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Evolution in Reverse Gear: The Molecular Basis of Loss and Reversal

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Three types of regressive evolution are reviewed: loss, reversal, and regain after loss. Loss refers to the loss of a physical entity, either a structure or an organ, whereas reversals apply to character states returning to plesiomorphic from apomorphic conditions. The regain of characters after their loss represents a third type of evolutionary character change. The reconstruction of multiple losses and gains of characters by mapping on phylogenies is often problematic because of a lack of information about the relative likelihood of losses and gains. A developmental genetic approach using morphological, developmental, and molecular analysis is therefore an extremely important adjunct to phylogenetic approaches in interpreting losses, reversals, and regains. The molecular developmental basis of character loss and reversal is gradually becoming better understood. Loss of organs can occur by gain-of-function mutations (suppression) and loss-of-function mutations (that often leave a vestigial structure). The regain of characters after loss may occur by regulatory capture (a gain-of-function mutation) or by loss of function in suppressor genes. Reversals may occur by cryptic innovation (the formation of a new structure that mimics the old structure by gain-of-function mutations) or by loss of gene function associated with the apomorphic state (although this may have pleitropic or neomorphic effects). The genetic landscape of reversal is illustrated by the reversal to polysymmetry from monosymmetry in flowers. The range of observed phenotypes, loss with vestige, cryptic innovation, and loss with neomorphism matches the range of changes predicted.

> "In plants with separated sexes, the male flowers often have a rudiment of a pistil; and Kolreuter found that by crossing such male plants with an hermaphrodite species, the rudiment of the pistil in the hybrid offspring was much increased in size; and this shows that the rudiment and the perfect pistil are essentially alike in nature" (Darwin 1859).

IMPORTANCE OF REGRESSIVE EVOLUTION

Evolution does not run backward in any literal sense: The second law of thermodynamics determines that. However, as the quote above from Darwin indicates, regressive evolution, such as the loss of something once possessed, leaving only a vestige, is a very important facet of evolution and one of the strongest pieces of evidence for the evolutionary process. A famous example is the human vermiform appendix, which is hard to explain unless humans evolved from ancestors possessed of a leaf-eater's caecum. In the first edition of the *Origin* (1859), not only does Darwin have a section entitled "Rudimentary, atrophied, or aborted organs," but he also mentions rudiments or rudimentary organs more than 100 times throughout the work. Another section of the *Origin* is titled "Reversion to long lost characters," further indicating the importance of regressive evolution to the development of his theory.

In the age of molecular biology, we have the opportunity to explain these phenomena at the level of genes and molecules, an endeavor that both deepens the evolutionary synthesis and strengthens the evidence for evolution that Darwin realized comes from regressive evolution. In the quotation above, Darwin refers to the experiments by Koelreuter (1775) on crosses between the dioecious red campion (*Silene dioica*), with vestigial sex organs, and the hermaphrodite white sticky catchfly (*Silene viscosa*). Darwin realized that the intermediate size of the organs in the hybrids was excellent evidence that vestigial traces were indeed rudiments of the sexual organs and not some different class of structure, thus forestalling any arguments from his opponents on this score. This must be the first example of the use of genetics for homology determination, and Darwin's line of thought harmonizes well with modern evolutionary developmental genetics.

SOME TERMS: LOSS, REVERSAL, AND REGAIN AFTER LOSS

If we are to dissect regressive evolution at the molecular level, it is necessary to be very clear about the processes with which we are dealing. The terms "loss" and "reversal" are often used interchangeably for two rather different evolutionary events. First, there is the loss of a physical structure, such as legs or pappus scales, and second, there is the loss of a derived character state, e.g., the loss of red flower returning to the ancestral state of blue flowers. For this chapter, I propose to restrict the term "loss" to the first example and to restrict the term "reversal" to the second.

The first example, the loss of a physical entity, could be called a reversal in that the physical entity must have evolved in the first place, so loss of legs in snakes could be regarded as a reversal to the primitive state of leglessness as found in the primitive vertebrate *Amphioxus*. However, there are two objections to this:

1. The biological context of leglessness is so different in *Amphioxus* and snakes that it is not very useful to consider that a snake has reverted to an *Amphioxus*-like condition. The biological context of legs in reptiles is that they have evolved from fins and so a reversal would, more properly, be a reversal to fins.
2. If a loss is considered a reversal, this implies that the primitive and derived absences are similar states. However, shared absences are a very weak form of similarity. Bacteria and snakes are not similar because they both lack legs. They both lack an infinite number of real and potential structures, but that does not make them infinitely similar.

The second example, red petals reverting to the ancestral blue condition, is only a loss in the sense that any character state change is a loss—a loss of what went before it could equally well be considered a gain in that it is a secondary gain of blue flowers. It is not very useful to call a gain a loss, especially if it is only a loss in the sense that all change involves loss. It is, however, certainly a reversal, and this term describes it precisely.

