Review

Reversing opinions on Dollo's Law

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Dollo's Law, the idea that the loss of complex features in evolution is irreversible, is a popular concept in evolutionary biology. Here we review how application of recent phylogenetic methods, genomics and evo-devo approaches is changing our view of Dollo's Law and its underlying mechanisms. Phylogenetic studies have recently demonstrated cases where seemingly complex features such as digits and wings have been reacquired. Meanwhile, large genomics databases and evo-devo studies are showing how the underlying developmental pathways and genetic architecture can be retained after the loss of a character. With dwindling evidence for the law-like nature of Dollo's Law, we anticipate a return to Dollo's original focus on irreversibility of all kinds of changes, not exclusively losses.

Pattern and process of Dollo's Law

The search for universal patterns that can be termed rules or laws has a long and checkered history in ecology and evolutionary biology [1,2]. Our desire to discover such patterns and use them to make robust predictions is strong, but law-like patterns are elusive. The modern version of Dollo's Law formulated by Simpson [3], the idea that complex characters (see Glossary) that have been lost in evolution cannot be regained, has great appeal, owing to its conceptual simplicity and the ease with which most of us can think of examples: whales and crown-group snakes appear to have never regained legs; ratites have not regained the ability to fly; and birds have not regained teeth. But are these popular ideas really true? Are complex features really never reacquired in lineages that have lost them? If they are not reacquired, why not? And what does the Dollo's Law framework contribute to studies of the reacquisition or the possibility of reacquisition of characters?

Dollo's Law is composed of two parts: (i) the observation of a phylogenetic pattern that lost complex characters are not regained, followed by (ii) the inference that characters cannot re-evolve because the genetic and/or developmental features underlying that unexpressed character accumulate mutations that are extraordinarily unlikely to be reversed. Here we discuss how advances in phylogenetic methodologies have shown that exceptions to this pattern are more common than previously acknowledged. Further, application of genomics and evo-devo methods is gradually eroding the idea that the genes underlying unexpressed characters are lost to random mutation. Finally, we highlight some ways in which old genetic pathways can be reactivated to reacquire characters. Of course, rules are made to be broken, but the growing number of exceptions to both parts of Dollo's Law suggests that its usefulness as an evolutionary principle might be coming to an end.

Documenting patterns of irreversibility

Although it is trivial to demonstrate that teeth have not been reacquired by modern birds, and that legs have not been reacquired by modern whales, demonstrating that a lost character has not re-evolved is not typically so simple. When characters vary across taxa, for example digits of lizards in the genus *Bachia*, in which several species have the ancestral number of digits whereas others have fewer digits [4], explicit phylogenetic analyses are necessary.

Phylogenetic character-state reconstruction is used to examine the frequency and direction of evolutionary change in a character of interest. The character states (i.e. feeding larvae versus no feeding larvae) are mapped onto a phylogenetic hypothesis of the relationships be-

Glossary

Bayesian analysis: phylogenetic inference that combines the prior probability of a phylogeny with the likelihood to produce a posterior probability distribution of trees. The posterior probability represents the probability that the tree is correct [40].
Character state: differences between homologous characters in different organisms.
Complex character: characters that are constructed of integrated parts.
Convergence: independent evolution of similar traits in different lineages. A case of convergence is the resemblance of flight structures in bats and birds.
Evo-devo: the comparative study of development in an evolutionary context.
Heterochrony: change in timing of expression of a feature.
Heterotopy: change in the location of expression of a feature.
Homology: the same feature shared by different organisms as a result of inheritance of that feature from a shared common ancestor.
Meristic characters: structures that occur in a series of repeated units. Examples of meristic characters are vertebrae, teeth and fins in fishes.
Modularity: characteristic of a system, which is divided into relatively autonomous units (modules) that interact with each other.
Node: branching point on a phylogenetic tree.
Parallelism: similar or identical traits that evolved independently starting from a similar condition, often via similar developmental pathways or due to similar selective regimes.
Parametric bootstrapping: sampling scheme with replacement to determine empirically the significance level of a statistic.
Parsimony: philosophy that accepts the simplest explanation of the data.
Pleiotropy: the contribution of a single gene to multiple phenotypic traits.
Quantitative trait locus (QTL): a region on a chromosome where a set of genes encodes a phenotypic (quantitative) trait. Variation in this region results in variation in the trait controlled by those genes.

