

Lost along the way: the significance of evolution in reverse

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Recently, researchers have begun to identify the prevalence of trait simplification, loss and reversal across all levels of biological organization. These studies have taken increasingly integrated approaches, incorporating phylogenetic, developmental and molecular methods, in the quest towards understanding the patterns and processes behind evolution in reverse. Here, we highlight the emerging interest in the reversibility of evolution by discussing a spectrum of studies examining both the genotypes and phenotypes of evolution in reverse. These integrative approaches have greatly increased our knowledge of the biological interactions that produce patterns of evolution in reverse and have led to promising new areas of research.

'Rudimentary, atrophied, or aborted organs. Organs or parts in this strange condition, bearing the stamp of inutility, are extremely common throughout nature' [1].

Evolution in reverse is a widespread phenomenon in biology; however, many researchers are only just beginning to take notice of the significance and prevalence of trait loss and/or simplification [2]. Part of this disregard is due to conflict among researchers about the validity of the concept of evolution in reverse. Many would argue that most commonly cited examples of REVERSE EVOLUTION (see Glossary) are actually *de novo* forms that have no relationship to ancestral states. Even if the concept is accepted, studies of the reversibility of evolution have been difficult to identify owing to confusion over what qualifies as 'reverse evolution'. Terms such as simplification, REGRESSION, and REVERSION all refer to some form of reverse evolution (Box 1). In the strictest sense, reverse evolution has been defined as the reacquisition by derived populations of the same character states as those of ancestor populations [3]. But, in many natural systems, the character state(s) of the ancestor population is unknown, making reversions under these criteria unidentifiable. Additionally, evolution in reverse has been identified at various biological levels of organization, including phenotypes (structure, function, or behavior) and genotypes (gene deletions and back mutations) both within and among populations (Box 2). By restricting 'reverse evolution' to a process occurring only within populations, many cases might be misidentified as reverse evolution when, in fact, what is being observed is simply shifting

allele frequencies, rather than the reversal of a fixed trait. Encompassing all of these related patterns, reverse evolution is an influential process in evolution, capable of forcing multiple diverged populations and species to converge on similar forms [4,5], overcoming evolutionary constraints that can impede diversification [6–8], and effectively 'pruning' unnecessary structures, functions and behaviors, enabling new evolutionary pathways to be explored [9].

With the identification of new patterns and processes of evolution in reverse, several questions have become major areas for discussion. First, there has been considerable debate over how long a group of organisms must travel an evolutionary path before evolution becomes irreversible. Within the boundaries where evolution is reversible, the questions become more mechanistic: to what degree does evolutionary history constrain reverse evolution?, and what are the genetics behind reverse evolution? [3]. Here, we explore these questions by providing case studies that investigate the probability, potential mechanisms and evolutionary implications of reverse evolution.

Is evolution reversible?

One of the underlying issues of reversibility is whether evolution is actually reversible. In other words, can an organism retrace a previously traversed evolutionary pathway? Although many researchers would not argue

Glossary

Convergent evolution: a process in which changes occur from different ancestral character states to the same descendent character state in independent evolutionary lineages [54].

Epistasis: the nonreciprocal interaction of nonallelic genes, for example where one gene masks the expression of another [53].

Genetic hitchhiking: the spread of a neutral allele through a population because it is closely linked to a beneficial allele and therefore is carried along as the gene that is selected for increases in frequency [53].

Pleiotropy: the phenomenon in which a single gene is responsible for a number of distinct and seemingly unrelated phenotypic effects [53].

Regression: relative to either the ancestral condition or closely related species, characters that are atrophied or degenerate and often without visible function as a result of an evolutionary change in lifestyle. Continued degeneration might lead to character loss. (also termed vestigialization) [55].

Resident genome: genome of a bacterium that lives in close, often intracellular, association with a eukaryotic host [33].

Reverse evolution: the change of a character state to a state similar in appearance to an ancestral state, encompassing patterns associated with both reversion and regression.

Reversion: in reference to fixed character states, when a derived state evolves to a state present in ancestral lineages.

