Relaxed selection in the wild

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Natural populations often experience the weakening or removal of a source of selection that had been important in the maintenance of one or more traits. Here we refer to these situations as 'relaxed selection,' and review recent studies that explore the effects of such changes on traits in their ecological contexts. In a few systems, such as the loss of armor in stickleback, the genetic, developmental and ecological bases of trait evolution are being discovered. These results yield insights into whether and how fast a trait is reduced or lost under relaxed selection. We provide a prospectus and a framework for understanding relaxed selection and trait loss in natural populations. We also examine its implications for applied issues, such as antibiotic resistance and the success of invasive species.

What is relaxed selection?

Environmental change often eliminates or weakens a source of selection that was formerly important for the maintenance of a particular trait. Such circumstances are often termed 'relaxed selection' (Box 1). Thus, eyesight is said to be under relaxed selection in lightless caves; a habitat free of herbivores or predators relaxes selection on previously evolved defenses against them; and the disappearance of toxins or pathogens results in relaxed selection for resistance. Any form of selection can be relaxed in this sense, and this change alters the fitness surface acting on the trait in one of various ways (Box 2).

Relaxing selection from one source leaves other sources to operate on the trait, so these cases are a subset of the ubiquitous occurrence of changed selection. Cases of relaxed selection are of particular interest because removing a previously important source of selection can increase the probability of a trait losing its contribution to the fitness of the organism and, consequently, lead to an increased probability of trait loss [1] (Figure 1). The question of what affects such probabilities requires a focused empirical and theoretical study of relaxed selection in its own right. The reason for this is that the consequences of such situations for the expression and evolution of a trait

are often less clear than typical cases of trait evolution. When an environmental change results in strong selection on a trait from a known source, a prediction for trait evolution often follows directly from this fact [2]. When such a strong source of selection is removed, however, no clear prediction emerges. Rather, the likelihood of various consequences can only be understood by integrating the remaining sources of selection and processes at all levels of trait control and expression. Moreover, the answers often have important implications, especially in cases that relate to problems in applied biology (Box 3).

Recent studies of natural systems has revealed the consequences of relaxed selection on particular traits in an ecological context. Several of these studies have provided insights into whether and how a trait is reduced or lost when an important source of selection is removed (Table 1). Here we integrate these insights into a general framework for understanding how an environmental change can relax a source of selection on a trait, and what happens to such a trait as a result. We summarize the factors that influence traits following the removal or weakening of a source of selection, and outline possible consequences for trait expression and evolution.

A spotty history

Until recently, evolutionary biologists devoted little direct attention to relaxed selection and the loss of adaptive value of a trait. Darwin observed that many traits persisted that had apparently lost their usefulness, and recognized that selection for energy economy would predict reduction of such traits over time. He also cautioned that traits could have multiple functions or could switch functions, and that these could maintain traits even if one function had been rendered obsolete [3]. George Romanes, Darwin's contemporary, argued that the decay of unused traits was best seen as resulting from evolution under either selective neutrality ('panmixia' or 'cessation of selection') or direct selection against the trait [4].

The 20th century saw the alternatives to direct selection crystallize into mutation accumulation and antagonistic pleiotropy [1,5–7], but few further advancements were made in theory or mechanism. The major works of the

architects of the new synthesis devoted little space to what they called 'degenerative' or 'regressive' evolution. This lack of consideration of relaxed selection and functional disintegration relative to positive selection and adaptation is unsurprising, given the traditional emphasis on progression and advancement in the history of biology [8]. Sparse empirical study on relaxed selection might also be due to the challenging practical demands involved in determining causes and mechanisms of evolution. Only recently have empirical studies been able to identify those mechanisms in actual cases.

Influences on a trait under relaxed selection
Direct and indirect fitness effects and neutral factors can be integrated into a general framework of influences on traits when a source of selection has been relaxed.

Direct fitness benefits and costs
The net effect of selection on a trait can be considered as a resultant vector that is the combination of the vectors of all individual agents of selection acting on that trait, with their different magnitudes and directions. Weakening or removing one source of selection on a trait essentially subtracts, or reduces the magnitude of, one vector among several. A main challenge is to determine the new resultant vector of selection with regard to the trait in question. Following environmental change, the remaining and new sources of selection can interact with the trait in various ways. Box 2 considers several unidimensional scenarios under initial stabilizing selection. More generally, the main possibilities with respect to selection are that current trait values provide net fitness benefits via an existing function or a new function, or that they incur net fitness costs.