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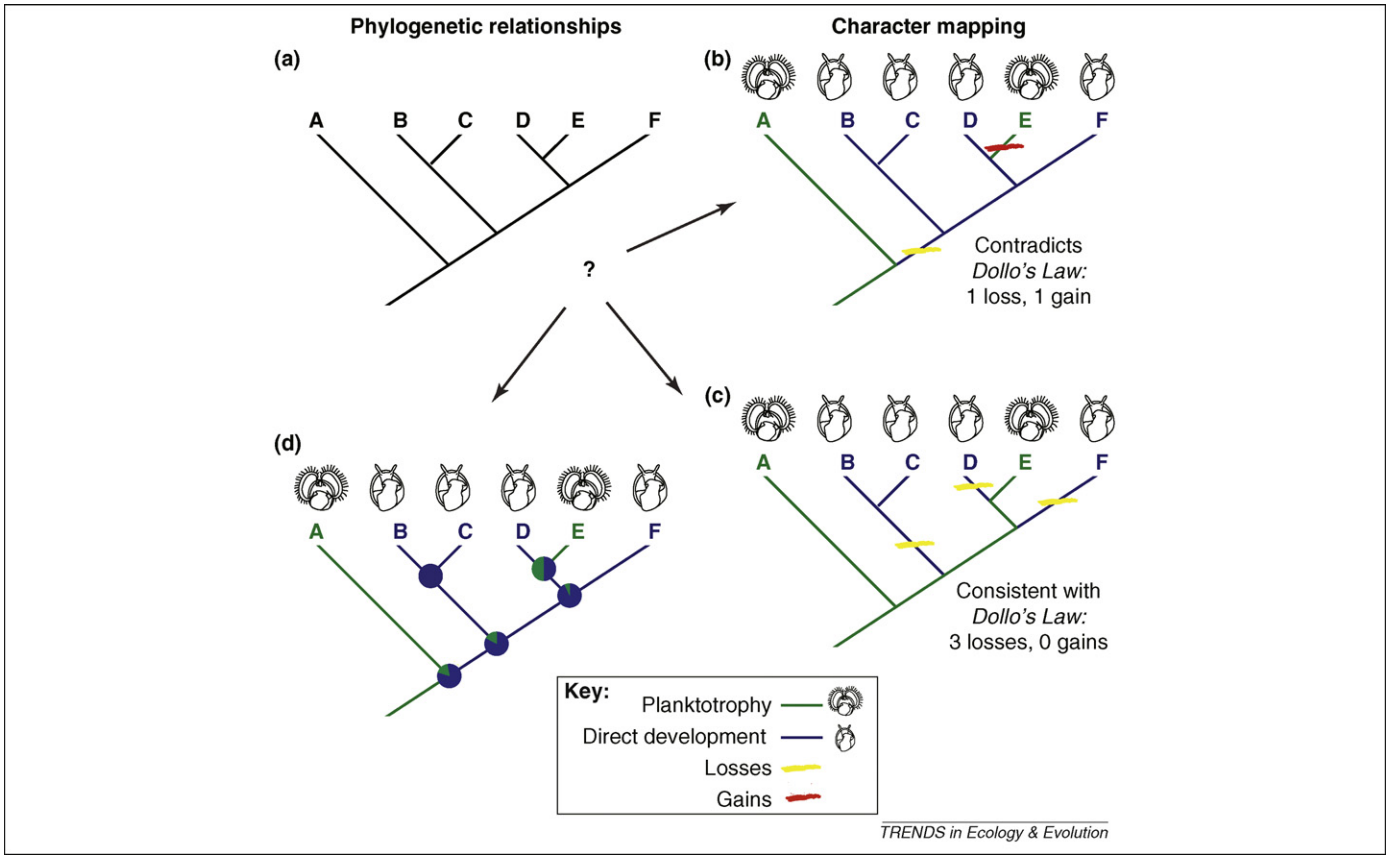

Figure 1. Steps to determine whether the pattern of character evolution is consistent with Dollo's Law. To determine whether a pattern of character evolution is consistent with Dollo's Law, the relationships of the species must be assessed to produce **(a)** a phylogenetic tree, and the state of the character in each species must be determined, in this case the presence (planktotrophy) or absence (direct development) of planktotrophic larvae in the life cycle. The pattern of character-state changes is then mapped onto the tree. Different methods could produce different patterns (see arrows; Box 1). The mapping in **(b)** consistent with equal-weighted parsimony shows a pattern that contradicts Dollo's Law, where the path from the bottom of the tree begins with the presence of planktotrophy, loses it, and then regains it for a total of a single loss and a single gain across the tree. The mapping in **(c)** shows a pattern derived from 3:1 parsimony weighting, where losses are scored as three times more likely than gains, and is consistent with Dollo's Law. Here, planktotrophy was retained in one lineage (lines in green), whereas it was lost in three other lineages (in blue). **(d)** Preferred methods (Box 1) usually portray the probabilities of each state at each node as a pie diagram. This highlights that, in many cases, neither state can be determined with statistical significance.

tween species (Figure 1). A phylogenetic pattern consistent with Dollo's Law can be demonstrated in two ways: (i) if after calculating the rates of character-state changes (gains and losses) across the tree, the calculated rate of gains is not significantly different from 0 and (ii) if reconstruction of states at specific sites on the tree show that a trait is lost and never subsequently regained. The first approach is global, showing that reacquisition is significantly unlikely across the entire group, and the second approach is local, demonstrating that in a particular instance the feature does or does not seem to have re-evolved.

