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PERSPECTIVE: REVERSE EVOLUTION

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Abstract.—For some time, the reversibility of evolution was primarily discussed in terms of comparative patterns. Only recently has this problem been studied using experimental evolution over shorter evolutionary time frames. This has raised questions of definition, experimental procedure, and the hypotheses being tested. Experimental evolution has provided evidence for multiple population genetic mechanisms in reverse evolution, including pleiotropy and mutation accumulation. It has also pointed to genetic factors that might prevent reverse evolution, such as a lack of genetic variability, epistasis, and differential genotype-by-environment interactions. The main focus of this perspective is on laboratory studies and their relevance to the genetics of reverse evolution. We discuss reverse evolution experiments with *Drosophila*, bacterial, and viral populations. Field studies of the reverse evolution of melanism in the peppered moth are also reviewed.

Key words.—Constraint, epistasis, experimental evolution, reversibility, selection, variation.

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The irreversibility of evolution has long been an important theme in evolutionary biology (Darwin 1859; Dollo 1893; Gregory 1936; Muller 1939; Simpson 1953; Lewontin 1966; Gould 1970; Maynard Smith 1970; Wright 1977; Lande 1978; Wagner 1982; Bull and Charnov 1985; Harvey and Partridge 1987; Wake 1991; Williams 1992; Marshall et al. 1994; Fong et al. 1995; Sanderson and Hufford 1996; Bell 1997; Ghiselin 1997; Gayon 1998; Crill et al. 2000; Teotónio and Rose 2000). Few evolutionary biologists would contend that long-term evolution is reversible at all levels of biological organization, because of the improbability of retracing numerous evolutionary events over long periods of time. The reversibility of evolution for particular phenotypes over shorter time spans is nevertheless still an open question.

After Darwin's *Origin of Species* (1859) was published, a major concern within the scientific community was whether natural selection would be immediately undermined by short-term reverse evolution (for a review, see Gayon 1998). Empirical observations indicated that in each generation the offspring phenotypic values tended to be closer to the mean of the parental population as a whole than to the midparent value. Thus, there seemed to be a regression to the species-specific type, also called the ancestral state. The explanation

given for this at the time was that species-specific spheres of variation forced a return to the ancestral type, generation after generation. Evolution, it was thought, could only take place through large-effect mutations, which would put the populations under new spheres of attraction. If there were such spheres of attraction, how could evolution occur by means of natural selection on extant variation? This paradox was resolved after Mendelism gave heredity a sound theoretical explanation and particularly with the demonstration that gene frequencies have no tendency to directional change in the absence of selection (Gayon 1998). This resolved 19th-century concerns about the power of natural selection over short time spans, and since then the reversibility of evolution has received comparatively less attention.

At present, the study of reverse evolution takes a considerably different form. Irreversible evolution is viewed as “an extreme type of evolutionary restriction” (Bull and Charnov 1985), and one of the main questions today is the degree to which evolutionary history constrains reverse evolution (cf. Maynard Smith 1970; Bull and Charnov 1985; Loeschcke 1987; Gould 1989; Williams 1992; Travisano et al. 1995; Bell 1997; Losos et al. 1998; Teotónio and Rose 2000). Related to this issue, but important in their own right, are the genetics of reverse evolution. While a comparative approach can sometimes yield important insights into these questions (e.g., Simpson 1953; Lande 1978; Bull and Charnov 1985; Wake 1991; Sanderson and Hufford 1996), especially by gen-