Traits that are maintained by several sources of selection might be expected to retain their advantage when one source of selection is removed. Thus, the fitness function of such general-purpose traits following putative relaxed selection from one source might be similar to that of the
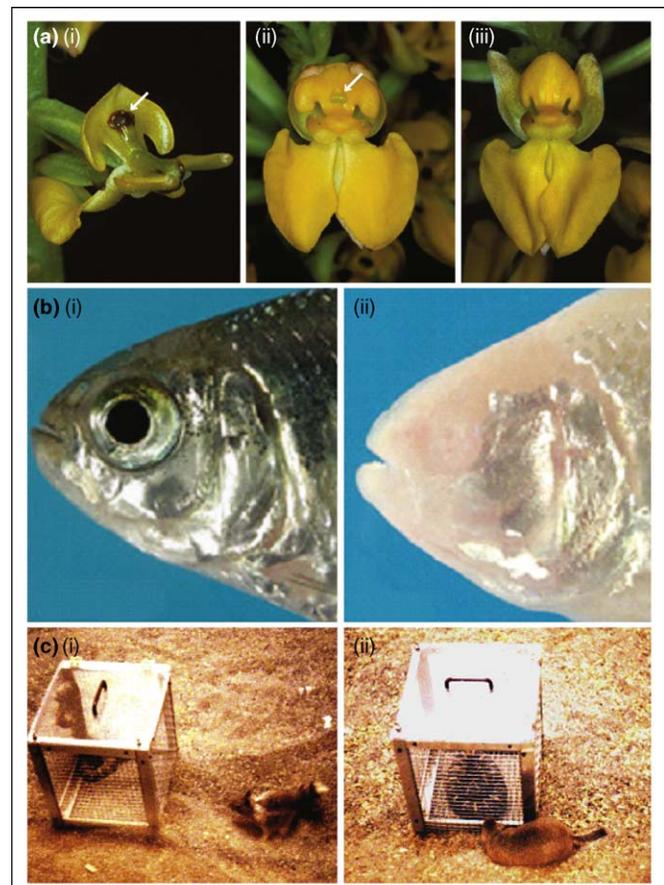
Figure 1. Examples of trait loss following relaxed selection in the wild. **(a)** The orchid *Ceratandra atrata* (i) is pollinated by oil-collecting bees, and has a brown oil-producing callus in the center of the flower (arrow). A more-derived species, *Ceratandra grandiflora*, is pollinated exclusively by beetles that do not collect oil, resulting in relaxed selection on oil production. In this species, flowers have either a vestigial oil gland (ii, arrow) or none at all (iii) [47]. **(b)** The teleost *Astyanax mexicanus* has diverged into a surface form (i) and a cave form (ii); the latter has lost both pigmentation and its eyes [23]. **(c)** A California ground squirrel *Spermophilus beecheyi* (i) from the Lake Tahoe basin, where predatory snakes are absent, harasses a rattlesnake by throwing sand at it. Squirrels from this area recognize snake predators despite experiencing relaxed selection for an estimated 70 000 years, which has resulted in nearly complete loss of physiological resistance to rattlesnake venom. By contrast, the Arctic ground squirrel *Spermophilus parryii* (ii), under relaxed selection from snakes for an estimated 3 000 000 years, exhibits a relaxed posture while inspecting the rattlesnake. This species shows no recognition of snakes as predators, and has no resistance to rattlesnake venom [22]. Reproduced, with permission, from K.E. Steiner (a), W.R. Jeffery (b) and R.G. Coss (c).

ancestral condition, or else somewhat weaker but with the same optimum [e.g. Box 2, case (c)]. This hypothesis has been proposed to explain the persistence of antipredator behaviors in animals with a history of multiple predators [9]. In another example, plants are often said to be under relaxed selection for defenses when introduced to regions without their native herbivores, but, in some cases, the defenses are so general (e.g. tannins) that they also function against new herbivores [10]. Some traits are graded or parceled in their expression, such as pollen production, vigilance and muscle mass. These traits might also continue to convey a fitness benefit when one of several sources of selection maintaining them are removed, but they might face selection for decreased size or intensity of expression [e.g. Box 2, case (d)] [11]. Functions that are novel or that were previously secondary can also maintain a trait after one function has been lost. To the extent that the new

Box 2. Ways in which an environmental change can relax a source of selection on a trait

Selection of any form (balancing, directional, etc.) can be relaxed. For illustrative purposes, we consider here a quantitative trait held by stabilizing selection at a particular value (e.g. of size or intensity of expression). A hypothetical environmental change could relax selection in one of several ways. In the most extreme case, selection could be removed completely, resulting in a flattening of the fitness landscape influencing the trait (Figure Ia). Alternatively, and perhaps more likely in a natural context, trait changes in one direction could remain deleterious (e.g. gain-of-function mutations), whereas changes in the other direction become neutral (Figure Ib) [75]. For example, predator escape behaviors might no longer be elicited in a predator-free environment and so be neutral, but increasing the probability of expressing these behaviors in the absence of predators would incur an energetic cost.

Another form of relaxed selection is continued but weaker stabilizing selection, resulting in a broadening of the fitness peak without a change in trait mean (Figure Ic). This could occur in polluted waters where turbidity compromises female assessment of male courtship displays in fish [76], or else if correlations with other functional traits cause current trait values to be maintained. Alternatively, the selective optimum of a trait might decrease owing to remaining sources of selection, including selection on correlated traits (Figure Id). An example might be relaxed selection for sexual reproduction in apomictic plants that still require pollen for endosperm fertilization. The removal of one of the two functions of pollen results in a shift of optimal pollen production to a lower level [11].