A variety of methods can map characters onto tree topologies [5–7] (Box 1) and can be used with phylogenies derived from molecular and/or morphological data. The traditional method of parsimony assumes an *a priori* relative frequency of gains and losses and uses this to reconstruct character-state evolution. For example, equal-weighted parsimony assumes that gains are as likely as losses, whereas Dollo's parsimony assumes that gains never occur. Therefore, parsimony is circular when used for calculating frequency of gains and losses. It can be used only in a limited way to map characters but not to statistically test hypotheses about character evolution [8,9]. Another drawback of parsimony is that it can be more easily misled than other methods when rates of character evolution are rapid and when the probability of gains and losses of a character are not equal [10]: exactly the cases where we expect to be investigating the possibility of irreversibility. Although useful for data exploration and still frequently used to portray graphically patterns of character evolution on phylogenies (e.g. [11]), parsimony should be avoided in studies of Dollo's Law to prevent circularity and because more robust methods that generate statistical confidence in character reconstructions are now available and widely applied.

Recent advances in phylogenetic methods have revolutionized our ability to test statistically hypotheses of character evolution (Box 1). These methods can be used to test whether the gain of a character is more or less frequent than the loss, and whether the frequency of gain is significantly different from 0. They can also be used to estimate the state of the character at any specific node in the phylogenetic tree and provide an estimate of statistical confidence in that reconstruction. Finally, they can determine whether a tree topology showing character re-evolution is a significantly better or worse fit to the data than topologies that do not support re-evolution. Recent applications of these methods have brought to light several surprising exceptions to the pattern predicted by Dollo's

Box 1. Phylogenetic methods to test for irreversibility

Patterns consistent with evolutionary reversals can be examined using several computational methods to estimate rates of character-state changes (in this case gains and losses) across the tree, and to reconstruct states at specific sites on the tree.

Maximum likelihood

Maximum likelihood (ML) methods use an explicit model of character evolution (incorporating the state of the characters on the tips of the tree, the rate of evolution and the length of internodal branches) to estimate the probabilities of character-state changes and the presence of each character state at each node [6,44,45]. ML estimates the probability of each character at each node so that a character-state reconstruction can be shown to be either statistically significant or uncertain using an ML ratio test. This is a major advantage over parsimony. An ML approach can also be used to determine whether the rate of reversals is significantly different from zero. This has been widely used to show exceptions to Dollo's Law [4,12–17]. The sensitivity of ML results to different rates of gains and losses can also be used to determine how unlikely losses must be before the re-evolution of a character is reconstructed on the phylogeny [46].

SOWH test

The Swofford-Olsen-Waddell-Hillis (SOWH) test is a topological test that uses parametric bootstrapping [47] to test whether the best tree topology (i.e. the most likely tree, if using ML criteria) on which one pattern of character evolution is reconstructed is significantly different from the best topologies that are consistent with alternate evolutionary patterns. For example, Kohlsdorf and Wagner [4] used the SOWH test to determine whether the best tree of *Bachia* lizards, which showed the gain of toes, is significantly better than topologies that show only a reduction in toe number.

Bayesian methods

Both maximum parsimony and maximum likelihood ancestral-state reconstruction treat phylogenies as error free, whereas Bayesian methods account for this phylogenetic uncertainty. Bayesian ancestral-state reconstruction averages all the possible character reconstructions over all possible trees sampled in the Bayesian search [5,7,48,49], weighed by their posterior probability. Likewise, forward and reverse rates of character-state changes are calculated over all possible states at each node of each tree and are not constrained *a priori*, although a prior probability distribution is specified [5]. This method has not to our knowledge been applied in the context of Dollo's Law.

Law, suggesting that stick insects can regain wings [12], lizards can regain digits [4], slipper limpets can regain a coiled shell [13], asexual mites can regain sex [14], frogs can regain tadpoles in their life histories [15] and marine snails can regain a feeding larval stage [16]. Possibly owing to publication bias, there is a single published study showing that a feature, self-incompatibility, has never been regained in a family of plants [17].

Although extremely powerful when appropriate data are available, there are several limitations to a purely phylogenetic approach to character-state reconstruction. These limitations are primarily based on the assumptions that the phylogeny is correct, good taxon coverage is obtained and characters are scored correctly. The importance of incorporating additional information into such analyses [15,17] has also been highlighted recently. In highly variable groups, incorporation of independent information about states at ancestral nodes can drastically alter the results compared with analyses excluding this information [17]. Likewise, the results of maximum likelihood analysis could give misleading reconstructions owing to the assumption that rates of character change do not vary across the phylogeny [15]. An available method that allows the rate to change locally [6,18] has not yet been widely used. This approach represents a direction of great promise for future progress, as does the development of new phylogenetic methods that incorporate additional data.