Finally, it may be helpful in emphasizing the distinction between loss and reversal to analyze the terms in the context of phenotype ontology (Gkoutos et al 2004). In this, a lost organ is an *entity* (E), whereas a changed character state is a *quality* (Q), and philosophically, the difference is a fundamental one. Qualities are factors that are inherent to a particular instance of an entity. There cannot be an instance of an entity that does not exist. The absence of an organ is therefore not a feature of that entity but of a higher-level entity, such as the organism (E), which can have the quality "lacks part" (the lacking part specified by a second entity, E2). This may seem like an unnecessary precision of language, but it is important that genes and pathways are annotated to phenotypes in ways that are logically consistent (Mabee et al. 2007).

POTENTIAL PROBLEMS

Having made the case for separating the loss of an entity from the reversal of a character state, it must now be admitted that the two evolutionary events have many overlapping features. For instance, the reversal of a character state may often result from the loss of a gene (loss of a physical entity at the molecular level).

Furthermore, many morphological entities (usually minor ones) whose presence and absence are "flickering" (Marshall et al. 1994) on and off in evolution can plausibly be said to be reversing. An example is provided by the seed hairs of the Acanthaceae (Manktelow et al. 2001). Seed hairs appear to have evolved as a synapomorphy for the Whitfieldieae/Barlerieae clade and were subsequently lost in three component genera, an example of flicker over comparatively short evolutionary timescales. However, the multiple loss of pappus scales in European daisies (Bellideae) (Fiz et al. 2002) cannot be considered a reversal, because pappus scales evolved originally from sepals. This therefore represents a loss of the entity sepals/pappus. A reversal would be pappus scales evolving back into sepals.

The regain of an organ or entity after its loss is a special type of "reversal" that is worth considering separately because such changes break Dollo's law (discussed below). Purported examples include the regain of wings that have been lost during the evolution of flightlessness (Whiting et al. 2003). This is distinct from other types of reversals in that it involves the abolition of the quality "lacks part" in a group of organisms in which the presence of the part is plesiomorphic, so the gain is not an innovation but a reversal.

In conclusion, it therefore seems useful, for the reasons given above, to maintain a distinction among (1) loss of a physical entity (character state changes of a special type: present to absent), (2) reversal (character state change from apomorphic to plesiomorphic, but specifically excluding presence/absence cases), and (3) regain of a lost entity (i.e., abolition of a quality "lacks part" in an organism). Within the general concept of regressive evolution, three distinct types can therefore be recognized: *l*oss, *r*eversal and *r*egain (LRR) from loss. These may conveniently be referred to collectively as LRR events. Importantly, although these three types of evolutionary changes are related, they represent potentially distinct processes that may have different molecular mechanisms underlying them.

SINGLE AND MULTIPLE CHARACTER LOSSES AND REVERSALS

Phylogenetic versus Developmental Approaches to Loss and Reversal

The incongruence of molecular and morphological data under cladistic analysis has been remarked upon many times. The molecular evidence (particularly if it is based on multiple genes) is often regarded as giving the best estimate of the phylogeny. Incongruence of the tree with the evolution of morphological characters is then explained by parallel evolution (homoplasy). Homoplasy can result from multiple character state gains (parallel innovation), a single loss/reversal/regain (LRR) event, or multiple LRR events.

It can be problematic to reconstruct multiple LRR events by character mapping on phylogenies because these can also be modeled alternatively as multiple gain events. It is easy to find which is most parsimonious (or most likely under maximum likelihood) assuming symmetrical probabilities of losses and gains. However, if losses are thought to be more likely than gains, or vice versa, the situation becomes more complex. Most studies deal with this by determining the level of asymmetry of change probability at which the scenarios switch. Thus, Oakley and coworkers (Oakley and Cunningham 2002) noted that eyes in ostracods can be interpreted as a single parallel gain or as some 30 losses. As the authors note, both scenarios are somewhat improbable. It is problematic that something as complex as a compound eye should be able to evolve twice, but there is similarly no explanation for the alternative scenario of a massively repeated evolution of eyelessness. Similarly, a study of winged and wingless stick insects concluded that there were multiple gains of wings from a wingless ancestor, because under a multiple loss scenario, losses would

have to be more than five times as likely as gains (Whiting et al. 2003). However, without evidence from developmental genetics, we have no means of knowing the relative likelihood of losses and gains, and such phylogenetics-only reconstructions will remain uncertain.

At this point, phylogenetic methods of homology assessment reach the limit of their usefulness, and character-based (developmental genetic) methods must take over (Cunningham 1999). In the example above, the single parallel gain hypothesis would imply that the ostrocod eye is not homologous to other compound eyes. In biology, the distinction between homology and analogy (coined by Richard Owen in 1843 [Boyden 1943]) is fundamental and determined by critical observation. If homology can be rejected at the level of similarity (whether molecular, developmental, or morphological), the two eyes are then (in Richard Owen's terminology) analogous, rather than homologous, and the parallel gain hypothesis is supported.

On the other hand, if molecular mechanisms can be discovered that support the relative ease of gains over losses (or vice versa), this is powerful evidence for constraining the asymmetry of gain/loss transition probabilities. It is one of the themes of this review that there has been an understandable overreliance on phylogenetic methods to study losses, reversals, and regains (driven by the ready availability of reliable phylogenies during the last two decades). However, the rise of molecular genetic and developmental understanding of character change permits a new, mechanism-based attack on the problems of losses and reversals.