Box 1. Straying from the path: the debate over evolution in reverse

Discussing reverse evolution as an influential evolutionary phenomenon is fraught with problems, the least of which is the debate about whether it actually exists. Furthermore, although the concepts of regression, reversal and loss are not contentious, the idea of these being interrelated processes is. However, we contend that reverse evolution is a phenomenon much like speciation, where different processes can result in a similar pattern of evolution. We therefore unite the processes of reversion, regression and loss under the term of reverse evolution and endeavor to outline their similarities and differences.

For our purposes, we define reverse evolution as a change in character state to one resembling an ancestral state. We recognize that, owing to diverse evolutionary histories, reacquisition of an exact ancestral state is improbable in many cases. However, even the approximation of an ancestral state, although perhaps novel in form, indicates an interrelated set of evolutionary processes. Although many terms have been used to indicate some form of reverse evolution, most of the observed patterns can be encompassed by three types of change: regression, loss and reversion. Regression is related to the ideas of simplification or vestigialization, and includes a gradual reduction in functionality whilst still retaining some form of the original feature. After a reduction in utility is underway, the character can remain in some vestigial form or can be lost completely. The difficulty for many in accepting this as evolution in reverse is that the reduced characters usually only resemble an ancestral state. Furthermore, is the loss of a structure the same state as an ancestral state lacking that structure? Although a structure might be lost, constraints might preserve developmental or genetic pathways that are not present in the ancestor [8,45].

In contrast to the reduction and loss of a trait, characters that evolve to known ancestral states are easier to accept as examples of reverse evolution. Not even this classification is straightforward, however, because reversion to an ancestral state is often accomplished using mechanisms that are not present in ancestral populations [38,39]. Phenotypic similarity might mask mechanistic or developmental differences, making the classification of evolution in reverse dependent on the level of organization being studied. However, although the similarity to ancestral forms can vary from exact features to mere approximations, the novel pathways and forms used to accomplish these similarities are what make studies of evolution in reverse worthwhile. Reverse evolution offers organisms alternative routes for moving around functional constraints and evolutionary dead-ends.

with the potential to retrace pathways over short evolutionary time spans (i.e. several hundred generations) [4,10], the longer the path traveled, the more difficult it becomes to return. Therefore, the longer the time span since diversifying from an ancestral state, the more researchers tend to accept the irreversibility of evolution [3]. Several recent studies focusing on opposite extremes of the reversibility spectrum with respect to time span have addressed this issue and have illustrated that evolution in reverse is achievable, both in short, experimentally controlled studies of populations and over long evolutionary histories encompassing the diversification of large groups of species.

In *Drosophila melanogaster*, the effects of several hundred generations of evolution within a particular environment on fitness-related characters were undone in as little as 20 generations after the ancestral environment was re-imposed [10]. This study provided empirical proof that, over short time spans, evolution can be reversed

Box 2. Experimental systems in reverse evolution

Because reverse evolution as a pattern of evolution has been identified across various levels of biological organization (genotype and phenotype), taxonomic diversity (microorganisms to vertebrates), and evolutionary groups (populations and species), by necessity, different terms and methodologies have been employed. Although these studies deal with similar issues, they are not necessarily directly comparable in terms of the form and function of reverse evolution. For example, owing to the differing reproductive strategies (asexual versus sexual) and genetic architecture, it would not be expected that microbial systems and vertebrate systems exhibit similar mechanisms of regressions and reversals. Also, studies of microbial systems have the unique advantage of having better characterized genomes, and changes at the molecular level can often be quantified directly at the phenotypic level. Perhaps the most important advantage to microbial systems, however, is the ability to control environmental factors and to produce replicates of a system of interest for comparative purposes [24,36,37]. This advantage can also be recognized in other laboratory systems (e.g. *Drosophila*) [4]. Although these tightly controlled experimental systems enable specific genetic interactions with the environment to be investigated and can provide indications of patterns and mechanisms, they might not be directly comparable to more natural systems, where replications under different environmental influences might not exist. In more natural systems, it becomes more crucial to find the appropriate controls for comparisons, for example populations of eyed surface fish versus eyeless cave populations in *Astyanax mexicanus* [19].