Do the genetic bases of a lost feature degenerate?

The second part of Dollo's Law is a statistical argument that predicts that complex characters will be difficult or impossible to reacquire because genes for unexpressed features, freed from stabilizing selection, are expected to accumulate deleterious mutations. The probability of subsequent back mutations reversing these mutations, to regain the original function, is very low. Presumably this neutral accumulation of mutations takes time, during which features could easily be reexpressed, accounting for the short-term reacquisition of some features [19–21]. This loss and regain of a character is not to be confused with reversals of character states (e.g. flower color changing from white to red and back to white). Therefore, reacquisition of lost features should occur in the short term, whereas Dollo's Law should apply to the period after gene function is predicted to be lost. However, as we discuss below, this line of reasoning, although reasonable for structural genes of limited or single effect, is a somewhat simplistic view that does not apply to genes with multiple effects.

The few published studies to date of these occurrences suggest that characters can be reacquired tens of millions of years (myr) after the initial loss: metamorphic development might have re-evolved 20–42 myr after it was lost [22] and coiling in slipper limpets re-evolved after more than 10 myr [13]. Most published exceptions to Dollo's Law have not dated the observed losses and gains. Estimation of such dates in more cases would be a useful starting point for understanding the potential mechanisms of reacquisition.

Structural genes of single effect

The absolute timescales over which structural gene function is lost can be predicted or estimated in several ways. Marshall *et al.* [23] estimated that the function of an unused protein-coding gene could be retained for up to 6 myr but would certainly be lost as a result of accumulated mutations after 10 myr. A different approach to the loss of unused genes, using the death of duplicated genes in whole and partial genomes of six eukaryotes, gave a similar but somewhat longer estimate for the loss of function [24]: the average half-life of duplicated genes is 4 myr. This implies that there is a good chance for a gene to be retained for 8 myr or more, but they will almost certainly not last longer than 16–24 myr.

These genetic data suggest that there is a large window during which it might be possible to avoid stochastic degradation of a single protein-coding gene (i.e. within the first 6–10 or even up to 16 myr). However, these estimates assume that the trait is neutral. This is not always the case, as genes underlying some lost structures, such as eyes in cave fishes, degenerate much more rapidly

as a result of selection [25]. In this case it has been shown that multiple steps in the developmental pathway can be lost in less than 1 myr.

The documented exceptions to Dollo's Law suggest reversals have occurred at the outer age limit of the retention of structural genes.

Pleiotropy

These estimates of the lifespan of unused genes are probably only relevant to a small percentage of genes: those that have only a single effect and are therefore unexpressed when the character of interest is lost. Some genes certainly seem to have only a single function in an organism (e.g. enamalin, hemoglobin), but these appear to be in the minority. Pleiotropy, in which a single gene contributes to multiple structural or developmental pathways, could retain the genes underlying lost features because selection on the other pathways will maintain gene function. Many signaling genes expressed in early development are expressed at multiple sites and during multiple stages of development [26]. The same holds for many structural proteins. For example, the gene encoding dentin matrix protein 1 is expressed in teeth and long bones, as well as in brain, pancreas and kidney tissues [27]. By contrast, there are few data on the strength and frequency of pleiotropic effects on gene regulatory regions. A single study has shown that the *cis*-regulatory region of a distal-less family gene from fishes that lost oral tooth expression 50 myr ago retains the capacity to drive normal oral tooth development [28], suggesting that its function has been maintained.

The question of how likely genes are to be retained comes down to how common pleiotropy is. Pleiotropy was predicted by Wright [29] to be universal. Bonner [30], however, suggested that pleiotropies are organized into networks held together by strong pleiotropic interactions but weakly connected to other such strongly connected networks. Modern quantitative trait locus (QTL) studies, gene expression studies and knockout experiments are rapidly generating data that suggest pleiotropic interactions are common. For example, ~50% of the QTLs contributing to stickleback body shape contributed to more than one aspect of shape [31]. Further empirical work in this area, as well as reviews that quantify the frequency, patterns and amount of pleiotropy, will contribute significantly to understanding the retention of unused genes.

How can genetic and developmental pathways of lost traits be reactivated?

Because development is modular, morphogenesis of many structures could result from the initiation of a common developmental module at a specific time and location. This is followed by differentiation or the development of structure-specific features. Such modularity is seen in the development of the vertebrate limb, where the same set of genes functions in both the fore and hind limbs, and in insects where similar genetic programs form legs and wings [32,33]. Likewise, the same program is present in diverse epithelial organs such as teeth, feathers and scales. Such a module underlying the development of teeth is present in birds despite the fact that teeth were lost in this lineage 60 myr ago (Figure 2; Box 2) and might be retained by selection on its function in the development of these other features.