Ecological and Developmental Factors Promoting Multiple Loss and Reversal

Multiple LRR events, like multiple convergent gains, are probably driven by strong selection in response to common ecological factors. Accessory olfactory bulbs in the brain, that function in responding to airborne pheromones, have been lost independently in the brains of sirenians, cetaceans, bats, and certain primates. The common ecological factor seems to be a departure from terrestrial habitats. In aquatic, aerial, or arboreal habitats, pheromones either are not dispersed or are too highly dispersed to function (Johnson et al. 1994).

In plants, the biochemical pathway of crassulacean acid metabolism (CAM) is an important mechanism for maintaining high rates of photosynthesis under drought conditions and is consequently commonly found in epiphytes. It appears to be a very labile character, gained and lost as plants adapt to shifting habitats. In Bromeliaceae, for instance, CAM has been gained and lost numerous times (Crayn et al. 2004). Breeding systems of plants are also labile traits because they are under very strong divergent selection for reproductive fitness (cross-pollination) or reproductive assurance (self-pollination) under different ecological conditions (Weller and Sakai 1999). A recent study of a small group of flax-flower plants, *Linanthus* sect. *Leptosiphon*, suggested four losses of self-incompatibility (SI) in response to conditions that favor partial selfing (Goodwillie 1999).

Finally, sexual selection functions as a very strong driver of reversals and losses (Omland and Lanyon 2000). Horn evolution in scarab beetles appears to have resulted in 25 separate gains and losses of five different horn types (Emlen et al. 2005). These labile and fast-evolving characters, under strong and opposing selectional forces, undergo what has been called evolutionary "flicker," i.e., relatively frequent gain and reversal or gain and loss in multiple lineages during a relatively short evolutionary timescale (Marshall et al. 1994).

In addition to ecological causes of multiple LRR events, it is possible to conceive that certain characters may be predisposed to multiple losses or reversals because of intrinsic developmental vulnerability. An example might be a character resulting from a gene network that is unstable for some reason. Conversely, other gene networks might be well buffered against change, thus preventing evolutionary loss or reversal. As the genetic basis of character change becomes increasingly well understood, it will be interesting to see whether examples of this sort emerge.

Multiple losses at the molecular level may also be due to mechanistic predisposition rather than ecological drivers. Intron losses provide a good example of basically stable characters that appear to be subject to repeated loss in some clades. Most flowering plants have an intron in their chloroplast *rpl2* gene, so this character is evolutionarily stable. However, in *Bauhinia*, a large genus of legumes, the intron is missing in many species and appears to have been lost independently in several different clades of *Bauhinia* (Lai et al. 1997). Similarly, there have been multiple losses of the rpoC1 intron from *Medicago* (Downie et al. 1998). In addition to molecular sequences that can undergo loss by deletion, secondary structures are another type of molecular feature that can undergo loss. A particular stem-loop structure in the mitochondrial genome has undergone as many as seven parallel losses (Macey et al. 2000).

IRREVERSIBILITY OF LOSS? DOLLO'S LAW AND GENE NETWORKS

In the introduction above, a distinction was made among (1) losses of organs, (2) reversals of character states, and (3) the regain of lost organs. The last of these is of particular evolutionary interest because it breaks Dollo's law. This law, in its modern form, states that a complex structure or organ, once lost, will not be regained. The Belgian palaeontologist Louis Dollo (1857–1931) articulated this law in 1893 as a result of his studies of fossil vertebrates. Stephen Jay Gould gave added prominence to the law in an influential essay (Gould 1970) and illustrated it with examples of the irreversibility of the loss of shell-coiling in the mollusca.

It has been suggested that there is molecular and developmental logic to Dollo's law, which is that the gene network underpinning a structure will rapidly decay when the structure is no longer phenotypically expressed and the underlying network is not subject to purifying selection. Marshall et al. (1994) suggested that silenced genes or lost developmental programs may be reactivated as long as this happens in under 6 million years. After 10 million years, they suggested that the chances are unrealistic of vestigial

gene networks still being intact enough for reactivation. A good example of this, at a biochemical level, is provided by the loss of the cyanidin branch of the anthocyanin biosynthetic pathway in *Ipomoea* (Zufall and Rausher 2004), which is a key step in the production of blue pigment. The block of the cyanidin branch is therefore important in the evolution of red flowers in species such as *Ipomoea quamoclit* from blue-flowered ancestors. There is evidence that the relaxation of selection that occurred after the initial blockage of the pathway will now make it very unlikely that blue flowers will reevolve in that lineage.

On the other hand, mechanisms have been proposed to explain the conservation of genetic pathways after loss. Conservation may occur if the network has another function and was merely coopted to create the character that was subsequently lost. There is then no reason that the network should not be coopted again to remake the original character. The reversal of loss is therefore akin to a multiple gain. An example of how this might work is provided by the wing spots in *Drosophila* (Gompel et al. 2005). *D. biarmipes* has evolved a prominent wing spot caused by novel expression of the pigment gene, *yellow*. The *yellow* gene has become wing-expressed in a highly specific pattern because it has come under the regulation of the conserved *Engrailed* (*En*) gene regulatory network, by gaining (as a result of random mutation) a *cis* element that interacts with *En*. It is easy to see that the wing spot could be lost by a loss of the *cis* element. However, neither the *En* network nor the *yellow* gene would be lost because they have other important functions in *Drosophila*. If the wing spot were to be lost, it could be regained by the same means. It would, however, represent an independent gain, and the *cis* element might be in a slightly different location.

