Vestigialization and Loss of Nonfunctional Characters

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ABSTRACT

Reduction and total loss of characters are common evolutionary phenomena. Vestigialization of any morphological, physiological, or behavioral feature can be expected upon relaxation of selection on the trait. Direct selection of vestigialization is rarely documented. Most explanations of evolutionary reductions invoke indirect selection through energy economy or antagonistic pleiotropy arguments, while some invoke the effects of accumulation of neutral mutations. A few documented cases of trade-offs between fitness and wing reduction or pesticide resistance in some insects, and between fitness and resistance to phages or antibiotics in bacteria suggest that indirect selection is a plausible mechanism for evolutionary reductions. Expression of presumably useless genes suggests that neutral mutation arguments require a longer time than is available for the observed reductions. Rapid decay of useless behaviors may require explanations in terms of trade-offs among neural pathways for information processing.

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INTRODUCTION

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

Charles Darwin 1859, p. 418

Darwin drew heavily on examples of new features, such as the vertebrate eye, to support his theory of natural selection (21); yet, as the quotation indicates, he was also impressed with the pervasive nature of rudiments or vestiges. Vestigialization begins when a trait is rendered nonfunctional, or becomes a selective liability outright, due to shifts in the environment. The feature then atrophies over time. Eventually it will persist at a simplified stage in which further reduction is maladaptive, or it will disappear entirely. The intermediate, reduced structure is the vestige. JBS Haldane (cited in 43, p. 61) allowed that probably for every case of progressive evolution in the sense of descendants being more complex in structure and behavior than their ancestors, there have been ten cases of regressive evolution. A cursory examination of introductory biology texts yields such examples as simplified morphology of parasitic animals, reduced number of digits in horses, loss of limbs in snakes, the vermiform appendix and coccyx of humans, vestigial wings of flightless birds, eye and pigment loss of cave-dwelling organisms, etc.

The vestigialization and loss of a structure can be of evolutionary significance. Rudiments of portions of the primitive reptilian jaw have evolved into critical components of the modern mammalian inner ear (79). In the salamander family Plethodontidae, the loss of lungs apparently freed the hyobranchial apparatus from serving as a buccal respiratory pump, thus allowing for the evolution of a projectile tongue, a specialized feeding mechanism that contributed to the extensive radiation of the family (54, 101).

Causes of Vestigialization

Modern evolutionary biology has emphasized the appearance of new structural or functional complexes (15). Theories of vestigialization and regressive evolution have assumed minor importance (see 6, 26, 69, 73, 109), except for evolutionary biologists studying cave-dwelling organisms (4, 17, 20, 107).

Vestigial or missing structures may be signs of evolutionary trade-offs. The evolution of elaborated extra-optic sensory structures of cave animals may require eye and pigment reduction due to energy economy in resource-poor environments such as caves (67, 68, 88). Eye and pigment loss frees up energy required for elaboration of extra-optic sensors. Similarly, compensatory sensory system trade-offs could be the most plausible explanation for eye reduction in cave animals (40). In the broader context, metabolic efficiency has received indirect support as the cause of flightlessness in some island-dwelling

birds (59). More generally, character loss may typically be a consequence of indirect selection (38, p. 41). At the molecular level no feature may be selectively neutral, because nonessential messages will produce "noise" in the biochemical pathways of an organism, and natural selection will eliminate unused features and the biochemical messages they produce (73).

Vestigialization and loss of structures may have nothing to do with adaptation, either directly or indirectly. Eye and pigment reduction in cave-dwelling animals may be the result of the relaxation of selection, particularly stabilizing selection (107). In essence, structures lose complexity due to the accumulation through genetic drift of selectively neutral mutations. Whether this process can result in the observed rates of loss has been questioned (4, 55), but plausible genetic models of eye and pigment loss by neutral mutation and drift can produce the observed rates (11, 17). Rates of evolution of even presumably adaptive characters often could be accounted for, in principle, by drift (56). Such nonadaptive explanations are of particular interest because they echo the question of the relative commonness of adaptive and nonadaptive evolution (36, 61, 99). Convergence is often cited as one of the strongest pieces of evidence for natural selection in evolution. If reductions occurring independently among diverse taxa, such as eye loss in cave animals, are simple consequences of accumulating neutral mutations, convergence may also reflect nonadaptive evolution (see also 80).