Combinations of the above phenomena (Figure Ib–d) are possible and are probably common. One such combination is illustrated in Figure Ie, which involves regions of neutrality in trait space (as in Figure Ib), lower fitness differences among trait values (as in Figure Ic) and a skewed weakening of selection resulting in a shifted optimum (as in Figure Id). Cavefish eyes might fit this pattern: they appear to be in a tradeoff with other sensory systems (e.g. taste), causing a reduction in the optimal expression of eyes in a lightless environment [23]. This selection is probably weaker than that on eyes of surface fish; and continued early development of cavefish eyes suggests that, below some threshold of expression, eye development is close to neutral.
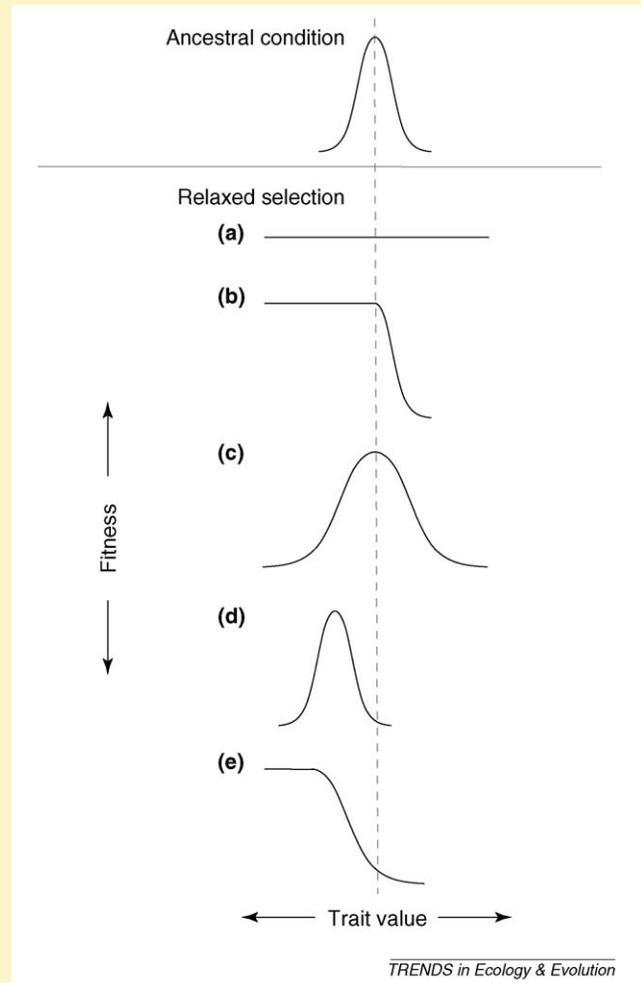
Figure I. Forms of relaxed selection on a quantitative trait under stabilizing selection.

primary function differs from the old, the trait is likely to undergo selection for modifications, as in birds whose wings are no longer needed for flying but have been co-opted for swimming (e.g. penguins) or paddling (e.g. steamer-ducks *Tachyeres* spp.) [1,12].

Selection against a trait can be considered in terms of fitness costs incurred in the maintenance or expression of the trait. Some costs are automatically incurred in the act of maintaining or expressing the trait ('constitutive' costs). By contrast, other costs are 'contingent,' or arise owing to particular features of the environment where the traits are expressed. These categories might not always be distinguishable, and the nature of a cost can evolve; nevertheless, they are instructive for identifying broad-scale patterns. In terms of Box 2, a trait incurs a cost wherever the fitness curve is not maximized [i.e. cases (d) and (e) at the vertical dotted line]. Any cost contributing to that reduction in fitness is constitutive if its effect on the curve is the same for all environments, and is contingent if it is environment dependent.

Constitutive costs, because they are automatically incurred, were present even in the ancestral environment where a trait was functional, but were overridden in that environment by the fitness benefits of the trait. In the absence of such benefits, selection is expected to act on those costs and dismantle the trait. The most general constitutive cost is the energetic demand of the trait, dubbed the 'Weismann principle' [13]. Plant defensive compounds are likely to incur such a cost, as evidenced by findings that a decay in the compound under relaxed selection is correlated with a shift of resource allocation to growth or fecundity [14]. Constitutive costs have been particularly well pinpointed in recent studies of tolerance and resistance traits in a variety of organisms. For example, in the absence of insecticide, resistance in *Culex* mosquitoes is often disadvantageous relative to the non-resistant wild type [15]. One mechanism by which resistance is achieved is through the production of modified acetylcholinesterase by the *Ace.I* gene. Although the modifications render this enzyme insensitive to insecticide, they also hamper its proper functioning in the central nervous system. These and other costs result in fitness-related changes, such as a lower adult size, lower overwintering survival and higher predation [15]. Because constitutive costs are borne even where the traits are useful, a plausible expectation is that, within some evolutionary window,

Box 3. Applications of the study of relaxed selection

Applying evolution to medical, agricultural, conservation and social issues often involves relaxed selection and its consequences for trait evolution. Here we list the major current issues, which fall into two categories according to whether relaxed selection is the solution or the problem:

(i) Humans have imposed selection and now seek to relax it to promote:

- Loss of resistance to antibiotics in pathogens [77].
- Loss of resistance to pesticides in insects and plants [16].
- Loss of adaptations to harvesting practices in fish and other wild-caught animals (e.g. young age and small size at maturation) [78].