for particular phenotypes. However, not all of the characters investigated reverted completely, if at all, demonstrating that phenotypic convergence on an ancestral form is not a universal possibility, even after only a few hundred generations of selection [4,10]. Additionally, even over short time spans, caution must be taken when labeling a feature as having returned to an ancestral condition. It is not always obvious whether the reversal is an actual return to a primitive state, or a *de novo* convergent form approximating the primitive state. In microbial systems, changes in genotype can be directly linked to phenotypic changes and can be observed over time, making an ideal study system for CONVERGENT EVOLUTION. Studies of the reversal of antibiotic resistance have found that, once adapted to the selective regime imposed by a particular antibiotic, microbes are unlikely to return to the ancestral fitness conferred by sensitivity to antibiotics, even after removal of the antibiotic [11,12]. Instead, fitness is recovered by compensatory rather than back mutations of the changes conferring resistance, making the 're-evolved' strain genetically distinct from the ancestor.

Even with the demonstration that evolution is reversible over several hundred generations, the possibility of evolution in reverse over longer evolutionary time periods or evolution of more complex characters would still be rejected by many. Yet, recent phylogenetic investigations of the Phasmatodea (stick insects) indicate that it is possible to 're-evolve' complex structures, as demonstrated by the multiple, independent re-acquisitions of wings in a group where the most recent common ancestor had lost its wings. Furthermore, detailed studies of wing morphology and flight-specific musculature and innervation suggest that the reacquired wings are a re-expression of basic insect wings, rather than of convergent *de novo* forms. The

studies of these insects illustrate that the basic blueprints for complex developmental structures can remain largely intact even over large evolutionary spans (i.e. radiations of higher level taxonomic groups), although the specific mechanisms of loss and/or reacquisition might differ between lineages [8].

The continuum of reversibility

Although evolution in reverse is often described only as a return to an ancestral character state, a much broader spectrum of processes fall into this category, from traits that are completely or partially reversible to a recent ancestral state, to those complex traits termed 'regressive' that have been reduced or even lost. The difference between these trends is the evolutionary distance between the extant organism and the ancestral state that it is returning towards. Because ancestral states are generally unknown entities, the most commonly studied form of evolution in reverse is phenotypic regression. Although many studies have been devoted to characterizing the observed patterns of regressive evolution in nature, investigations have begun, more recently, to look at the underlying genetics of these systems. Here, we discuss several examples of systems that have investigated not only phenotypic, but also the genetic, molecular and developmental mechanisms of evolution in reverse to illustrate the similarities and disparities between different forms of reversibility.

Phenotypic regression

The most frequently studied form of evolution in reverse is phenotypic regression, the vestigialization of morphological, physiological, or behavioral traits. Some of the most commonly cited examples include the structural simplifications of parasites, loss of limbs in snakes, flightlessness in birds and insects, and the loss of photosynthetic ability in parasitic plants. More recent work on sexual selection indicates that losses of elaborate male traits are taxonomically widespread, with several well studied groups (e.g. tanagers and dabbling ducks) containing loss:gain ratios as high as 5:1 for elaborate traits [13–15].

Perhaps the most powerful example, however, is observed in cave-dwelling organisms. The worldwide convergence of form found in the cave environment, exhibited in structural, functional and behavioral regressive changes across diverse taxonomic groups has fascinated and perplexed biologists since Darwin (Figure 1) [5,16]. Termed 'troglomorphy', this suite of changes includes reduction in pigment and eye size, hypertrophy of nonoptic sensory organs and a reduced metabolic rate [9,17]. One of the best studied cases is the teleost *Astyanax mexicanus*, a fish species that includes both eyed surface and eyeless cave-dwelling populations [18–20]. Although adults lack eyes to varying degrees, cavefish embryos develop small optic primordia, which degenerate during ontogeny [20] via a series of steps involving both molecular and developmental mechanisms (Box 3). Furthermore, although several of the major developmental steps involved in cavefish eye degeneration are understood, whether selective or neutral forces underlie these steps is still ambiguous. Studies of the interaction between the expression of the master eye control gene,