The interesting aspect, and the rub, of evolutionary reductions is not that they are too difficult but rather that they are too easy to explain in theory. Distinguishing among various theories of regressive evolution is hampered by lack of empirical information and by experimental limitations posed by many of the organisms in question. Little is known of the genetic basis for many of the characters in question or of the energetic cost of their development and maintenance. Estimates of rates of evolutionary change are rarely attempted. Further, little is known about the relationship between character reduction and its effects on fitness. We review here some empirical case studies and theoretical perspectives on evolutionary reductions, evaluating recent progress and suggesting potential avenues for future work.

REDUCTION AND LOSS OF DIGITS AND LIMBS IN TETRAPODS

Reduction and loss of digits and limbs is a repeated theme in many lines of tetrapods, including amphibians, reptiles, birds, and mammals (45). Reduction of digits and limbs generally follows an orderly sequence, proceeding proximad from distal elements in amphibians (1) and reptiles (33). Analyses of patterns of limb formation in many tetrapods, especially amphibians (see 63, 87), have greatly clarified the developmental processes underlying digit and

limb reduction. Such structural changes can proceed rapidly under weak selection (45); otherwise, our understanding of the ultimate evolutionary causes of digit and limb reduction is far from complete. Simplification of bony elements in the limbs and skull of some salamanders may be a consequence of responses to selection for miniaturization (37). The reduction of limbs in squamate reptiles was likely preceded by elongation of the body (33). Whereas limb reduction may have occurred multiple times in lizards such as skinks and legless lizards, leg loss may have occurred only once in snakes (33, 74). Instead of providing a catalog of limb reduction and loss in tetrapods, we concentrate on one case for which sufficient data allow a critical review: the evolution of flightlessness in birds.

Flightless Birds

Loss of sustained flight has occurred repeatedly in birds and is associated with reductions in the size of skeletal elements in the wings and of the flight muscles (28). Flightlessness is found among 26 families in 17 orders of birds (53). In reviewing the distribution of extant and recently extinct (within 15,000 years) flightless species in ten orders, Roff (78) estimated that flightlessness has evolved independently at least 30 times.

Most considerations of flightlessness in birds have emphasized flightless birds on islands. The argument is that, in the absence of predators, there is relaxed selection to maintain flight for predator avoidance, and that reduction of wings along with pectoral muscle mass results in energy conservation (see 59). The association between flightlessness and insularity is statistically significant for rails, in which all 17 flightless species, as compared to only 18 of 105 volant species, are found on islands. Each of these 17 species probably represents an independent transition to flightlessness (78). The basal metabolic rates of several flightless rail species are significantly lower than their volant congeners, and basal metabolic rate is inversely correlated with pectoral muscle mass relative to total mass (59). This seems to support the idea that flightlessness evolved as a response, manifested as reductions in wing size and pectoral muscle mass, to selection for energy conservation through lowered metabolic rate. The critical and unproven assumption is that lowered metabolic rate results in increased fitness in rails.

The association of ratites with islands is suggested by the fact that 25 of the 41 species are/were from New Zealand and Madagascar. If the ratites had evolved from a single flightless ancestor, their higher frequency of occurrence on islands than on continents might not indicate that insularity is necessary for the evolution of flightlessness (78), but rather that flightless species adapted well to the islands, or that flightlessness led to a high speciation rate with a higher proportion persisting on predator-free islands than on continents.

Excluding rails and ratites, Roff (78) estimated 15 transitions to flightless-

ness for the other birds: 8 transitions involve insular species, 4 involve coastal species, and 3 involve species of inland waters. The coastal transitions include penguins, the great auk, steamer ducks, and a few extinct California coast ducks, while 4 of the insular transitions include species, such as the Galapagos cormorant, that forage off the coast of the islands. For at least the extant species in both groups, the shortened stout wings play an important role in propulsion during dives; in addition, steamer ducks use their wings as oars for propulsion on the water (see 51–53). Not surprisingly, steamer ducks have high relative pectoral muscle mass (59). Thus wing reductions in these birds may be the direct result of selection for enhanced locomotory performance in water at the expense of aerial flight capability. The three inland water transitions occur in three species of grebes living in isolated low-latitude mountain lakes. Flightlessness in grebes, which are foot-propelled divers, is associated with structural reduction and loss of locomotory capacity of their wings; energy conservation is again the mechanism invoked for their wing reduction (50). Constancy and high productivity of the habitat are proposed as reasons for relaxation of selection for maintenance of aerial flight on steamer ducks (53) and flightless grebes (50).