A similar example appears to be provided by the developmental genes *Notch* (*N*), *Distalless* (*Dll*), *En*, and *Spalt* (*Sal*) in butterflies, which are responsible for body patterning, are expressed at the eyespot, and may be coopted as part of the eyespot regulatory pathway (Beldade et al. 2005). An eyespot loss mutant has been shown to have reduced *N* and *Dll* expression (Reed and Serfas 2004). However, if eyespots are lost, the underlying network will not be because it is of vital importance to the whole organism, and eyespots can potentially be regained. Regulatory capture of this sort may prove to be common in evolution.

In the example above, loss of *cis* elements causes loss of gene expression and thus loss of a feature. This is a loss-of-function mutation, which would be genetically recessive. Alternatively, the loss of an organ or feature may be caused by active suppression as a result of a gain-of-function mutation in a suppressor gene. Such a mutation would be genetically dominant. An example of this appears to be the loss of bracts in the Brassicaceae, including *Arabidopsis*. In most eudicots, the inflorescence branches are subtended by bracts. However, bracts are usually missing from the inflorescences of the Brassicaceae, although there is vestigial expression of bract-specific genes where bracts would be expected. These vestiges have been called "cryptic bracts." The leaf developmental gene *JAGGED* is required to form bracts (Dinneny et al. 2004), and this is excluded from the inflorescence of *Arabidopsis* by the action of the *BLADE ON PETIOLE* (*BOP*) genes acting in concert with the developmental gene *LEAFY* (Norberg et al. 2005). Thus, bracts are actively suppressed in *Arabidopsis*. Loss-of-function mutations in the suppression gene network allows bracts to form, after having been evolutionarily lost, by desuppression. Because the suppressed gene has an important role in leaf development elsewhere in the plant, there is no likelihood that the *JAGGED* gene will degenerate, making the reoccurrence of bracts impossible.

The key feature of both loss by suppression and regulatory loss is that the gene that is no longer expressed in a particular place is expressed elsewhere in the organism. What is therefore important is a reduction in expression domain of a gene that has pleiotropic effects and not an elimination of expression. Such a gene will still be exposed to purifying selection, and the original expression pattern could be readily regained by either desuppression or regulatory capture, thus breaking Dollo's law. In such cases, the main factors preventing evolutionary recall may be ecological conditions that continue to select for loss.

Breaking Dollo's Law by Heterochrony

The reduction in expression domain may be in space (heterotopy) or time (heterochrony). Gastropods have lost shell-coiling multiple times, but two purported occurrences of the reevolution of shell-coiling have also been identified (Collin and Cipriani 2003). Because many gastropods without coiling in the adult have planktonic larvae with coiled shells, it is probable that the genes for shell-coiling continued to be exposed to purifying selection in juveniles, having been eliminated from adults by suppression or regulatory loss. It is therefore unsurprising that these genes could be reexpressed in the adult by either desuppression or regulatory capture, in which they would come under the control of adult developmental gene networks. Ironically, this study of the reversibility of the loss of shell-coiling is in direct contrast to Gould's earlier use of shell-coiling to illustrate Dollo's law (Gould 1970).

EVOLUTIONARY–DEVELOPMENTAL MECHANISMS OF LOSS AND REVERSAL

A Genetic Landscape of Loss and Reversal

In the introduction, a distinction was made among character loss, character state reversal, and regain after loss. These different evolutionary phenomena may have different processes underlying them. Another axis that is relevant to dissecting the evolutionary developmental mechanisms underlying loss and reversal is the gain-/loss-of-function axis. These two axes are used to produce a landscape of loss and reversal in 12 categories as shown in Figure 1.

A loss may be due to a gain-of-function (dominant) mutation in a gene, or genes, that causes active suppression of a trait. Similarly, a reversal may result from a "cryptic innovation" that produces what appears to be a reversal but is in fact an innovation caused by a gain of gene function.

	Gain of function	Reduction of function	Loss of function in pleiotropic gene	Loss of function in specific gene
LOSS OF ORGAN	Suppression	Loss with vestige	Loss with possible neomorphism	Perfect loss
REVERSAL OF CHARACTER STATE	Cryptic innovation	Reversal with vestige	Reversal with possible neomorphism	Perfect reversal
REGAIN FROM LOSS	Recapture of conserved network	Partial desuppression	Desuppression with secondary phenotype	Desuppression

Dominant mutations — Recessive mutations

Figure 1. Genetic landscape of reversal and loss.

Another means of generating losses and reversals is through recessive loss-of-function mutations. If the loss of function is not complete, as it would be if a protein were to be merely down-regulated (and not completely eliminated as by a knockout mutation), then vestigial traces of a character are to be expected. This is the phenomenon of loss-with-vestige. Vestigial characters may be reduced forms of the original organs or more subtle traces. A common example of reduced organs is found in staminodes, which are stamens whose development was arrested very early.