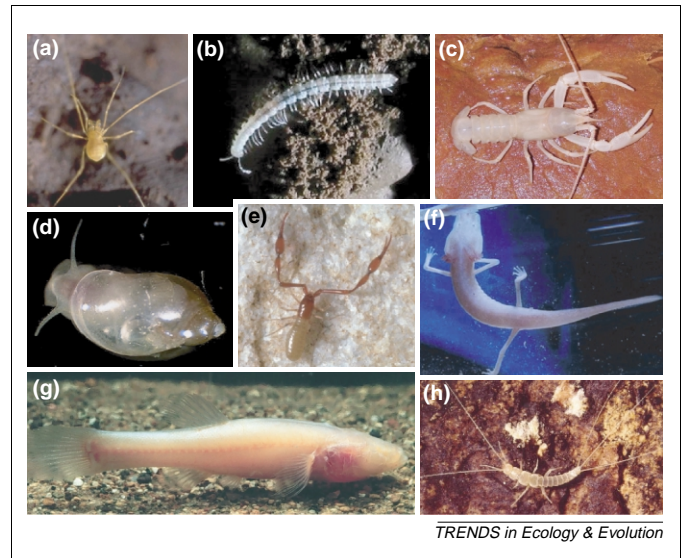


Fig. 1. Examples of convergence across diverse taxonomic groups owing to similar subterranean habitats. (a) harvestman, *Texella reyesi*; (b) millipede, *Speodesmus* n.sp.; (c) crayfish, *Orconectes stygocaneyi*; (d) snail, *Physa spelunca*; (e) pseudoscorpion, *Australinocreaagris grahami*; (f) cave salamander, *Eurycea rathbuni*; (g) cave fish, *Amblyopsis spelaea*; and (h) silverfish, *Texoreddellia texensis*. Reproduced with permission from W.R. Elliott (a,c,e), W.R. Elliott and J. Ivry (h), J. Jasper (b), J. Krejca (f), H.H. Hobbs III (g) and M.L. Porter (d).

Pax6, and midline-signaling genes, such as *sonic hedgehog* (*shh*), imply that the constructive changes in enhanced midline signaling activity might be important factors in controlling *Pax6* expression and therefore cavefish eye degeneration [19,21].

Genomics in reverse

At the molecular level, studies of evolution in reverse have investigated both reversions, in the form of microbial evolution in relation to antibiotic resistance and alternating host environments, and the regression of entire genomes. In microbial evolution, many have questioned whether environmentally induced genetic transformations can return to an ancestral genotype. Investigations of viral adaptation have provided some of the few clear cases of genotypic reversion [22–24]. When the bacteriophage ϕ X174 was switched between *Escherichia coli* and *Salmonella* hosts, original fitness levels were recovered by reversion of two to three substitutions in the major capsid gene controlling host recognition, rather than by compensatory mutations [24]. The natural host range expansion of parvovirus shows the same pattern as the experimentally controlled bacteriophage host alternations. An originally feline parvovirus jumped hosts to canines in the early 1970s as a result of up to five substitutions in the capsid protein [25,26]. After host range expansion, the canine parvovirus regained the ability to infect felines owing to changes in the same region of the capsid gene [27,28].

At the genome level, evolution in reverse takes the form of 'streamlining' or molecular noise suppression, where the transmission of redundant information is diminished by reduction, inactivation, or elimination of unimportant genetic material [29]. The plethora of parasitic and symbiotic intracellular microorganisms and the multiple, independent occurrences of endosymbiotically derived

Box 3. The genetic and developmental mechanisms behind regressive evolution in *Astyanax mexicanus*

One of the best studied systems of phenotypic regression is the Mexican tetra *Astyanax mexicanus*. Because *A. mexicanus* contains both epigeic and at least 29 different populations of cave-adapted forms, studies of drastic morphological changes in isolated populations of the same species are possible [18]. These changes include both constructive changes (i.e. increased complexity of feeding apparatus and the mechanosensory system of cranial neuromasts) and regressive changes (i.e. loss of eyes, pigmentation and aggressive behavior) [19]. Of this suite of characters, particular attention has been paid to eye degeneration in the cave-adapted form of *A. mexicanus*, whose embryos form eye primordia that later degenerate and sink into the orbit [46]. Based on morphological, biochemical and phylogenetic studies, the different cave populations comprise at least two genetically distinct lineages with similar eyeless phenotypes, resulting from independent invasions from surface populations [47]. Additionally, crosses between geographically isolated cavefish populations can produce progeny with a greater degree of eye development than that exhibited by either parent, indicating that different cave populations have evolved different mechanisms of degeneration [19]. Within a single population, studies have shown

that the genetics of regression are multifactorial, with at least three quantitative trait loci mapped to reduced eye size [9].