Two generalizations emerge from the bird data. First, there was relaxed selection for maintenance of flight due to release from predation on islands, release from migration due to constancy and high productivity of the habitats, or both. Second, there are at least two patterns of reduction. In the penguins and steamer ducks, for example, the smaller wings probably resulted from direct selection for improved performance in an aquatic medium. In other words, flightlessness is misleading in these birds, they just fly under water. In the rails, grebes, and ratites, the causes of flightlessness and wing reduction are not as clear. The only evidence for the energy economy argument is the observed lower metabolic rate of a few flightless rails compared to volant congeners with larger flight muscles, yet the lowered metabolic rate could be a consequence of, rather than the reason for, flightlessness. Data on intraspecific variation in wing size and flight muscle mass and in fitness components, such as egg or clutch size, are needed.

LUNG LOSS IN PLETHODONTID SALAMANDERS

It is generally accepted that lunglessness in plethodontid salamanders is a derived condition evolved from lunged ambystomatid-like ancestors (24). Assuming cool, swift, oxygen-rich, upland Appalachian streams in the late Mesozoic as ancestral habitats, lung loss in plethodontids is hypothesized as an adaptation for increased ballast, and thus decreased risk of downstream drift, in concert with increased reliance on cutaneous instead of pulmonary respiration (105, also see 5, 7). According to this hypothesis, selection for

maintenance of lungs in ancestral plethodontids was weak because cutaneous respiration was sufficient, and the presence of lungs was maladaptive due to buoyancy. The first point is supported by the fact that both lunged and lungless salamanders show low energy demands as reflected by metabolic rates (27), and that even in lunged salamanders, cutaneous respiration accounts for a large percentage of total oxygen consumption at low temperatures (102, 103). The second point is problematical. Although larvae of *Ambystoma maculatum* with reduced lungs are less buoyant and thus drift for significantly shorter distances compared to larvae with larger lungs (7), the selective advantage is unclear. Reduced downstream drift may increase larval survival and population stability for species with lengthy larval stages (5), but whether the larval duration of ancestral plethodontids was short or lengthy is debatable (83).

Alternatively, plethodontids could have a terrestrial origin because cool, oxygen-rich, upland Appalachian streams may not have existed in the late Mesozoic—much of the area was purported to be a peneplane, and the climate was subtropical (82). In such a scenario, lunglessness is hypothesized to be a consequence of reduced buccal volume due to selection of narrower heads (82), or of the evolution of terrestrial courtship and mating (72). The first hypothesis is based on the observation that in lunged ambystomatids narrower-headed species show a greater reliance on cutaneous respiration than do wider-headed species (103, 104) because they have lower capacity for pulmonary respiration due to reduced tidal volume of the buccal pump. Selection of narrower heads in ambystomatid-like ancestral plethodontids resulted in less reliance on pulmonary respiration, although the selective advantage of narrower heads is unclear. This hypothesis assumes weak or no selection for maintenance of lungs and assumes that the presence of lungs was not maladaptive per se. The second hypothesis assumes strong selection for shifting courtship and mating activities of ancestral plethodontids from water to land, in order to escape the costs of migration from land to water and competition for mates at breeding ponds. The energetic demand of courtship and mating activities in slow-flowing to stagnant, relatively hypoxic breeding ponds required pulmonary respiration to supplement cutaneous respiration. Shifting such activities to land resulted in energetically less expensive breeding activity in an environment with greater oxygen supply, with a consequent relaxation of selection for pulmonary supplement to cutaneous respiration. This hypothesis also assumes weak or no selection for maintenance of lungs and that lungs were not maladaptive per se in ancestral plethodontids. Thus, if plethodontids did originate on land, their lunglessness is essentially a case of the evolutionary loss of a nonfunctional character. The already low energy demand of salamanders as a group also suggests that energy economy arguments may have little significance as the ultimate cause of lung loss.

SUBTERRANEAN ANIMALS

Cave-Dwelling Organisms

Among the most distinctive features of animals from caves and other subsurface habitats are the reduction and loss of eyes and pigment. These have occurred independently across many taxa, such as arachnids, insects, crustaceans, fish, and salamanders, with literally hundreds of documented cases (see reviews in 17, 34, 98). The ubiquity of these reductions is underscored by the estimated 50,000 to 100,000 obligate cave-dwelling species in the world (18), most of which are eyeless.