(ii) Humans have relaxed selection and now seek to avert, reverse or cope with:

- Loss of antipredator defenses in animals in conservation-oriented captive breeding programs (such animals can suffer higher mortality upon reintroduction to the wild) [79].
- Loss of migratory or other movement patterns in animals, owing to restrictions on movement (e.g. dams) or practices that remove its benefit (e.g. food supplementation) [80].
- Spread and competitive superiority of invasive species, owing to release from ancestral sources of selection resulting in either immediate (plastic) or evolutionary responses in the new environment [81].
- Loss of biodiversity, owing to human interference with diversifying or sexual selection (e.g. compromising species recognition and mate choice) [76].
- Accumulation of harmful mutations in humans, owing to our insulation from many sources of selection that would have acted in our past [82].

older traits incur lower costs because compensatory mutations are more likely to have arisen. For example, in a New England population of the potato beetle *Leptinotarsa decemlineata*, one of several costs of resistance declined between 1999 and 2005, apparently owing to compensatory genetic changes [16].

Contingent costs, by contrast, arise owing to particular environmental features and are not incurred in all environments. In particular, costs could arise from the environmental change causing relaxed selection. For instance, natural selection for efficient locomotion probably selected against legs when the ancestors of whales increased the time that they spent in oceanic environments, and when the progenitors of snakes began to burrow [17]. Likewise, some subterranean rodents are thought to face increased risk of eye damage relative to their surface relatives [18]. In the threespine stickleback *Gasterosteus aculeatus*, reduced fish predation in lakes relative to the ancestral ocean environment is thought to relax selection on lateral armor plates. These plates incur a contingent cost as fish grow more slowly when developing plates in fresh water but not in salt water [19], possibly due to reduced ion availability for plate construction in the former [20].

Trait correlations: indirect benefits and costs
The influences of correlations among traits can be integrated into this cost–benefit framework. Whether a correlated trait leads to a cost or a benefit will depend on the polarity (positive or negative) of the correlation, and the fitness function of the other trait in the new environment. In general, positive correlations with functional traits, for

instance through shared genetic and developmental pathways, result in an indirect selective benefit to current trait values [7]. We call this phenomenon 'buttressing pleiotropy,' in the sense that the function of the correlated trait is buttressing or maintaining values of the focal trait. This effect has not been demonstrated directly in a natural population, but could be likely for behavioral traits, in that the neural and endocrinological bases of perception and cognition are often shared among many traits (mating, predator avoidance, resource acquisition, etc.) [21,22].

By contrast, positive correlations with other traits that are also under relaxed selection act as costs. This situation is likely to occur when a suite of traits shares both a functional and a genetic or developmental basis. In *Astyanax* cavefish, for instance, the eyes as well as the cranial cavities surrounding them are controlled by lens regulatory genes, and both are under relaxed selection in dark environments; therefore, any cost to eyes in caves will be pleiotropically extended to the cranial orbits, and vice versa [23].

Negative correlations with functional traits, which include all the tradeoffs described so far, can also be interpreted as costs. In fact, any cost of a trait can be interpreted in this way, if we include a negative correlation with all traits, or with fitness itself. Particularly striking are examples where the tradeoffs are based on cases of antagonistic pleiotropy that could not have been predicted without knowledge of the genetic bases of the traits. For instance, selection for early fecundity in *Drosophila melanogaster* causes a correlated reduction in starvation and desiccation resistance, so when such resistance is no longer favored (as under laboratory conditions), the tradeoff between early fecundity and stress resistance drives a decline in resistance [24]. In *Astyanax*, enhanced *sonic hedgehog* (*shh*) signaling promotes eye degeneration by inducing lens apoptosis but also increases the number of taste buds [23]. Thus, if selection favors more taste buds for foraging efficiency in the cave environment, antagonistic pleiotropy between sight and taste might promote loss of eyes in cavefish.

Neutral factors
The accumulation of neutral mutations was long considered a major factor in the decay of nonfunctional traits. For instance, Julian Huxley described the process of trait loss as follows: 'harmful mutations are not weeded out; they accumulate, and genetic degeneration sets in' [25]. Classically, neutral trait loss has been expected to result from recurrent mutation together with genetic drift; this would result in a flat fitness surface with respect to the trait, at least in the direction of trait reduction [Box 2, cases (a) and (b)]. Laboratory experiments of mutation accumulation have provided estimates of trait decay rates and have recently begun to elucidate trait-specific and species-specific mutational biases [26]; however, little information is available from natural populations. Two recent studies were the first to document mutation accumulation rates in the field: one on a radish, *Raphanus raphanistrum* [27], and the other on trembling aspen *Populus tremuloides* [28]. Both studies tested for an effect of mutation accumulation on sexual function. In the radish, variance in sexual

