhibit significant pleiotropic consequences; furthermore, it appears that epistatic effects on fitness are also common. These conclusions favor Wright's convictions [34] that pleiotropic effects and genetic interactions are ubiquitous and must be taken into account in any model of adaptive evolution.

It is also reasonable to consider the connection between these negative pleiotropic effects and the positive function of the genes in question. One conclusion that can be taken from the genetic studies is that barriers to gene exchange between closely related species are typically caused by changes in a large number of genes and that each such change has only a small pleiotropic effect on hybrid fitness. If these effects bear any connection to the positive phenotypes for each of these substitutions, this conclusion is consistent with the neo-Darwinian view of evolution. Nevertheless, because these studies address only the negative pleiotropic effects of allele substitutions, the relevance of these results for our understanding of the genetics of adaptive change can be debated.

Conclusions

Given the current interest in using the candidate gene approach to investigate evolutionary change, and given the fact that the established paradigm for evolutionary change in natural populations would argue against this approach, it is perhaps surprising that more studies have not been carried out to characterize the genetic architecture underlying phenotypic variation both within and between species. One explanation for the lack of research in this area is that high-resolution genetical analyses have only become feasible in recent years. The recent discovery of strains that produce fertile hybrids between *Drosophila melanogaster* and *Drosophila simulans* raises the hope that the tremendous resolution of *D. melanogaster* genetics can be brought to bear on the study of fixed differences between species [57•].

Another reason for the lack of data in this area is simply the inertia inherent in different disciplines: developmental geneticists are trained to think in terms of candidate genes as a feasible means of addressing the connections between genotypes and phenotypes and have continued to do so during recent forays into evolutionary investigations. Population geneticists are trained to think in terms of diffuse genetics and small fitness differences and have tended to study either the evolution of genotypes or phenotypes but have often failed to connect the two empirically. The modern field of developmental evolution offers an opportunity for both groups to find common ground, as we explore the genetic and molecular details of the evolution of developmental programs.

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45. Mackay TFC: **The genetic basis of quantitative variation: numbers of sensory bristles of *Drosophila melanogaster* as a model system.** *Trends Genet* 1995, **11**:464–470.

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The authors show that natural populations of *Drosophila* harbor polymorphisms at the *Ubx* gene that affect the developmental stability of the regulatory identity of the third thoracic segment. Specifically, certain *Ubx* alleles, which can be identified using DNA polymorphisms as markers, increase the frequency with which an environmental perturbation (exposure to ether vapor) effects a partial transformation of the third thoracic segment towards the identity of the second thoracic segment. Hence, there appears to be functionally relevant, extant genetic variation for the determination of thoracic segment identity and this variation maps to the obvious candidate gene (identified initially as a mutation of large effect during genetic screens).

49. Ffrench-Constant RH, Steichen JC, Rocheleau TA, Aronstein K, Roush RT: **A single-amino acid substitution in GAMMA-aminobutyric acid subtype A receptor locus is associated with cyclodiene insecticide resistance in *Drosophila* populations.** *Proc Natl Acad Sci USA* 1993, **90**:1957–1961.

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51. McKenzie JA, Batterham P: **The genetic, molecular and phenotypic consequences of selection for insecticide resistance.** *Trends Ecol Evol* 1994, **9**:166–169.

52. Endler JA: *Natural Selection in the Wild.* Princeton: Princeton University Press; 1986.

53. • Akashi H: **Inferring weak selection from patterns of polymorphism and divergence at 'silent' sites in Drosophila DNA.** *Genetics* 1995, **139**:1067–1076.

Synonymous codon usage in many species is biased toward a subset of preferred codons, which generally code the most abundant tRNA(s) for each amino acid. Selection on synonymous DNA mutations in *Drosophila* is investigated here by comparing the evolutionary dynamics of preferred versus non-preferred changes in codons for numerous genes. In the *D. simulans* lineage, unpreferred changes are sometimes polymorphic within species but have rarely gone to fixation during evolution, confirming the action of selection at silent sites. This selection appears to have been relaxed in the *D. melanogaster* lineage. Synonymous DNA changes appear to be subject to extremely weak selection in *Drosophila*.

54. Palopoli MF, Wu C-I: **Genetics of hybrid male sterility between *Drosophila* sibling species: a complex web of epistasis is revealed in interspecific studies.** *Genetics* 1994, **138**:329–341.

55. • Davis AW, Wu C-I: **The broom of the sorcerer's apprentice: how many genes cause reproductive isolation between sibling species?** *Genetics* 1996, in press.

In this study, approximately 10% of the euchromatic portion of the *Drosophila mauritiana X* chromosome was introgressed into a *Drosophila simulans* genetic background. Males harboring these introgressions are always sterile and suffer from hybrid incompatibilities at a minimum of four loci. As hybrid genetic analyses have increased in resolution, the number of recognized factors that reduce hybrid fitness have increased dramatically, confirming recent suggestions that even closely related species have experienced huge numbers of substitutions with functional relevance.

56. Wu C-I, Palopoli MF: **Genetics of postmating reproductive isolation in animals.** *Annu Rev Genetics* 1994, **28**:283–308.

57. • Davis AW, Roote J, Morley T, Sawamura K, Herrmann S, Ashburner M: **Rescue of hybrid sterility in crosses between *D. melanogaster* and *D. simulans*.** *Nature* 1996, **380**:157–159.

The authors have discovered strains of *Drosophila simulans* and *Drosophila melanogaster* that produce fertile female hybrids. Species identity for each, as well as the identity of hybrids, was confirmed by multiple criteria. By passing genes between these two species, it may be possible to bring the high resolution of *D. melanogaster* genetics to bear on the study of fixed species differences.