Developmental studies have shown that both reduced expression of the *Pax6* gene, a crucial controller of eye development, during early eye development and apoptosis of the lens contribute to eye regression. In an elegant study of eye degeneration, *A. mexicanus* lens tissue was reciprocally transplanted between developing surface and cavefish embryos. In cavefish eyes receiving a surface fish lens, eye structure was recovered, whereas in surface fish with a cavefish lens, degeneration was observed [20]. This study illustrates that the lack of an inductive signal emanating from the lens is a major cause of eye regression in cave populations [18,20]. The current understanding of the developmental steps in eye regression are the following: (1) *Pax6* expression is reduced at the anterior midline; (2) potentially as a result of this altered expression, a smaller lens and optic vesicle/cup are formed; (3) cavefish lenses undergo apoptosis instead of differentiation; (4) in the absence of lens signaling, further eye structures fail to develop; and (5) the eye collapses into the orbit and is covered by a flap of skin (Figure 1) [19].

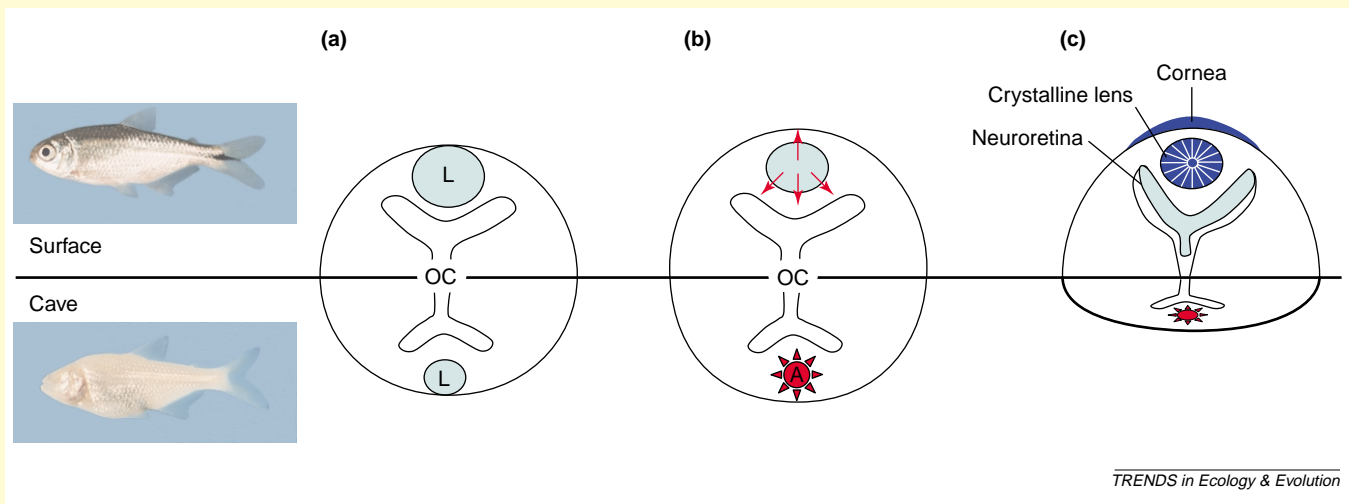


Fig. 1. Eye development in surface versus cave populations of *Astyanax mexicanus*. (a) Smaller lens and optic cup form in cavefish embryos; (b) cavefish lenses undergo apoptosis, whereas surface fish functional lenses exhibit normal signaling; (c) surface fish lens signaling plays a role in inducing development of eye structures, including the cornea, iris, pupil and retinal photoreceptors, whereas in the absence of signaling, cavefish eyes degenerate. Abbreviations: L, lens; OC, optic cup; A, apoptosis; Reproduced with permission from [18] and W.R. Jeffery.