Table 1. Recent studies of relaxed selection leading to trait reduction or loss in the wild

| Organism	Cause of relaxed selection	Resulting trait evolution	Insight	Refs
Plants				
Aleppo pine *Pinus halepensis*; Black spruce *Picea mariana*	Reduced herbivory by squirrels	Decline in cone defenses against squirrels but increase in defenses against crossbills	Trait decay facilitates coevolution with crossbills; comparable evolutionary changes in three different populations	[59,60]
White campion *Silene latifolia*	Escape from herbivores and parasites	Decline in defenses but increase in growth and reproduction	Compensatory evolution provides evidence for an energetic tradeoff	[14]
Swamp loosestrife *Decodon verticillatus*	Cold environment at range margin	Loss of sexual reproduction	Several independent losses; common developmental mechanism: pollen tube growth failure, although timing varies across populations	[57,61]
Arabidopsis thaliana	Range spread into warmer areas	Decline in freezing tolerance	Genetic basis: multiple mutations in both regulatory and coding regions in a cold-acclimation gene family	[51]
Orchid *Ceratandra* spp.	Pollinator shift from bee to beetle	Partial to full loss of floral reward (oil) and its secretory gland	Three species in the genus at three different points on the trajectory of trait loss	[47]
Invertebrates				
Oligochaete *Limnodrilus hoffmeisteri*	Removal of cadmium pollution	Loss of cadmium resistance	Evolutionary mechanism includes gene flow from neighboring nonresistant populations	[30]
Copepod *Eurytemora affinis*	Invasion of freshwater from saline environment	Loss of high salinity tolerance, increased low salinity tolerance	Tradeoff between adaptations to two environments; correlated evolution of developmental timing	[62]
Damselflies *Enallagma* spp.	Escape from predatory fish	Loss of recognition of fish as predators	Ten species tested; trait loss concordant with speciation in at least two events	[63]
Noctuid moths	Escape from predatory bats	Various levels of decline in auditory sensitivity	Neurological basis: partial degeneration of A2 sensory cell	[64]
Waterflea *Daphnia magna*	Decrease in predatory fish	Loss of negative phototaxis in presence of fish odor	Loss of a plastic behavioral trait over a 10 year period	[65]
Bushcricket *Poecilimon intermedius*	Asexuality, lack of acoustic signaling	Decrease in hearing organ size and receptor cell number	Partial loss, with some persistence of hearing, perhaps for predator avoidance	[33]
Parasitoid wasp *Leptopilina clavipes*	*Wolbachia* infection causing parthenogenesis	Decline in male fertility	Genetic basis: 47% of variation attributable to a single quantitative trait locus	[52]
Vertebrates				
Notothenioid icefishes	Cold, oxygen-rich water	Loss of hemoglobin and red blood cells, partial loss of myoglobin	Genetic basis in globin loci; compensatory cellular and circulatory system evolution	[66]
Cave tetra *Astyanax mexicanus*	Lightless habitat	Loss of eyes, but maintenance of retinal circadian rhythms	Developmental genetic basis: lens genes have a major regulatory role	[23,67]
Threespine stickleback *Gasterosteus aculeatus*	Escape from fish predators	Loss of lateral and sometimes pelvic bony armor	Multiple events enable tests of parallelism and predictions of trait evolution	[19,50]
Medium ground finch *Geospiza fortis*	Supplementation of food by humans	Increase in variation and loss of bimodality in beak size	Relaxed selection on a trait correlated with reproductive isolation hinders diversification	[68]
Village weaverbird *Ploceus cucullatus*	Escape from brood parasitic cuckoos	Decline in egg color variation and precision, persistence of recognition	Egg color decays before recognition does (behavioral trait persists longer than does morphological trait)	[42,69]
California ground squirrel *Spermophilus beecheyi*	Escape from predatory snakes	Persistence and loss of antisnake behavior, loss of rattlesnake venom resistance	Venom resistance decays before antipredator behavior does	[22]
Tammar wallaby *Macropus eugenii*	Escape from predators	Loss of group size dependence in vigilance, weakening of recognition [a]	Presence of even a single predator can maintain antipredator behavior	[9]

function increased and the mean decreased nonsignificantly over nine generations. In the aspen, clones of different ages did not differ in the variance of sexual function, but they did differ in the mean to an extent that would translate into a decline in sexual fitness of 0.0001–0.001 per year, as determined by counts of viable pollen.

Neutral evolutionary mechanisms could contribute to the decay of nonfunctional traits even if they are not the sole explanation. Founder effects have been suggested to aid trait loss by amplifying drift [29], especially if the ancestral population exhibits a range of trait values. Gene flow can also accelerate trait loss in a population. In

Limnodrilus oligochaete worms, for instance, cadmium resistance declined precipitously within 9–18 generations of the cleanup of pollution at the study site [30,31]. The cleanup also reduced the size of the worm population, and substantial immigration of nonresistant genotypes from outside the cleanup area is likely. In this system, gene flow might have operated alongside selection.