Box 2. Lost but not forgotten – the reexpression of hen's teeth

Teeth were lost in the lineage that led to modern birds over 60 million years ago and teeth have not been reacquired in any known species. Stephen J. Gould [50] used the reported production of enamel and dentine by grafts of chick epithelium with mouse mesenchyme [51] as an example of the amazing 'latent capacity of genetic systems.' This result was subsequently shown to be an experimental artifact, but recent studies exemplifying many of the ideas discussed here have continued to show that chicks do indeed retain an extraordinary capacity to initiate tooth development.

The amniote tooth developmental program, as exemplified by modern mammals and crocodiles [43,52], begins when the oral epithelium forms a dental lamina (see Figure 2). This invaginates (or evaginates in some reptile teeth) into the underlying mesenchyme and becomes dental epithelium, while the mesenchyme condenses to form tooth buds. Extracellular matrix proteins (enamel-associated proteins and dentin) are deposited between the two layers by epithelial ameloblasts and mesenchymal odontoblasts [43,53].

In normal chick embryos, tooth development is almost completely lost: a ridge similar to the mammalian dental lamina forms but development goes no further [42,53]. However, examination of gene expression and embryonic manipulations show that almost the entire pathway remains intact and can be reactivated.

Many genes that play a role in defining the odontogenic region of mice are normally expressed in a similar manner in the chick mandible [42,53] (Figure 2). Others, vital in tooth development, are not expressed, resulting in a breakdown in epithelial–mesenchymal interactions and failure of invagination and subsequent development. When two of these gene products, BMP4 and FGF4, are applied, the pathway is jump-started [42] (see Figure 2), suggesting that the early morphogenic pathways are retained intact and could be naturally reactivated with a relatively small change in *Bmp*4 expression or contact with BMP-expressing tissue [43]. Pathways involving the same genes and genetic interactions, which are not tooth specific, are expressed in several other epithelial appendages (e.g. feathers and scales) [42], and therefore they are likely to have been retained intact owing to expression in other parts of the embryo.

Most important for Dollo's Law is that this latent genetic capacity can be reactivated naturally in *talpid*^{2} mutant chicks [43]. These embryos developed conical outgrowths, strikingly similar to early alligator teeth, at the oral–aboral boundary of the mandible, the normal location of tooth development [43], and displayed gene expression similar to crocodiles and mice (see Figure 2). Histological evidence of matrix formation and early odontoblasts indicates a tooth-specific pathway, as does the expression of *Pixt2*, which is not known to occur in epithelial organs other than teeth [43].

Retention of much of the genetic architecture of early induction and morphogenesis begs the question: if *ta*^{2} was not a lethal mutation, how much further could these epithelial buds differentiate and produce characters diagnostic of teeth? The available evidence is equivocal. For example, some dentin genes are retained via pleiotropic expression in bones [54]; by contrast, the red jungle fowl genome shows that genes encoding tooth enamel proteins have been lost [55]. This suggests that chicks could grow teeth using much of the ancestral developmental pathway, but these teeth, lacking enamel, would be noticeably different from other tetrapod teeth, supporting Dollo's original proposal that organs or complex structures cannot return to the identical condition shown by an ancestor, but nevertheless showing a clear reacquisition of most features.

In this case, there was *a priori* little expectation that the pathways for tooth development would have been retained in birds for so long. Such retention might be more common than expected and, in the future, biologists might marvel at the numerous ways in which such pathways are commonly retained and reactivated.