plastids provide molecular equivalents to organismal habitat shifts inducing rudimentation. The establishment of permanent parasitic or symbiotic relationships usually leads to massive gene loss, resulting in the smallest known genomes for cellular organisms [30]. These gene-level deletions occur because of a relaxation of constraints for functions that are no longer needed or that are already performed in the new host environment, host partitioning of population structure and the inaccessibility of foreign DNA as a source of gene acquisition (Box 4) [30–33]. Studies of DNA in ‘nongreen’ (e.g. parasitic) plants show that the plastid genome is being streamlined, exhibiting an extreme reduction in gene content and an increase in the rate of evolution of the remaining genes [34]. In the nonphotosynthetic plant *Epifagus virginiana*, only 17 of the 30 tRNA genes and 21 of the 79 protein genes normally found in angiosperm plastid DNA remain in the genome [35]. In the case of microbial genome shrinkage, massive gene losses become irreversible owing to the

specialized environment and the lack of encounters with foreign DNA [30].

How does evolution back up?

Many mechanisms have been proposed for affecting the reversibility of evolution, including both impeding (lack of genetic variation, small population sizes, EPISTASIS and novel genotype-by-environment interactions) and facilitating (PLEIOTROPY, GENETIC HITCHHIKING and mutation) factors [3,9]. However, the emerging picture of the processes responsible for evolution in reverse is one of contingency. In short-term experimental studies of *Drosophila*, complete reversions are not universal and the incomplete reversions are not due to either lack of genetic variation or epistatic influences, because hybrids exhibit similar patterns of reversibility [10]. Instead, the degree of reversion is highly sensitive to environmental conditions and, at least partly, dependent on previous selective histories (i.e. genotype-by-environment interactions) [4].

Box 4. Aphids and *Buchnera*: studies of genomics in reverse from a mutualistic endosymbiont

One of the best studied systems of regressive genomic reduction comes from the coevolved mutualistic system of aphids and their primary endosymbiont bacteria, *Buchnera*. Evolved from a single bacterial infection of an ancestral aphid 100–250 million years ago [48], the symbiotic relationship between aphids and *Buchnera* is obligate for both species and neither can reproduce without the other [48]. As a result of this long association, *Buchnera* exhibit characteristic features of 'RESIDENT' GENOMES, including severe genome reduction, extreme adenine-thymine bias and fast sequence evolution at all loci [29]. In particular, the regressive genome reduction in *Buchnera* shares similarities with other reductive genomes, including the loss of loci encoding DNA repair, recombinase functions and nonessential amino acid biosynthesis pathways [31,49]. Most of the reduction in genome size (65–74%) occurs soon after the establishment of the symbiosis but before the diversification of the major lineages of extant aphids, suggesting that genome reduction proceeds at an exponentially decreasing pace (Figure 1) [31]. This is also supported by the conserved genetic architecture of *Buchnera*, with no chromosome rearrangements or gene

acquisitions having occurred within at least the past 50–70 million years [50]. However, the reductive process is ongoing as evidenced by lineages with further genome reductions, such as the recent 25-kb deletion of an already greatly reduced *Buchnera* genome from the aphid *Cinara cedri* [29].

Rather than adaptive changes, these major reductions are attributed to degenerate genome evolution via a mutational bias favoring deletions, reflecting a lack of effective selection for gene maintenance [30]. The combination of rampant deletions and lack of selection is exacerbated by a suite of factors associated with the specialized host environment, including genetic isolation, small effective population sizes and the loss of DNA repair mechanisms [30,31]. Genetic isolation and the loss of recombination pathways effectively reduce the probability of gene acquisition to zero, whereas small effective population sizes enable an irreversible accumulation of deleterious mutations owing to drift. In *Buchnera* species, these reductive processes have led to some of the smallest genomes yet described (Figure 1) [29].