Vestigial structures are very common mainly because when a structure becomes minute, the selective pressure to eliminate it further drops: At a certain size, it has not selective consequences. However, many structures are eliminated to a much greater degree and are only visible as vestiges by microscopic investigation very early in development. *Arabidopsis* lacks bracts in the inflorescence but "cryptic bracts" are distinguishable as patches of gene expression where bracts would be expected.

If the reversal results from the knockout of a gene that has other pleiotropic effects (as is the case in many artificially induced mutants), neomorphic characters are likely. Neomorphisms are new phenotypes produced as by-products of gene mutations, in addition to the main phenotypic effects. A good example of a neomorphism is hexamerous flowers commonly produced as a result of knockout of the floral symmetry gene *CYCLOIDEA* (*CYC*).

If there are no vestiges or neomorphic side effects, it may then be possible to get a "perfect" loss or reversal, although this appears to be rare. An example of loss-without-vestige (Cronk 2001) is found in the stamens of the Detarieae (Tucker 2001), in which, rather than being reduced to staminodes, certain stamens are lost without any ontogenetic trace. Such a phenomenon may result from the clean deletion of an ontogenetic pathway or from very complete but progressive reduction.

Reversal from loss, the regain of a structure or organ once lost, is a particularly interesting type of reversal because it is not degenerative but involves a new innovation. If the original loss was by a gain-of-function or suppressive mutation, regain is then easy—it just requires a desuppression by a loss-of-function mutation of the suppressing genes. However, if the original loss was a loss of function, then a gain of function will be needed to reinnovate the structure. This is more problematic because it involves reevolution of a character. In the case of complex structures such as eyes and wings, this is intuitively implausible. However, if the evolution of complex characters can occur by the capture of regulators by a control gene that is then expressed ectopically to produce the structure anew, the process may be more plausible. An example of this mechanism of evolution is found in the wing spots of insects as discussed in the section above, under Dollo's law.

Soft- versus Hard-wiring

Endress (2001) proposed that early in the history of an innovation, reversals are common, but over time, the innovations become "deeply rooted genetically in the organization" and so are less prone to reversal. In support of this interesting idea, similar to the concept of canalization, Endress cites the distribution of flowering plant characters on plant phylogenies. He tentatively suggests that character innovations in flowering plants such as sympetaly and tenuinucellate/unitegmic ovules may have begun as minor changes with, at first, frequent reversals. If this is correct, it implies that the gene networks underlying character innovations are at first "soft-wired," i.e., there is little developmental canalization (Waddington 1957). However, as the networks develop, they become buffered against reversals, and thus development is more canalized (hard-wired).

Figure 2 shows a simple graphic representation of this as a genotype of a lineage changing over time, across a critical threshold from phenotype change. At the start, away from the critical threshold, the phenotype is stable at A. Near the critical threshold, the phenotype can change rapidly as the genotypic "walk" crosses and recrosses the critical threshold. Later, when the walk has moved away from the critical threshold, the phenotype is again stable but at state B. This might be a useful analogy for Endressian soft-

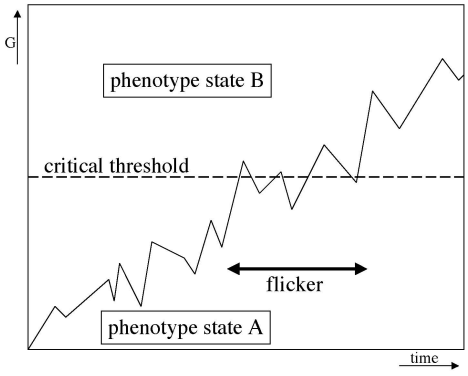

Figure 2. Graphic illustration of the concept of "flicker." Phenotypic traits when they first appear may not be stable, but they may acquire evolutionarily stable expression in later diverging lineages. In this diagram, the evolution of a lineage through time (*x* axis) is plotted with the *y* axis representing change in some feature or features of the genome.

wiring if there is a critical threshold in the evolution of a particular developmental gene network.

Endress' idea resonates with the idea of "flicker" put forward by Marshall et al. (1994), interpreting Shaffer's study of Mexican ambystomatid salamanders. In this study, metamorphosing salamanders, neotenic salamanders, and facultatively neotenic salamanders were found to exist in a complex phylogenetic pattern, apparently indicating frequent evolutionary change and reversals. Similar reversals have also been postulated in plethodontid salamanders. These have evolved from having a larval life stage to direct development, and in some of the direct-developing lineages, larvae have apparently reevolved (Mueller et al. 2004).

Under this interpretation of a labile character undergoing change and reversal, in appears that rapidly alternating character state changes can occur over relatively short evolutionary time periods. However, it has been argued that over longer periods, one of the character states will be irretrievably lost by mutational decay of the relevant gene networks and the time course of mutational decay of gene function has been modeled (Marshall et al. 1994).

The test of these ideas requires a good understanding of the developmental networks underpinning traits so that their evolution can be reconstructed and the likelihood of response to perturbation modeled.