Wilkens (107), working with the characin fish *Astyanax fasciatus,* argued that neutral mutation is responsible for eye and pigment loss in cave animals. The argument is that without light the presence or absence of eyes and pigment has no effect on fitness. Most mutations affecting a complex system such as an eye are likely to be degenerative (17), and ultimately some of these mutations will be fixed in the population by genetic drift. Others (e.g. 4, 55) have objected that the process of neutral mutation and genetic drift is too slow to account for rates of eye and pigment loss; *A. fasciatus* populations probably have been isolated in caves for fewer than 10,000 generations. Nonetheless, there are plausible scenarios for eye loss resulting from mutations at a subset of loci of a polygenic system (17), or from high rates of mutation from functional to nonfunctional alleles (11). F_1 hybrids between *A. fasciatus* populations have larger eyes than either parental population (106), indicating that eye reduction involves different genes in different populations. Data on protein electrophoretic variation also support the hypothesis of independent isolation in different caves (3). Wilkens (108) pointed out that there should be a relaxation of stabilizing selection accompanying isolation in caves. He argued that the increased variability of eyes in recently isolated populations supports this view, but he did not attempt to measure stabilizing selection directly. Culver et al (19) measured stabilizing selection in a cave-dwelling population of the amphipod *Gammarus minus* and failed to find any reduction in stabilizing selection relative to spring-dwelling populations. However, stabilizing selection was rare in both habitats.

Although neutral mutation is probably an important force in eye and pigment loss, it is not the only factor. In particular, energy economy and pleiotropy can be invoked to couple the reduction in eyes and pigment with the increase in extra-optic sensory structures (20, 29, 67, 88). There is evidence of direct selection for small eyes in different cave-dwelling populations of *G. minus,* even when selection for extra-optic sensory structures is taken into account (41). In these cases, instead of energy economy, there may be an evolutionary trade-off at the level of neurological connections in the brain. Compensatory

innervation of parts of the optic processing areas of the brain by projections of neurons from olfactory or tactile sensory organs are observed in mutant anophthalmic mice (42) and in cave fish (100). Finally, the standardized rate (see 56) of increase of extra-optic sensors (antennae in particular) is nearly an order of magnitude less than the standardized rate of loss of eye characters in *G. minus* (20). The estimated rates of reduction for two eye size characters are 2.4×10^{-5} and 1.5×10^{-5}, whereas rates of increase for six antenna size-characters ranged from 8.9×10^{-7} to 1.1×10^{-6} (20, p. 179). There are of course other factors that may be involved, such as differences in the number of loci involved, but the consistent differences in rates of change of optic and extra-optic sensory structures suggest that both selection and neutral mutation are important in the evolution of reduced eyes of *G. minus*. Extra-optic sensory structures increase in size as a result of directional selection. Optic structures decrease in size as a result of directional selection and neutral mutation.

Mole Rats

The eyes of the mole rat *Spalax ehrenbergi* are minute and covered by skin, and they are operationally blind, as light flashes evoke no action potential along the optic pathway (see review in 62). The atrophied eyes of the mole rat, however, do seem to function in photoperiodic entrainment of circadian activity and thermoregulatory rhythms. The mole rat also shows severe reduction in the thalamic and tectal areas of the brain, which discern form and motion, but this is accompanied by the hypertrophy of structures that serve photoperiodic functions (14). It is suggested that such a highly specific set of reductions and hypertrophies in the brain indicate that eye reduction in mole rats, which, in effect, eased a costly metabolic burden, cannot be the simple consequence of relaxed selection for vision, but rather, it may be part of a highly complex set of adaptations to the underground environment (14). Nevo (62) pointed out that because of the tight coupling of photoperiodism and reproductive cycles in mammals, even atrophied eyes are important in photoperiod detection, so it is not surprising that there are no completely eyeless subterranean mammals.

INSECTS

Not surprisingly, a taxonomically diverse group such as insects exhibits many cases of character loss and vestigialization. For example, adult mayflies (Ephemeroptera) spend their brief existence mating and do not feed, and they have nonfunctional vestigial mouthparts (25). Nasute soldiers have evolved independently in two phyletic lines of termites (26). Instead of using their mandibles for defense, as is common in primitive taxa, these soldiers are convergent for use of a "squirt gun," a prolonged portion of the head that ejects irritating

fluids; and they are also convergent for nonfunctional vestigial mandibles (26). While these examples further illustrate the pervasive nature of vestigialization, we concentrate on two cases that can shed light on the causes of character reduction. These are the loss of flight and the loss of hearing.

Flightlessness and Wing Reduction

The loss of flight and of wings from winged flight–capable ancestors has evolved numerous times in the insects (see review in 77). The incidence of flightlessness is correlated with the mode of metamorphosis. Flightlessness is almost nonexistent in the hemimetabolous orders with naiads as immatures, is uncommon among holometabolous orders with larvae as immatures, and is common in the paurometabolous orders with nymphs as immatures. Roff (77) suggested that, relative to orders with the other two types of metamorphosis, flightlessness is more likely to evolve in paurometabolous insects in which the immatures are more mobile and have feeding habits similar to those of adults. In other words, there is relaxed selection for maintenance of flight for dispersal. The incidence of flightlessness is positively correlated with latitude and altitude, and is high in woodland, desert, ocean surface, and aquatic habitats, while it is low in stream, river, and pond margins. The data suggest that habitat persistence is conducive to the evolution of flightlessness (22, 77, 78), which also implies relaxed selection for the maintenance of flight.