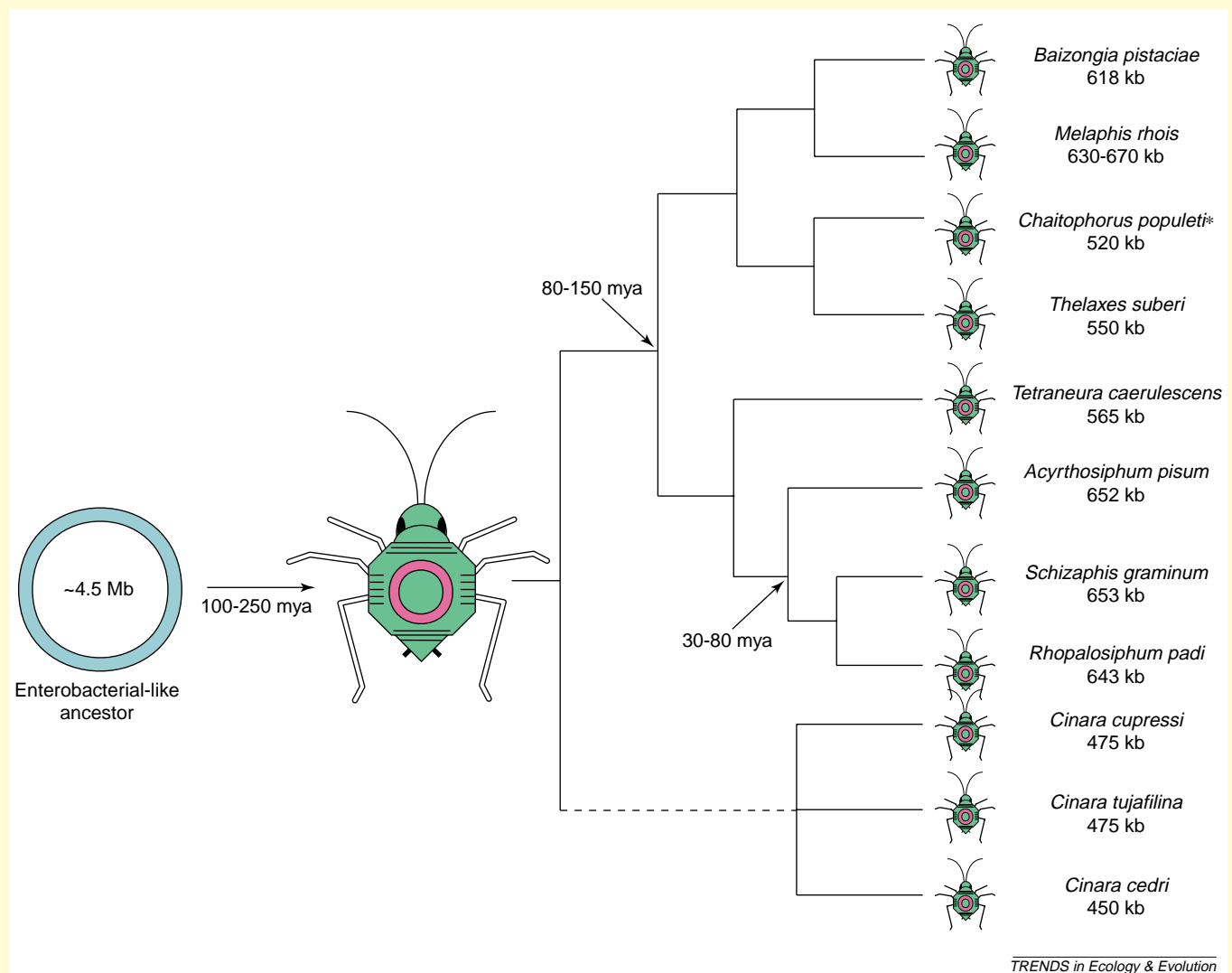


Fig. 1. Evolutionary history of aphids and their endosymbiont bacteria, *Buchnera*. Divergence times are indicated for selected nodes (Data from [31,48,51]). *Buchnera* genome size is given under the name of each host species (Data from [29,31,52]). The phylogeny is based on maximum likelihood analyses of *Buchnera* 16s rDNA sequences presented in [48]. The dashed line indicates positioning on the tree based on other taxa from the same family, and from analyses of aphid sequences. **Chaitophorus populeti* is placed on the tree based on representative species from the same genus.