AN EXAMPLE: REVERSALS FROM MONOSYMMETRY TO POLYSYMMETRY IN FLOWERS

It is thought that angiosperms ancestrally had spirally arranged floral parts. However, in the eudicots, the petals are typically inserted at the same level and are definite in number (typically five) arranged radially. These radial whorls are therefore "polysymmetric," having rotational symmetry and five planes of reflectional symmetry. In some clades of eudicots, however, asymmetry has developed along the adaxial–abaxial axis, with the adaxially oriented petals assuming a different form from the abaxially oriented petals. This results in the petal whorl being monosymmetric, having no rotational symmetry and only one plane of reflectional symmetry (Cubas 2004). This change of symmetry is analogous to the change from typical polysymmetric (pentamerous) sea urchins to monosymmetric heart urchins. Polysymmetric and monosymmetric petal whorls are illustrated in Figure 3.

The transition to monosymmetry from polysymmetry is well studied in the snapdragon (*Antirrhinum*). It results from the adaxial expression of *CYCLOIDEA*-like genes (*CYC* and its close paralog *DICHOTOMA* [*DICH*]) (Luo et al. 1996), which not only directly affect the morphology of the domain in which they are expressed, but also activate a second gene *RADIALIS* (*RAD*) that has a greater effect (Corley et al. 2005). In *Antirrhinum*, the expression domain of RAD is somewhat greater than the expression domain of *CYC* probably due to movement of the *RAD* transcript (Corley et al. 2005). The asymmetric expression of *CYC* and *RAD* in the adaxial domain leads to asymmetric petal morphology and hence the transition from polysymmetry to monosymmetry.

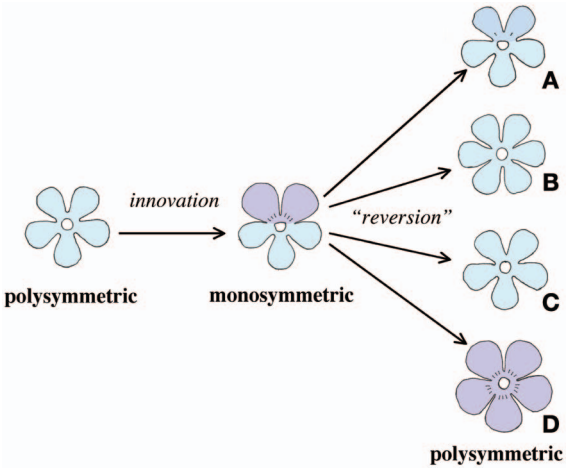

Figure 3. Different types of reversals from monosymmetric to polysymmetric flowers. (*A*) Reversal with vestige, (*B*) reversal with neomorphism (hexamery), (*C*) reversal without vestige, (*D*) cryptic innovation.

Many clades of flowering plants are characteristically monosymmetric and reversals seem to be rare. Nevertheless they do occur, both in nature and as horticulturally important mutants. Thus, floral symmetry is potentially a useful model for studying character reversal in a trait (reversal of monosymmetry back to polysymmetry) at the molecular level. Examples occur in the pea family (Leguminosae), for instance, all species of the peculiar genus *Cadia* (discussed further below), and horticultural varieties of the butterfly pea (*Clitoria ternatea*). Other examples occur in the snapdragon family (Scrophulariaceae), as in the so-called "peloric" mutants of snapdragon and toadflax (*Linaria*) and in the related African violet family Gesneriaceae, for instance, all members of the genus *Ramonda* and horticultural varieties of African violet (*Saintpaulia*) and gloxinia (*Sinningia*).

Not all reversals to polysymmetry are morphologically equivalent, and subtle differences may be found (see Fig. 3). Some polysymmetric revertants are hexamerous, as opposed to the original pentamerous state. This condition is found in the peloric snapdragon and in the horticultural polysymmetric *Sinningia*. Hexamery here is a "neomorphism" that results as a by-product of the reversion to polysymmetry (Fig. 3C). In the snapdragon, such mutants result from the elimination of the expression of *CYC* and *DICH*. This eliminates adaxial identity. Thus, all corolla lobes have default abaxial identity and are identical, leading to a polysymmetric flower. However, the elimination of *CYC*/*DICH* expression leads to the neomorphism of six corolla lobes. This implies that these genes have an additional, pleiotropic, role (that of meristic control) in addition to their role as identity genes. Because this mutation involves a loss of function, it is recessive.

Other polysymmetric revertants have barely noticeable vestigial monosymmetry. This is true of polysymmetric *Saintpaulia*, in which the mature flower appears nearly perfectly polysymmetric, but the early stages of stamen development are asymmetric. In also occurs in *Ramonda*,

in which the petal shape is polysymmetric, but small differences in pigmentation at the base of the corolla lobes are asymmetric. Such reversion might be caused by partial loss of function of *CYCLOIDEA*, or loss of function of downstream genes in the *CYC* pathway, so that the direct effects of *CYC* on floral zygomorphy are still expressed, giving a vestigial phenotype. Both scenarios result from recessive (loss-of-function) mutations, although neither is as extreme as the complete *CYC* loss-of-function mutation detailed above.

Cadia and Cryptic Innovation

The original innovation to give monosymmetry involved a specific novel expression of a pathway in the adaxial (dorsal) part of the flower. It is this difference between the dorsal and ventral part of the flower that has to be abolished to return to polysymmetry. There are therefore two mechanisms to revert to polysymmetry. The polysymmetric petals may be equivalent either to what were formerly the abaxial petals or to what were formerly the adaxial petals.