Consequences for trait expression and evolution

The nature of the influences on a trait following the relaxation of a source of selection determines whether the trait will persist, be reduced to a vestige or be lost. In
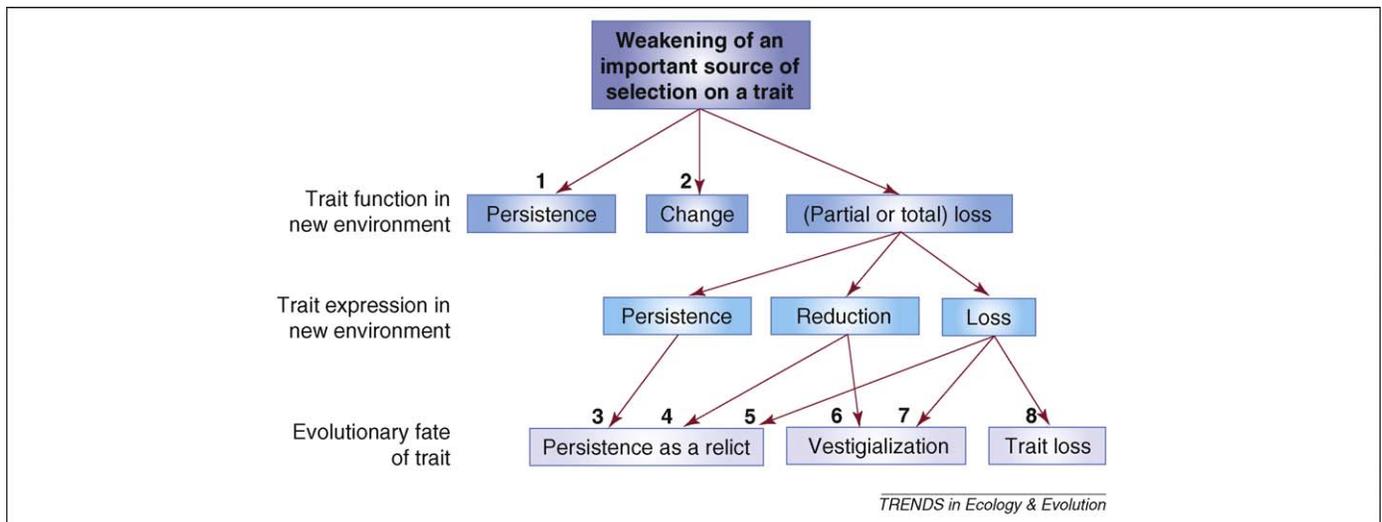
Figure 2. Consequences of relaxing a source of selection on a trait. When an environmental change weakens an important source of selection, a trait is likely to follow one of eight pathways with regard to its function, its level of expression and its evolution over a given period of time. Although these pathways could follow any environmental change, the weakening of an important source of selection is distinctive in that it increases the likelihood that the trait loses its function or adaptive value. A central question in the study of relaxed selection is which path a trait will take and why. These alternatives are presented as mutually exclusive alternatives for clarity, but are in fact on a continuum, and the outcome can change over time.

addition, trait loss can take multiple forms, from genetic changes to plastic loss that could be reversed if the selective agent were reinstated (Figure 2). These alternatives are on a continuum, and the outcomes can often change with time. For instance, a trait might persist for a while before being reduced, a vestige might decay slowly until it is lost, or a trait at any point on this continuum might gain a new function. Recent empirical studies in natural populations have provided probable examples of all eight of the pathways illustrated in Figure 2.

Trait persistence or functional modification
Traits can persist and continue to be expressed for long periods of time after a source of selection is removed (Figure 2, paths 1–3). In terms of the influences described above, persistence is expected when the benefits of a trait outweigh the costs. The simplest explanation for trait persistence is maintenance by remaining sources of selection for the same or a different function [32]. Thus, asexual *Poecilimon* bushcrickets no longer need to hear to find mates, but their hearing ability has persisted; it might be that they still use hearing to avoid predatory bats [33] (Figure 2, path 1). In flightless *Tachyeres* steamer-ducks, wings no longer function in flight, but have been modified for their new function of oaring on the water surface [12] (Figure 2, path 2).

Traits that persist intact despite having apparently lost any ecological role provide some of the most perplexing cases in the study of relaxed selection, although putative cases should be treated with caution. One problem is that new and previously secondary functions are not always easily identified (i.e. distinguishing path 3 from 1 or 2 in Figure 2 is particularly difficult). One way to test for either cryptic functionality or buttressing pleiotropy is to look for evidence of selection on genes of major effect that underlie the trait. For instance, genes underlying photosynthesis in the parasitic plant *Cuscuta* appear to be under strong purifying selection [34]. By contrast, increased variance in the size of a structural trait, and particularly in its

fluctuating asymmetry, can suggest a lack of function. Evidence for this trend comes from known vestigial features, such as unused wings [35] and rudimentary digits [36]. One candidate for a relict is the feeding morphology, in particular the beating cilia and oral groove, of several species of nonfeeding annelid larvae, despite the loss of the remainder of their digestive systems [37]. Another possibility is that compensatory mutations have reduced the costs of antibiotic (chloroquine) resistance in some populations of *Plasmodium* malaria parasites to such an extent that resistance has and will continue to persist following suspension of chloroquine use [38].

Besides positive correlations with functional traits, nonfunctional traits might persist because they lack standing genetic variation, incur little cost or experience gene flow from populations where the traits retain a function. These circumstances would increase the amount of evolutionary time required for a trait to decay ('evolutionary lag'), although no cases provide definitive evidence for these mechanisms of trait persistence in nature.