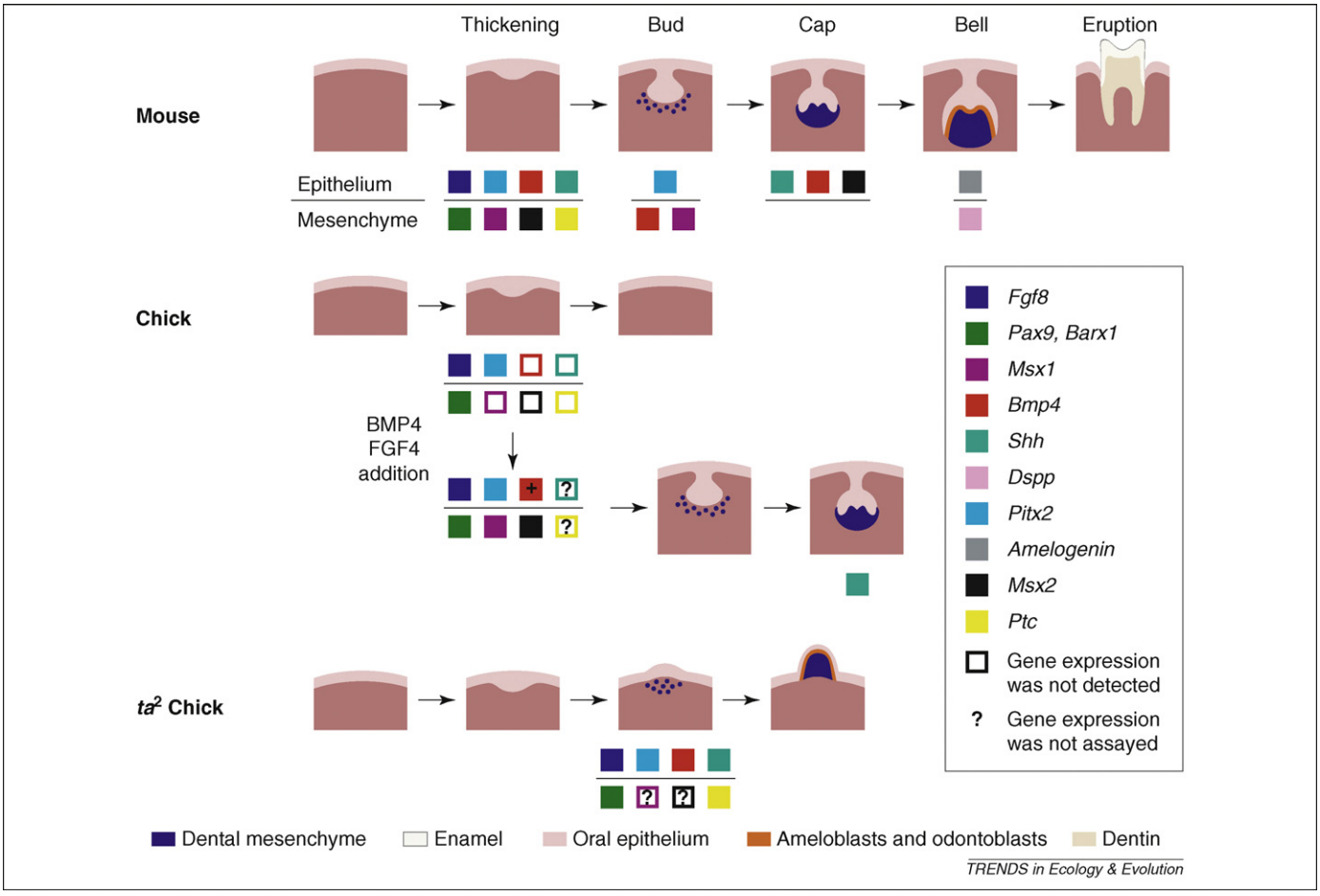

Figure 2. Gene expression in development of mammalian teeth, normal chick and *talpid*^{2} mutant chick. Tooth development in mammals (top) begins with a thickening in the oral epithelium, which overlies the neural-crest-derived mesenchyme. The epithelium invaginates into the mesenchyme, the dental mesenchyme condenses and a dental papilla, or tooth bud, is formed. During the subsequent cap and bell stages, extracellular matrix proteins are deposited between the two layers, with epithelial ameloblasts depositing enamel-associated proteins and mesenchymal odontoblasts depositing dentin. Specific gene expression in both the epithelium and mesenchyme are indicated below each stage [41]. In the 5-day-old chick a transient thickening forms concomitant with *Fgf8*, *Pax9*, *Barx1* and *Pitx2* expression but does not develop further [42]. Addition of exogenous BMP4 and FGF4 induces further events in the signaling cascade typical of mammalian tooth development and results in expression of *Shh*, *Msx1* and *Msx2* as well as epithelial invagination and some folding similar to the bud and cap stages. The expression pattern of *Ptc* was not examined but was absent in later stages [43] of normal chicks. Gene expression studies of *ta*^{2} mutant chicks at day 10 show that the tooth pathway is activated naturally and *Bmp4*, *Shh* and *Ptc* are expressed in the location of the presumptive teeth. By day 16 of development, evaginations develop along the mandible, similar to early crocodile teeth, and show a cavity and possible odontoblasts [42].

Many of the published examples of reacquired characters are explained by heterotopic shifts in such modules underlying meristic characters such as toes and teeth. If a developmental program already produces three toes, the complete developmental module could be co-opted in a different location to produce a fourth or fifth toe. Despite the fact that no tetrapods with more than five toes have evolved since at least the Permian, polydactyly is not uncommon in humans or guinea pigs, suggesting that developmental machinery does not limit the number of digits. There are several phylogenetic studies with results suggesting digit reacquisition (see Ref. [4]). Surprisingly, heterochrony, a major force in morphological evolution, has been hypothesized to explain only a single reacquisition: coiled shells might have been reacquired in uncoiled limpets by a heterochronic shift in the expression of the coiled larval shell [13]. Future studies are likely to uncover more examples of both mechanisms for re-evolution.