The incidence of flightlessness is higher among females than males, which Roff (77) suggested allows more resources for egg production. The hypothesis is that fecundity selection is constrained by selection to maintain flight but can proceed when selection for flight is relaxed, and thus flightlessness is indirectly adaptive. This trade-off hypothesis is supported by data from wing dimorphic insects. Among 22 wing-dimorphic species, the short-winged morph is more fecund and can reproduce sooner than the long-winged morph (75). The flightless short-winged morph of the cricket *Gryllus rubens* is also more fecund than the long-winged morph and is more efficient at converting assimilated nutrients into biomass, a potential result of lower respiration costs by vestigial flight muscles (60). Finally, in the crickets *Teleogryllus oceanicus* and *Gryllus firmus,* experimental removal of wings upon metamorphosis increases egg production (76). Wing dimorphism is also found in water striders (93). In some species flightless females have shorter preoviposition periods and produce more eggs over the initial reproductive stages (92). The case with water striders, however, is complicated by recent evidence indicating that wing dimorphism and short-wings may be ancestral conditions in some genera, and that there exist two types of short-wing morphs that develop through different morph determination mechanisms (2).

These insect data are illuminating in two respects. First, trade-offs between flight maintenance and fitness components are observed. Second, there is

evidence for a physiological mechanism underlying the observed trade-offs. Thus, when selection for flight is relaxed, flightlessness and associated wing and especially muscle reduction in insects probably evolved as correlated responses, through energy economy, to selection for enhanced fitness components.

Loss of Hearing

Tympanal ears have evolved repeatedly in insects (32). In some groups, especially noctuid moths, tympanal ears are tuned to between 20 kHz to 50 kHz and primarily function as detectors of foraging bats. The echolocation calls of bats trigger evasive aerial maneuvers by the insect (89). That insect hearing is an effective strategy against bat predation is underscored by the evidence that some bats shift echolocation frequencies above or below the most sensitive frequencies of tympanal ears (84).

Female gypsy moths (*Lymantria dispar*) are winged but are not exposed to predation by bats because they do not fly and are less sensitive to high frequency sound compared to males that fly (8). Noctuid moths endemic to bat-free islands of French Polynesia (a region that bats apparently never colonized) show reduced sensitivity to frequencies above 35 kHz compared to moths in bat-inhabited areas, and deafness in these moths is probably derived from ancestors with full auditory capacity (31).

Praying mantises show widespread sexual dimorphism in ultrasound sensitivity and in ear morphology (110). It is always the females that show reduced hearing, ranging from mild loss to complete deafness. Except in cases of mild loss, hearing reduction is associated with ears that are structurally different from those of the male. These mantises are also dimorphic in wing length, which is highly correlated with dimorphism in auditory sensitivity. Short-winged flightless individuals have reduced hearing compared to functionally winged mantises of the same species and mantises usually fly at night. There are also strong indications that wing reduction and hearing loss in female mantises are derived conditions (110).

In all these cases predation by echolocating bats is probably the selective agent behind the maintenance of functional tympanal ears. Full to partial deafness is always associated with release from bat predation. Although the commonness of this phenomenon among noctuid moths is unknown, it is common in the Mantodea. Structural dimorphism, which reflects differences in hearing sensitivity, occurs among 63 of 183 genera of mantises suitable for anatomical comparison between sexes, and hearing sensitivity dimorphism should be more widespread because it is not always associated with structural dimorphism. These 63 dimorphic genera span 3 families and 11 subfamilies within the large Mantidae (110). Thus, hearing loss associated with release from bat predation has likely evolved multiple times within the Mandotea and

certainly within the insects. Assuming that full auditory acuity or fully developed tympanal ears in the absence of bat predation is not maladaptive per se, reduction of these features is another indication of the prevalence of vestigialization of nonfunctional characters.

The dimorphism of wing length in mantises is identical to the patterns of wing reduction discussed in the previous section. It is probable that flightlessness and wing reduction in the female mantises also can lead to an increase in fitness components. This result, however, confounds the interpretation of the evolution of hearing loss; i.e. do hearing loss and associated simplified tympanal structures also represent energy savings that contribute to fitness gains? We suggest that a phylogenetic analysis may permit the teasing apart of the two phenomena.