Plastic loss of expression
Changes that occur during the development of an organism can act as an alternative to evolutionary trait reduction (Figure 2, paths 4 and 5), or interact with it (Figure 2, paths 6 and 7). In other words, a single genotype can result in trait expression in some environments and not in others, often in accordance with the usefulness of the trait. In some cases, expression can be lost or reduced because environmental changes have eliminated cues or learning opportunities important for the development of a trait. Thus, laboratory rearing of *Poecilia reticulata* guppies from habitats differing in predation risk has shown that population differences in some antipredator behaviors are largely the result of plasticity [39]. In another example, moose (*Alces alces*) reared in habitats where predators have been extirpated are less vigilant, even if they encounter predators as adults. However, vigilance rapidly intensifies in mothers who lose calves to recolonizing wolves

[40], revealing the plastic nature of the reduced behavioral expression (Figure 2, path 4). Plastic changes are not always reversible as in these cases; moreover, they can involve a complete loss of expression rather than just a reduction. For example, environmentally induced changes in gene regulation pathways can inhibit the expression of unused traits through a permanent developmental change, as in wingless castes of ants [41] (Figure 2, path 5).

Combinations of evolutionary and plastic changes are particularly likely in cases where traits are organized into functional suites, or when behavior is involved. For instance, village weaverbirds (*Ploceus cucullatus*) introduced to islands without their native cuckoo brood parasite lost some portion of their ability to recognize and reject alien eggs, but this was not owing to evolution of a behavioral or perceptual system. Rather, their recognition system remained intact, but they lost the opportunity to use it because the distinctiveness and consistency of their egg colors decayed through evolutionary change [42] (Figure 2, path 6). In another example, the cave form of the molly *Poecilia mexicana* lives in complete darkness, and its eyes have been reduced through evolution; however, cave mollies have been shown to see and even to exhibit visual mate choice in a laboratory setting [43]. In many instances when the expression of a trait has been lost, particularly in behavior, the data are not available to determine whether the change has involved plasticity, evolution or both.

An important feature of plastic trait reduction or loss is that unexpressed traits will not be subject to direct selection except for costs associated with maintenance of the latent trait or its plasticity. Consequently, such traits can persist for a longer period of time relative to traits without such plasticity [44]. Nonfunctional behavioral traits might then be expected to persist longer than morphological traits, to the extent that the former are more plastic. The hypothesis proposed above, that behavioral traits exhibit more buttressing pleiotropy, also leads to this prediction. Research on suites of traits involving both morphology and behavior meet this expectation in stickleback [44], phrynosomatid lizards [45], village weaverbirds [42] and *Spermophilus* ground squirrels [22]. Thus, traits under relaxed selection might be an important exception to the general rule [46] that behavior tends to evolve before morphology.

Trait loss or vestigialization

When relaxed selection results in a loss of function, the most general expectation is that the trait bears some cost, and is also subject to neutral evolutionary processes, so it will decay eventually. Evolutionary losses of traits following the removal of a source of selection occur by various mechanisms, are highly variable in evolutionary rate and can be either partial (leading to a vestige; Figure 2, paths 6 and 7) or complete (Figure 2, path 8). Figure 1 and Table 1 illustrate cases of relaxed selection in natural populations where traits have decayed partially or fully. In some systems, certain populations or species have experienced partial trait loss or vestigialization and others more complete loss of the same trait. Among the more striking examples are *Ceratandra* orchids, where a range of forms in a clade lie on a trajectory of loss of the oil gland that once

functioned in rewarding bee pollinators [47] (Figure 1a), and stickleback, where lacustrine populations vary in the extent to which armor has been lost [48].

Whether and how quickly a nonfunctional trait decays depends partly on the mechanisms responsible. The fitness functions in Box 2 provide a basis for predicting how quickly a trait should decay. Decay should be slowest or even nonexistent when positive correlations with functional traits result in continued maintenance of the trait [case (c)], although any costs of the trait would select for a decoupling of the correlation, resulting in a change in the fitness function. In cases where a reduction in the trait value is selectively neutral [cases (a) and (b)], neutral evolutionary mechanisms will predominate, and trait decay should be gradual and slow. Decay should be rapid when current trait values bear a significant cost, including antagonistic pleiotropy [cases (d) and (e)]. Cases of vestigialization or partial loss might result from decay of a trait up to the point where the cost of further decay begins to exceed the costs of maintaining or expressing the trait. Testing these predictions with data from natural populations is currently precluded by an insufficient quantification of costs, and often of the time frame over which relaxed selection has occurred.

The rapidity and extent of trait loss is also affected by the particular mutations that have arisen, and how they affect trait development. Perhaps the most significant variable is the extent to which mutations in structural versus regulatory genes are responsible for the trait loss. Trait loss in both cave tetra and stickleback involve several major changes, and the genetics of both broadly adhere to expectations of a few genes of large effect and many genes of small effect, with widespread pleiotropy [49,50]. Likewise, decay of freezing tolerance in *Arabidopsis* has been caused by several mutations involving both regulatory and coding regions in a related group of genes [51]. Several other cases involve relatively simple genetic changes that demonstrate how rapid and episodic trait loss can sometimes be. When males of the parasitoid *Leptopilina clavipes* are cured of their sterility-inducing *Wolbachia* infection, they differ widely in their residual fertility, and a single quantitative trait locus explains nearly half of this variation [52]. The loss of a wing spot in wild *Drosophila* species was shown to be attributable to regulatory mutations on a single gene, involving only two to seven nucleotide substitutions [53].