An intriguing example of how lost features can be recovered after more than a million years was recently described in the blind cavefish *Astyanax mexicanus* [25,34–36]. Blindness in these fish is based on degeneration of the development of both the lens and retina. Loss of sight is caused by the disruption of many genes and has been mapped to 12 QTLs which show a rapid loss of function due to selection [25]. Crosses between blind fishes from two different populations produced sighted offspring via complementation. This is a previously unexplored mechanism for the reacquisition of lost characters [35].

Alternate hypotheses

Studies of Dollo's Law generally focus on bolstering evidence for the scenario of interest and give little attention to alternate hypotheses. There are four possible alternate combinations of pattern and process (Figure 3) for character loss and regain, of which Dollo's Law explicitly considers only two scenarios: re-evolution and no regain due to genetic or developmental constraint. The first alternative to be examined is whether the phylogenetic pattern of character evolution shows regain of the character. Once this has been determined, the different scenarios must be distinguished using information from

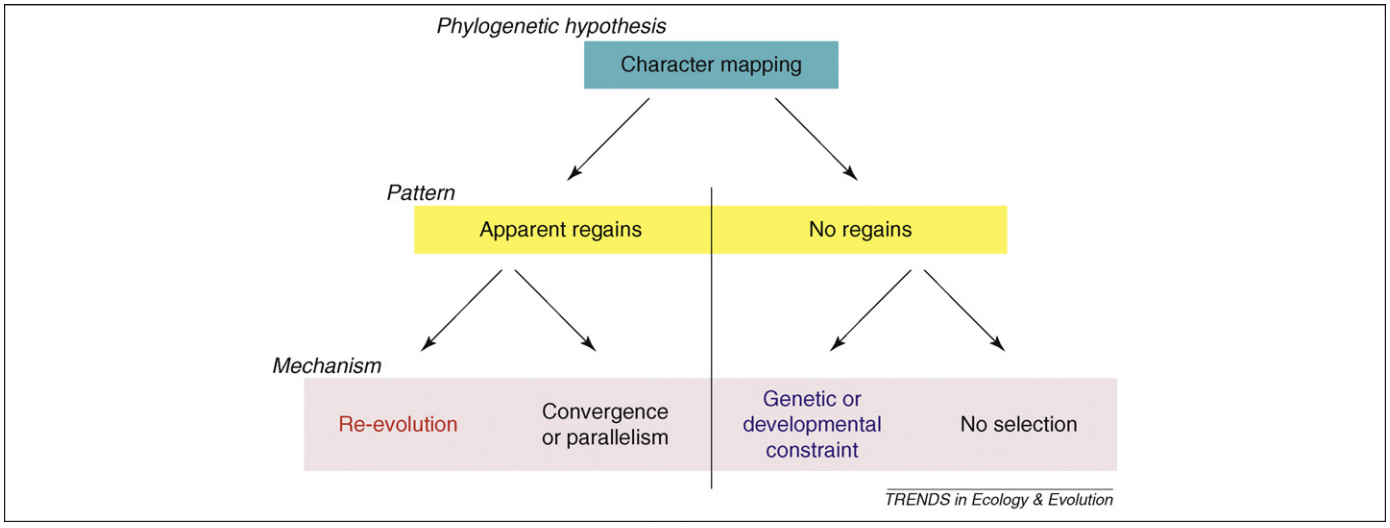

Figure 3. Decision tree showing the path that leads to Dollo's Law and alternate outcomes. The two parts of Dollo's Law are illustrated. The pattern showing a loss and subsequent reacquisition of a character is evaluated using phylogenetic comparative analyses. The pattern showing the gain of a lost character could be the result of two alternate mechanisms, reexpression of the lost character (counter to Dollo's Law), or convergence or parallelism. A pattern showing that the lost character has not been regained could likewise result from alternate mechanisms: loss of genetic or developmental pathways could prevent the reacquisition of the character, or natural selection could simply have not favored it.

comparative morphology, embryology, genetics, paleontology and ecology.

Interpretation of a phylogenetic pattern that shows the loss and subsequent re-evolution of a character comes down to a discussion of homology (Box 3). The pattern can represent two different situations: (i) an exception to Dollo's Law where a structure has re-evolved identical to the lost ancestral condition, and therefore presumably shares the same gene regulatory networks (i.e. is homologous according to Wagner [37]; see Box 3) or (ii) a convergence or parallelism, where similar structures have come about in different ways. It is not always easy to distinguish between these two possibilities without detailed genetic information, and it is important not to confuse the character and the character state. For example, the re-evolved digits of *Bachia* are clearly homologous as a character to other vertebrate digits, as opposed to, for example, convergent structures made from modified wrist bones. However, they have a different phalangeal formula than the ancestral digits in this genus and therefore have a different character state. Because almost any feature can be found to differ slightly from the ancestral condition, this criterion of the original formulation of Dollo's Law is not particularly useful. If the phylogenetic pattern shows that the feature has been reacquired, it is probably more useful to distinguish between characters that are homologous to the ancestral character or non-homologous to the ancestral character, rather than focusing on variation in the character states.