REVERSAL OF RESISTANCE

Much applied research has concentrated on the evolution of resistance to antibiotics in bacteria and to pesticides in agricultural pests and arthropod disease vectors, because of severe public health and economic consequences. Studies of the evolution of resistance are germane to our focus because reversal of resistance in the absence of the antibiotic or pesticide is analogous to the evolution of vestigialization and loss of nonfunctional characters.

Important pest control agents include insecticidal protein toxins derived from *Bacillus thuringiensis,* a common soil bacterium (see review in 96). These proteins act by binding to the brush border membrane of the insect midgut epithelium causing swelling and lysis of the cells. Resistance has recently evolved in the field in the diamondback moth *Plutella xylostella* and in nine insect species selected for resistance in the laboratory. The primary mechanism of resistance is reduced binding of the toxin to the midgut membrane. Strains of *P. xylostella* selected for resistance at levels 25 to 2800 times that of unselected strains show reversal of resistance to levels of unselected strains were not exposed to *B. thuringiensis* for many generations. Restoration of binding of the toxin to the midgut is the mechanism of reversal of resistance (97). Rapid reversal to sensitivity at pre-selection levels is also observed in the cockroach *Blattella germanica* resistant to pyrethroids (12) and in other insects (see review in 58).

Adverse pleiotropic effects on fitness-correlated life-history characters resulting from evolution of resistance has been hypothesized (64, 71, 86) and has been observed in a few cases (9, 35, 57). For example, strains of *P. xylostella* resistant to *B. thuringiensis* show reduction in survival, egg hatching, and fecundity compared to sensitive strains (35). That such trade-offs between fitness and resistance are observed infrequently may result, with continued

exposure to the insecticides, from selection to ameliorate the adverse effects on fitness (see 9, 81).

Amelioration of fitness costs associated with the evolution of resistance is observed in strains of *Escherichia coli* resistant to the virus T4 (47, 48) and in strains of *Bacillus subtilis* resistant to the antibiotic rifampicin (13). *Escherichia coli* resistance to T4 is accompanied by reduction of competitive fitness compared to sensitive strains. The competitive fitnesses of resistant strains grown in the absence of T4 can be restored to the level of sensitive strains, but they do not revert to sensitivity. Restoration of fitness resulted from selection directly for genetic changes that counter the adverse pleiotropic effects of resistance. In the case of *B. subtilis,* adverse pleiotropic effects of resistance were ameliorated through two mechanisms. The first is through replacement of mutations conferring resistance at high fitness cost with ones at lower cost. The second is through selection for modifiers at other loci that compensate for the fitness cost of the original mutations. These results can shed light on the energy economy and pleiotropy hypotheses of regressive evolution. Lenski (48) notes that trade-offs due to competition for limited energy or material should intensify with further adaptation, while trade-offs due to disruption of genetic integration should diminish. Even genetic trade-offs that persist can be difficult to detect, depending on the relative frequencies of loci that acquire resources and those that allocate resources to the traits involved in the trade-off (39).

VESTIGIAL BEHAVIOR

Decay of behavioral traits presumed to be selectively neutral, though rare, has been documented. Vestigial behavior can be especially significant in the study of mechanisms of vestigialization because a "useless" behavior can be elicited only under appropriate experimental conditions. Such a trait is not expressed in natural settings and thus is not subject to selection directly, nor can any pleiotropic effects or energetic costs of its expression be subject to selection. Two plausible explanations of the decay of such behavior involve the accumulation of mutations underlying the genetic basis for the behavior or trade-offs at the level of neural circuitry.

Decay of Sexual Behavior

All-female strains of *Drosophila mercatorum,* selected for parthenogenesis from bisexual wild ancestors, were maintained in the laboratory for over 20 years without males, thus rendering female mating behavior a nonfunctional trait (10). The parthenogenetic strains, upon exposure to males, showed greatly reduced mating propensity, measured as mating speed, compared to females of sexual strains. Because female mating behavior is elicited only in the

presence of males, this trait was not expressed while the parthenogenetic strains were maintained without males and thus was not subject to selection. Carson et al (10) suggested accumulation of neutral mutations as the genetic mechanism for these reductions.

Behavior of the Cave Fish Astyanax

The cave and epigean forms of the characin fish *Astyanax fasciatus* have been subjects of intensive studies (see 107). Cave forms differ from the epigean form in several behavioral features that have been demonstrated, through crosses among populations, to be under polygenic control. Specifically, cave forms show reduced intensity or complexity of aggressive behavior (66), schooling behavior (65, 66), fright reaction to alarm substances (30), and feeding behavior (85). Wilkens (107) ascribed accumulation of neutral mutations as the mechanism for these reductions.