In *Cadia* and *Clitoria*, the five rather large petals in the polysymmetric petal whorl appear to be equivalent to the adaxial or flag petal. This is not, strictly speaking, a reversal because the adaxial petal identity is an innovation in the origin of monosymmetry, so a true reversal would be a return to abaxial identity for all petals. A return to polysymmetry by a spread of adaxial identity is therefore a "cryptic innovation" rather than a reversal. Such cryptic innovation can result from gain-of-function mutations in *CYC*, for instance, in *cis*-regulatory elements, to expand the expression domain from adaxial regions to throughout the whole flower so that every petal takes on an adaxial identity. This is what has happened in *Cadia* (Citerne et al. 2006). Such mutations would be expected to be dominant and would be immediately exposed to selection. They would therefore only persist if a preexisting floral biology niche existed for such mutants to occupy. In the case of *Cadia*, a shift to bird pollination from bee pollination seems to have been involved.

It is conceivable that a perfect return to polysymmetric petals is possible, all with abaxial identity and no vestige, neomorphism, or "cryptic innovation." However, such perfect reversals appear to be nonexistent, or at least very rare. It would require partial inactivation of the genes responsible for monosymmetry, eliminating their promotion of adaxial identity and leaving untouched their other pleiotropic functions. It would thus require very specific and targeted loss of gene function. This might, however, be possible through natural selection over long periods of time, progressively eliminating all vestigial traces of monosymmetry. However, selection coefficients against vestigial characters is generally thought to be low, because such characters are likely to have negligible adaptive significance. The four types of polysymmetric revertants—reversal with vestige, reversal with neomorphism, cryptic innovation, and perfect reversal—are shown diagrammatically in Figure 3. These also correspond to the four types of reversals given in Figure 1.

DISCUSSION: REASSESSMENT OF LOSSES AND REVERSALS AT THE MORPHOLOGICAL AND MOLECULAR DEVELOPMENTAL LEVEL

The subject of evolutionary developmental genetics often, and rightly, focuses on the origin of innovations. However, every loss and reversal represents a challenge for the science of evo-devo. More specifically, the limitations of the phylogenetic pattern-based approach to distinguishing multiple gains from multiple losses means that a developmental, or process-based, examination of losses and gains is needed.

The phylogenetic method seeks to study character state changes by reconstructing ancestral states, generally using maximum likelihood or parsimony, of characters mapped on trees. However, such phylogenetic methods are prone to problems of interpretation, given that the asymmetry in the probabilities of character gain and loss are not known a priori. Ultimately, information about the relative ease of different transitions is needed, information that can only come from knowledge of the mechanisms underlying the processes. Thus, developmental genetic methods involving morphology, development, and molecular mechanism are required.

The first step in a developmental genetic study of loss is first-rate morphological and developmental descriptions. If multiple gains are postulated over multiple losses, differences in phenotype and development should be evident from minute morphological inspection, which should be confirmable by examining similarities and differences at the level of molecular process. Furthermore, much can be inferred about the molecular mechanism from morphological clues. Of particular importance are neomorphisms resulting from gene knockout and various vestiges left from incomplete loss of function. If what are apparently reversals are cryptic innovations, this too may be evident from careful morphological examination.

Finally, detailed understanding of the molecular mechanisms involved in loss, reversal, and regain after loss should be the most telling. It will be a challenge, although not impossible, to place probabilities on the occurrence of particular molecular events, but it is easy to distinguish between those events that are likely to be exceedingly rare and those events that are likely to happen repeatedly in evolutionary time.

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REFERENCES

Beldade P, Brakefield PM, Long AD. 2005. Generating phenotypic variation: Prospects From "evo-devo" research on *Bicyclus anyana* wing patterns. *Evol Dev* **7:** 101–107.

Boyden A. 1943. homology and analogy a century after the definitions of "homologue" and "analogue" of Richard Owen. *Q Rev Biol* **18:** 228–241.