Vestigial traits are often topics of great curiosity and lead to speculation as to why trait loss did not proceed to completion. For instance, rival hypotheses for the persistence of the human appendix are that it contributes to the immune system [54], and that further reduction compromises blood flow to the organ and increases the likelihood of appendicitis [55]. In general, developmental studies of decayed traits show that vestigialization and trait loss lie on a continuum. In only the most extreme cases does the entire developmental sequence relating to the trait disappear; this characterizes tooth loss in cypriniform fish, for example [56]. In other cases, early development of the trait proceeds but is eventually halted, such that vestiges of lost traits can be detected in early development. For example, in sterile populations of the clonal loosestrife *Decodon*

verticillatus, the development of sexual function proceeds normally until pollen tube formation eventually fails, at various stages depending on the population [57]. One remarkable but common pattern is early development of a trait followed by regression or resorption, as occurs for example in the eyes of cave tetra [23], the hind limbs of dolphins [17] and the webbing between the digits of land vertebrates [58]. The pattern of development of a lost trait, including whether and when vestiges are left, probably depends on the developmental timing of both the manifestation of costs and the expression of the genes whose mutation caused the trait loss.

Conclusions

Relaxed selection and trait loss are common phenomena in nature, and yet have been understudied and misunderstood. Little more than a decade ago, for instance, the selective neutrality of nonfunctional traits was a standard assumption, and a gradual decay of traits was generally assumed [1]. Contrasting with these views are two novel and distinctive conclusions from recent research. First, a role for selection in trait reduction or loss, either through constitutive or contingent costs, is pervasive enough to have been found in most studies that have specifically looked for it. Second, trait loss can sometimes occur rapidly, especially when hastened by strong selection, and in large increments, particularly when major regulatory genetic changes are involved; in fact, these instances might provide the most significant exception to Darwin's generalization that evolution proceeds by small steps [3]. Moreover, during the past decade, research has revealed at least some aspects of the genetic basis for trait reduction or loss in a variety of organisms; and in a few systems, such as the loss of eyes in cave tetra and loss of armor in stickleback, understanding of trait evolution now extends from genetic changes, through developmental patterns, to selective regimes in different habitats.

The framework proposed here for understanding relaxed selection involves three components: (i) recognition of relaxed selection in the sense of an environmental change that weakens a particular source of selection that was previously important in maintaining a trait; (ii) analysis of the selective and neutral influences on the trait following the environmental change; and (iii) assessment of the fate of the trait in terms of function, expression and evolution. Ideally, the cost–benefit structure of the trait (broadly defined so as to encompass trait correlations), including other functions and both constitutive and contingent costs, would yield a fitness function for the trait such as those in Box 2. The nature of that function and how it changes through time, together with the operation of neutral evolutionary mechanisms, the degree of trait plasticity and the pattern and effect of genetic changes that arise, will determine the route the trait takes among the eight general possibilities in Figure 2.

As outlined in Box 3, understanding the process of evolution following the removal of a source of selection on a trait should facilitate more informed action on several environmental, agricultural and public health problems. In fact, those problems have stimulated much of the work described here. Research on agricultural pests, invasive plants, parasites and systems where pollution or other anthropogenic disturbances have changed selective regimes has increased our knowledge of evolutionary processes. However, the many emerging model systems for relaxed selection and trait loss in the wild, including clonal and parasitic plants, nonfeeding larvae, cave and subterranean animals, parasites, hosts and prey animals, indicate that information can flow in the reverse direction as well, as natural populations provide insights with applied relevance.

The major issues in the study of relaxed selection are whether and how quickly a trait will decay when a source of selection is relaxed, and how the answers to these questions relate to the particular traits and mechanisms involved. Future research will be helpful in addressing these questions if it aims to identify and explain phenomena that are still poorly known, such as trait costs and correlations, cases of trait vestigialization or persistence, and neutral evolutionary mechanisms. In addition, replicate events are especially valuable; comparing genetic, developmental and phenotypic changes in different populations or species is probably the best way to assess the generality of existing hypotheses and observations. Finally, theoretical work is needed to integrate the various factors influencing trait evolution following the relaxation of a source of selection, and to flesh out and predict relative probabilities for the pathways and mechanisms outlined here. For instance, existing population genetic theory could be developed into models that integrate the influences on traits and produce predictions of waiting time to trait loss versus the likelihood of new functions arising. Adaptive landscape models could also be used to represent the cost–benefit structures of traits and how these structures change when a source of selection is removed.

In sum, just as pathologies have made contributions to physiology, and mutations have aided genetic research, we are likely to gain a better understanding of evolution if we attend not only to cases where a trait confers a particular functional advantage but also to where that advantage has been removed. Instances of relaxed selection are thus analogous to 'knockout' experiments, in that removing a source of selection can provide insights into the role of that source of selection as well as the nature of trait interactions and how those interactions affect fitness.

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