If phylogenetic methods show that a character has not re-evolved, there are two possible reasons. Such a pattern could result from the degeneration of the developmental and genetic basis of the character as posited by Dollo's Law, or it could simply be that the feature has not been selected for. Snakes might not have reacquired legs or birds regained teeth, not because they did not retain the genetic pathways but because there was no selective pressure to do so either because it was not advantageous or because it was so infrequently expressed, or had such low heritability that selection was too weak to have an effect [21]. Future studies should give explicit attention to the evidence for or against all four possibilities.

Prospectus

Dollo's Law has come a long way from Dollo's original statement that an organism is unable to return, even partially, to an identical condition expressed by an ancestor. As support for this view became untenable, various caveats were added and the focus moved from reversals in general to reversal of character loss. Superficially, this seems to be a restriction to a special subset of situations that seem to be intuitively less likely. However, it actually changed the hierarchical level of discussion from changes in character states to complete loss of the character. The idea was further limited to complex characters, because simple characters often appear to be gained and lost quite frequently. Now, with the growing number of phylogenetic studies showing patterns consistent with re-evolution of characters, and genetic data showing that developmental pathways can be maintained for tens of millions of years, is it time to give up Dollo's Law? Perhaps.

Studies in the context of Dollo's Law led to work on character evolution that is interesting in its own right. Unfortunately, the modern emphasis of Dollo's Law on the loss of characters as opposed to irreversibility of any change reduces emphasis on the roles of natural selection and convergence. It also overshadows one very interesting direction for future work: different character states have different evolutionary potential for reversals. For example, phylogenetic studies show that some pollinator syndromes in flowers appear to be 'dead ends' and not subject to reversals, whereas others appear free to vary [38]. A similar pattern is found in development of marine snails, where some types of direct development seem free to return to the ancestral condition, whereas others are not

Box 3. Homology and Dollo's Law

The problem of homology is a thorny one in evolutionary biology [19,37,56–62], and many different types and definitions of homology have been proposed. Here we review a few points that are directly relevant to Dollo's Law.

The criterion of gross overall similarity is often used as a preliminary indication of homology, and phylogenetic, genetic, developmental [57,59] and paleontological information are included if they are available. A strict phylogenetic definition of homology implies that a structure was present continuously in a lineage from ancestor to descendent [58] regardless of the various states taken by the character (i.e. a bat wing and a human hand are homologous characters despite the very distinct states or morphologies of the character). By this definition, a re-evolved character could never be homologous to the ancestral condition it resembles because it has been lost in the lineage in between.

A hierarchical view of homologies, where underlying genetic continuity is also considered, has been proposed [58]. This approach allows for homologous genetic or developmental pathways that lead to non-homologous adult structures as well as non-homologous developmental pathways that lead to homologous adult structures. The shared pattern of gene expression in early tooth and feather morphogenesis is an example of the former situation, where homologous genetic pathways lead to non-homologous structures. The second situation, referred to as developmental system drift (DSD), is illustrated by the evolution of vuval cells in nematodes [11], the vertebrate lens [58] and vertebrate neurulation [19]. In these cases, traits that appear to be expressed stably through evolution have undergone shifts in their underlying genetic and developmental pathways. The fact that traits and their developmental pathways can be modified independently is highly relevant to the identification of exceptions to Dollo's Law, as evidence from such pathways is often used to distinguish between re-evolved (assumed to have the same developmental pathways) versus convergent or parallel characters (assumed to have divergent developmental pathways; but see Ref. [62] for discussion).

Wagner [37] has combined these concepts to propose that historical continuity of 'character identity networks' underlies morphological homology. These character identity networks are types of gene regulatory networks that control the developmental program that specifies the identity of a character. They do not include all parts of the developmental pathway, and therefore this theory also accommodates DSD. Using this definition, re-evolved characters can be homologous if they utilize the same character identity gene networks as the ancestral character, but not if they have evolved using different networks. This provides an explicit way to identify re-evolved characters and is consistent with the less explicit thinking about homologies of re-evolved characters in the older literature.

[39]. An integrative approach similar to that applied to the loss and regain of characters could be useful in understanding what accounts for the differing evolutionary potential of character-state changes and duration of such changes in potential [21].

One major advantage of the Dollo's Law framework is that by directly proposing a developmental and genetic mechanism for a macroevolutionary pattern, it encourages the truly integrative application of cutting-edge genetic, evolutionary and developmental methods to single systems. Proposed mechanisms of few other macroevolutionary patterns are testable, and it would be interesting to see this diverse approach applied to reversals or irreversibility in character-state changes [20,21]. Until this becomes more common, such a framework is important and useful, not as a law, but as a way to make explicit the link between phylogenetic patterns and underlying mechanisms.

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