However, the process of reverse evolution can drive the convergence of multiple populations with different life-history and genetic changes to a common character state [4], similar to the widespread convergence of form observed in troglomorphic taxa.

In subterranean-adapted organisms, developmental and linkage studies point to pleiotropy as a significant process involved in reverse evolution [9,21]. In quantitative trait loci (QTL) studies of the cave-dwelling *A. mexicanus*, close linkages were found between a regressive and a constructive trait in two cases. These close linkages suggest that the regressive evolution of pigmentation and eye loss might be influenced by either pleiotropy or genetic hitchhiking [9]. Concordantly, the potential interaction of the *shh* gene with *Pax6* also indicates pleiotropy as an important mechanism [19,21].

In all of these examples, the most important factor is the interaction of traits and their underlying genetic backgrounds with the environment: in the *Drosophila* studies, returning to an ancestral environment after the evolution of divergent genetic backgrounds led to the differential reversibility of traits; in studies of *A. mexicanus*, selection pressure leading to adaptations to the subterranean environment (e.g. enhanced midline signaling) appear to interfere with the expression of genes involved in the development of the eye. In a particularly elegant study of the costs of horn production in *Onthophagus* beetles, Emlen [6] demonstrated that there is a tradeoff between the development of exaggerated horns and the reduction of neighboring morphological structures (antennae, eyes, or wings). Because the *Onthophagus* beetles inhabit different physical environments, the position of the exaggerated horns in each species is correlated with the reduced structure that imposes the smallest cost.

By contrast, studies of asexual lineage evolution, where there are more direct correlations between genotypic and phenotypic changes, seem to be more influenced by epistatic interactions [36,37]. Studies of microbial antibiotic resistance lead to a return towards ancestral phenotypic characters through epistatic compensatory mutations rather than via back mutations [38,39]. These epistatic intermediate-fitness compensatory mutations are more common than are higher fitness revertants, owing to the higher rate of compensatory mutations and the characteristic population bottleneck dynamics of parasitic and symbiotic microbes [11].

The future of reversibility

The studies highlighted here illustrate the importance of reverse evolution as an influential evolutionary phenomenon. Ignoring patterns of reversibility might have important consequences for evolutionary analyses, particularly in phylogenetic reconstructions. Studies of dabbling ducks and orioles caution against using these types of character, particularly with parsimony methods that minimize homoplasy, in constructing phylogenies in general and against equally weighted gain:loss ratios in parsimony-based ancestral state reconstructions [14,40]. Acknowledging the reality of evolution in reverse, however, necessitates incorporating the often confusing terminology encompassing reverse phenomena into a more comprehensive

framework for investigating this process. Further detailed genetic studies of a wide array of examples of reverse evolution are therefore needed to explore the interaction of ancestral evolutionary distance (time span) and reversibility. The next step in many studies of evolution in reverse is to identify the underlying genetic changes accompanying observed phenotypic reversions and/or regressions. Once the crucial genetic transformations in regressed systems have been identified, these candidate loci can be used for detecting the operational selective forces [41–43]. In studies of reversion, the direct comparison of ancestral and derived experimental populations is impossible in most natural settings [4,10]. Additional natural systems could be investigated using molecular methods, such as network approaches, which have been developed to deal specifically with intraspecific data, providing insights into the patterns involved in population-level evolutionary processes [44].

Furthermore, with genotype-by-environment interactions identified as one of the crucial factors affecting trait reversibility, several studies are already devoted to investigating the importance of the genetic architecture component of this interaction. But this interaction also needs to be approached from the opposite perspective, investigating the environmental effect in reverse evolution. By researching the occurrence of reversibility across a wide range of ecological types, the similarities and disparities across systems in terms of the effects of factors such as epistasis, pleiotropy, genetic variation and compensatory mutations can be investigated. Similarly, additional experimental studies of the effects of adaptation to varied ecological regimes on the reversion of similar genotypes are warranted.

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