Citerne HL, Pennington RT, Cronk QC. 2006. An apparent reversal in floral symmetry in the legume *Cadia* is a homeotic transformation. *Proc Natl Acad Sci* **103:** 12017–12020.
Collin R, Cipriani R. 2003. Dollo's law and the re-evolution of shell coiling. *Proc R Soc Lond B Biol Sci* **270:** 2551–2555.
Corley SB, Carpenter R, Copsey L, Coen E. 2005. Floral asymmetry involves an interplay between TCP and MYB transcription factors in *Antirrhinum*. *Proc Natl Acad Sci* **102:** 5068–5073.
Crayn DM, Winter K, and Smith JAC. 2004. Multiple origins of crassulacean acid metabolism and the epiphytic habit in the neotropical family Bromeliaceae. *Proc Natl Acad Sci* **101:** 3703–3708.
Cronk QC. 2001. Plant evolution and development in a post-genomic context. *Nat Rev Genet* **2:** 607–619.
Cubas P. 2004. Floral zygomorphy, the recurring evolution of a successful trait. *Bioessays* **26:** 1175–1184.
Cunningham CW. 1999. Some limitations of ancestral character state reconstruction when testing evolutionary hypotheses. *Syst Biol* **48:** 665–674.
Darwin C. 1859. *On the origin of species by means of natural selection,* 1st ed. Murray, London.
Dinneny JR, Yadegari R, Fischer RL, Yanofsky MF, Weigel D. 2004. The role of *JAGGED* in shaping lateral organs. *Development* **131:** 1101–1110.
Downie SR, Katz-Downie DS, Rogers EJ, Zujewski HL, Small E. 1998. Multiple independent losses of the plastid *rpo*C1 intron in *Medicago* (Fabaceae) as inferred from phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer sequences. *Can J Bot* **76:** 791–803.
Emlen DJ, Marangelo J, Ball B, Cunningham CW. 2005. Diversity in the weapons of sexual selection: Horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). *Evolution* **59:** 1060–1084.
Endress PK. 2001. Origins of flower morphology. *J Exp Zool* **291:** 105–115.
Fiz O, Valcarcel V, Vargas P. 2002. Phylogenetic position of Mediterranean Astereae and character evolution of daisies (*Bellis*, Asteraceae) inferred from nrDNA ITS sequences. *Mol Phylogenet Evol* **25:** 157–171.
Gkoutos GV, Green EC, Mallon A-M, Blake A, Greenaway S, Hancock JM, Davidson D. 2004. Ontologies for the description of mouse phenotypes. *Comp Funct Genomics* **5:** 545–551.
Gompel N, Prud'homme B, Wittkopp PJ, Kassner VA, Carroll SB. 2005. Chance caught on the wing: *cis*-regulatory evolution and the origin of pigment patterns in *Drosophila*. *Nature* **433:** 481–487.
Goodwillie C. 1999. Multiple origins of self-compatibility in *Linanthus* section Leptosiphon (Polemoniaceae): Phylogenetic evidence from internal-transcribed-spacer sequence data. *Evolution* **53:** 1387–1395.
Gould SJ. 1970. Dollo on Dollo's law: Irreversibility and the status of evolutionary laws. *J Hist Biol* **3:** 189–212.
Johnson JI, Kirsch JA, Reep RL, Switzer RC 3rd. 1994. Phylogeny through brain traits: More characters for the analysis of mammalian evolution. *Brain Behav Evol* **43:** 319–347.
Koelreuter JT. 1775. Lychni-cucubalus: Novum plantae hybridae genus. Novi comment. *Acad Sci Imp Petropol* **20:** 431–448.
Lai M, Sceppa J, Ballenger JA, Doyle JJ, Wunderlin RP. 1997. Polymorphism for the presence of the *rpL2* intron in chloroplast genomes of *Bauhinia* (Leguminosae). *Syst Bot* **22:** 519–528.
Luo D, Carpenter R, Vincent C, Copsey L, Coen E. 1996. Origin of floral asymmetry in *Antirrhinum*. *Nature* **383:** 794–799.
Mabee PM, Ashburner M, Cronk QCB, Gkoutos GV, Haendel M, Segerdell E, Mungall C, Westerfield M. 2007. Phenotype ontologies: The bridge between genomics and evolution. *Trends Ecol Evol* **22:** 345–350.
Macey JR, Schulte JA, Larson A. 2000. Evolution and phylogenetic information content of mitochondrial genomic structural features illustrated with acrodont lizards. *Syst Biol* **49:** 257–277.
Manktelow M, McDade LA, Oxelman B, Furness CA, Balkwill MJ. 2001. The enigmatic tribe whitfieldieae (Acanthaceae): Delimitation and phylogenetic relationships based on molecular and morphological data. *Syst Bot* **26:** 104–119.
Marshall CR, Raff EC, Raff RA. 1994. Dollo's law and the death and resurrection of genes. *Proc Natl Acad Sci* **91:** 12283–12287.
Mueller RL, Macey JR, Jaekel M, Wake DB, Boore JL. 2004. Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proc Natl Acad Sci* **101:** 13820–13825.
Norberg M, Holmlund M, Nilsson O. 2005. The *BLADE ON PETIOLE* genes act redundantly to control the growth and development of lateral organs. *Development* **132:** 2203–2213.
Oakley TH, Cunningham CW. 2002. Molecular phylogenetic evidence for the independent evolutionary origin of an arthropod compound eye. *Proc Natl Acad Sci* **99:** 1426–1430.
Omland KE, Lanyon SM. 2000. Reconstructing plumage evolution in orioles (*Icterus*): Repeated convergence and reversal in patterns. *Evolution* **54:** 2119–2133.
Reed RD, Serfas MS. 2004. Butterfly wing pattern evolution is associated with changes in a Notch/Distal-less temporal pattern formation process. *Curr Biol* **14:** 1159–1166.
Tucker SC. 2001. The ontogenetic basis for missing petals in *Crudia* (Leguminosae: Caesalpinioideae: Detarieae). *Int J Plant Sci* **162:** 83–89.
Waddington CH. 1957. *The strategy of the genes*. Allen and Unwin, London.
Weller SG, Sakai AK. 1999. Using phylogenetic approaches for the analysis of plant breeding system evolution. *Annu Rev Ecol Syst* **30:** 167–199.
Whiting MF, Bradler S, Maxwell T. 2003. Loss and recovery of wings in stick insects. *Nature* **421:** 264–267.
Zufall RA, Rausher MD. 2004. Genetic changes associated with floral adaptation restrict future evolutionary potential. *Nature* **428:** 847–850.