Decay of Egg-Rejection Behavior

Egg-rejection behavior in the Village Weaver, *Ploceus cucullatus,* a colonial nesting bird from Africa, had probably evolved to counter brood parasitism by the Didric Cuckoo, *Chrysococcyx caprius* (see 16). Village Weavers were introduced to Hispaniola in the eighteenth century, where they were not exposed to brood parasites until the arrival of the Shiny Cowbird, *Molothrus bonariensis*, in the early 1970s. Hispaniola Village Weavers are less discriminatory and reject a lower percentage of foreign eggs than do their African counterparts (16). The low level of rejection behavior probably is not an adaptation to parasitism by the Cowbird because of the recent contact between the species, and intraspecific brood parasitism is unknown in this population. Cruz & Wiley (16) concluded that egg-rejection behavior is selectively neutral in the absence of brood parasitism, and that the low level of rejection observed in Hispaniola Village Weavers represents the vestige of a nonfunctional trait.

VESTIGIAL GENES

Regal (73) noted that a biological molecule may be used in a variety of physiological systems and thus the same symbol (molecule) may encode different messages, providing, in the cybernetic sense, the potential for noise in the system. He argued that natural selection will favor the reduction or elimination of unused features because this will reduce or eliminate erroneous messages in physiological pathways, a process he called "streamlining evolution." He noted that this could be accomplished by either eliminating the genetic material from the genome or by "turning off" sections of the DNA. Li (49), in a similar context, suggested that pseudogenes and other forms of

"nonfunctional" DNA may remain in the genome as long as they do not impede the cellular physiology.

How much streamlining of the genome actually occurs is an open question. The discovery in the late 1970s of noncoding intervening sequences (introns) within the protein coding regions of the genome of many eukaryotes has led to a debate over their origin and significance. Doolittle (23), among others, argued that introns arose early in evolution, perhaps as parts of inefficiently organized pre-cellular genomes, and he suggested that their absence in modern prokaryotes might be a consequence of selection for more efficient organization and expression in rapidly growing cells. This hypothesis is in the spirit of streamlining at the molecular level. More recently, however, Doolittle and colleagues (94) have concluded that introns arose late in evolution as insertions in the structural genes of eukaryotes. Because most introns have no known function, yet their messages are transcribed and must then be spliced out prior to translation, a late origin for introns is contrary to the hypothesis of streamlining evolution.

The occasional appearance of atavistic features indicates that the genes for reduced or lost morphological characters are often retained in the genome. Chimeras of chick epithelium and mouse molar mesenchyme can result in tooth development in which enamel matrix proteins are secreted by the chick epithelium (44). The opsin gene, which encodes a visual pigment protein, is actively transcribed in the early development of the blind cave-dwelling characin *Astyanax fasciatus* (46), and sequence data suggest that this gene is capable of producing a functional protein (111). Thus, toothlessness in birds and eye regression in *A. fasciatus* appear to be consequences of disruptions of developmental or regulatory pathways rather than disruptions of specific protein-coding regions of the genome.

Maintenance of expression of a "useless" gene, such as the opsin gene in *A. fasciatus,* may reflect the recency of cave isolation and eye reduction in these populations (111). Moles and mole rats, however, have a long evolutionary history in subterranean habitats and, presumably, a long evolutionary history of eye reduction, yet they may still retain genes for three and one lens-specific crystallin proteins, respectively (70). With evolutionary times for eye degeneration to occur of 25 million years for the mole rat and 45 million years for the mole, the crystallin genes should, in the absence of selective constraints, be expected to have accumulated enough mutations to have been rendered silent (70). Their continued expression is an enigma.

The consequences of vestigialization at the molecular level are unclear. Some structural genes involved in the development of vestigial structures have been turned off, such as the gene for enamel protein in birds. Others, such as the crystallin protein genes of blind burrowing mammals, continue to be expressed, opposing the noise suppression hypothesis. Further, the hypothesis

of a more recent origin for introns is counter to the view of streamlining at the molecular level. The difficulty is that the lack of a known function for introns is not conclusive proof that they are useless. Likewise, the mere fact that crystallin genes have been conserved in moles and mole rats is suggestive of some unknown selective advantage (70). Pseudogenes that occur in many multi-gene families, produce no protein, and appear to have no other pheno-typic consequences would seem to be examples of the outcome of streamlining evolution. Even here the issue is not clearcut. Many pseudogenes exhibit rates and patterns of mutation that suggest that these nonfunctional sequences are selectively neutral (see 49), supporting the idea of streamlining evolution. Recent studies on some *Drosophila* pseudogenes (95), however, have revealed slower evolutionary rates, and patterns of codon and substitution bias that indicate as yet unknown evolutionary constraints on these sequences. How vestigialization is manifested at the molecular level requires a more thorough understanding of the functioning of genetic and developmental systems.

CONCLUSIONS

Although we have concentrated only on selected case studies of vestigial characters, there should be no question that evolutionary reduction and loss of characters is common. In addition, we have not even discussed any case of character reduction in plants, such as the loss of photosynthetic ability in parasitic plants (e.g. Indian Pipe), and there should be many examples. Generally if there is relaxation of stabilizing or directional selection on a character, whether morphological, behavioral, or physiological, vestigialization can be expected. A focus on vestigialization can provide a different perspective on evolutionary phenomena. Recent debates on the flight capabilities of *Archae-opteryx* have centered on whether its small flight muscles could have generated sufficient power for sustained flight (see 90). An indicator of aerodynamic function in birds is vane asymmetry of the flight feathers, with the central shaft being closer to the leading edge, which is necessary to generate lift (see 91). Vane asymmetry of *Archaeopteryx lithographica* is not different from that of modern flightless birds but is significantly lower (more symmetrical) than that of flying birds, suggesting that *A. lithographica* was incapable of flight (91). The interesting point, from our perspective, is the regression of vane asymme-try to the ancestral state in the flightless birds. Although wings and feathers are smaller in flightless birds and may represent energy savings, variation in the position of the central shaft in the feather probably does not reflect differ-ences in energy expenditure. The selective advantage of a more symmetrical feather in flightless birds is also unclear. The answers may come from exami-nations of pathways of feather development or from studies of vane symmetry

and thermal properties of feathers. Our point is that many more interesting examples of vestigialization in nature may be revealed from such a perspective.

Understanding of the causes of vestigialization and loss of characters is incomplete. If the reduction of a character is obviously adaptive, then vestigialization is of interest only as another example of evolution through natural selection. Adaptive reduction in such a direct sense is exemplified by the short and stout wings used for swimming by penguins and steamer ducks. Even when selection does operate on a vestigial character, as in the case of direct selection against eyes in the cave-dwelling amphipod *Gammarus minus,* precisely how selection works can still be unknown. However, most examples of evolutionary reduction are of interest because they resist explanations as adaptations per se. Most explanations of character reduction invoke indirect selection in terms of energy economy or antagonistic pleiotropy arguments, although what is meant by energy in such a context is usually unstated, and few, if any, such arguments are framed as testable hypotheses. Furthermore, such arguments generally rest on the assumption that trade-offs among populations also operate within populations. In this light, the documented trade-off between fitness components and wing size and wing muscle mass in the cricket *Gryllus rubens* is significant, especially because a physiological mechanism underlying the trade-off can be inferred. The many cases of documented trade-offs between fitness components and flightlessness in other insects, and especially between fitness and resistance to pesticides, indicate that this is a plausible mechanism for character reduction; the more general implication is that pleiotropy is widespread. More analyses of trade-offs between fitness components and vestigial structures within populations are needed to ascertain whether it is a general explanation for character reduction.

It is more difficult to explain vestigial behavior in terms of energy economy and pleiotropy, because the normal behavior is not expressed and the vestigial behavior is elicited only under experimental conditions. Indeed, in all cases of vestigial behavior the investigators invoked the accumulation of neutral mutations to explain the behavioral decay. The neutral mutation argument is attractive in its simplicity, yet it is difficult to test. The expression of presumably useless genes, either naturally as in the case of opsins in the cave fish and in mole rats, or experimentally induced as in the case of enamel proteins in birds, suggests that very long evolutionary times are needed for mutation to destroy the genetic basis of a character. Yet the decay of the egg-rejection behavior of the Village Weaver, and especially the sexual behavior of *Drosophila mercatorum,* have proceeded rapidly. Trade-offs involving the neural circuitry responsible for the processing of information connected to the vestigialized behavior and other neural functions may explain this phenomenon.

Finally, we note that studies of vestigial characters generally make three assumptions. The first is that the reduced state is derived, and the second is

that the character is nonfunctional. Although these are usually valid assumptions, the cases of the ancestral short-winged condition in water striders and of the photoperiodic function of the vestigial eyes in mole rats indicate caution must be exercised. The third assumption generally equates uselessness with selective neutrality per se. That this assumption is not always valid is reflected by the debate over whether the rates of evolution of pseudogenes are constrained by selection.

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