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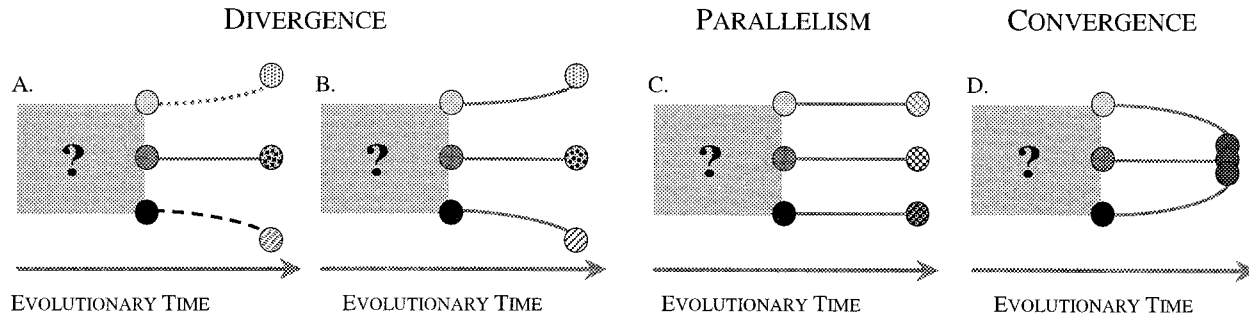


FIG. 1. Differentiation of populations (shaded and patterned circles) subject to different environmental conditions (lines) since their origin from an unknown ancestor. Repeatable evolution can be referred to as the attainment of the same final character state, or the undergoing of the same evolutionary processes by different independent lineages. In terms of process, C and D are equivalent (repeatable), although the pattern is different: C represents parallel evolution, while D shows convergent evolution. Divergent evolution, represented by A and B, will exacerbate initial phenotypic and genetic differences between populations, either under different (A) or similar (B) environmental conditions. With parallel evolution, those same differences will be maintained (see Cohan 1984).

erating new hypotheses, it cannot reveal the specific genetic mechanisms involved in reverse evolution. However, the time span of the experimental evolution approach is necessarily shorter than that of comparative evolution. Both approaches have their distinctive limitations, but the scientific issues of interest today are more tractable in experimental studies of evolution.

In this perspective we will not be concerned with evolutionary transitions that involve genomewide rearrangements, such as the reverse evolution of diploidy from polyploidy or haplodiploidy or the reverse evolution of homomorphic sex chromosomes from a heteromorphic condition. Such cases have been adequately analyzed by Bull and Charnov (1985) among others (e.g., Muller 1939; Harvey and Partridge 1987), and we will not repeat their reviews here. Instead, we focus our attention on cases where the course of reverse evolution is determined by changes in gene frequency, by the evolution of new gene interactions, or by the evolution of new genotype-by-environment interactions. (We do not suppose that these changes are mutually exclusive.) We will review several examples of experimental reverse evolution, with both sexual and asexual organisms, and one example of reverse evolution in nature.

DEFINITIONS

Following Bull and Charnov (1985), reverse evolution can be defined as the reacquisition by derived populations of the same character states, including fitness, as those of ancestor populations. Reverse selection can be defined as the reimposition on derived populations of the same selective pressures as those of recent ancestor populations. As a consequence of these definitions, the process of reverse evolution does not always need reverse selection to occur.

It is important to make the distinction between *reverse* and *relaxed* selection. The term “relaxed selection” is usually applied to artificial selection experiments, where the reproducing individuals are no longer chosen by the experimenter before each generation (see Wright 1977; Falconer and MacKay 1996). Reverse evolution can occur with relaxed selection. Studies of relaxed selection are often different from studies of reverse selection because the selection pressures imposed upon the population are specifically imposed in the

latter case, whereas in the former case they are not specifically determined.

These definitions have at least one important operational consequence for the experimental study of reverse evolution. They require that the phenotypic states and selective conditions of the ancestor population be known. Furthermore, reverse evolution is only detectable when convergence to an ancestral phenotypic state can be measured; because the impact of secular environmental changes is usually unpredictable, the ancestral population (or an equivalent representative) needs to be available for comparison with the derived populations to infer convergence to the ancestral condition (see Fig. 1).

GENETIC MECHANISMS OF REVERSE EVOLUTION

Two types of genetic mechanism need to be distinguished when discussing reverse evolution: those mechanisms that facilitate the process of reverse evolution and those that are responsible for its irreversibility or for its partial reversibility, with no full convergence to the ancestral states.

The genetic mechanisms impeding reverse evolution may be the same as those that restrict any kind of adaptive or nonadaptive evolution. A general lack of relevant genetic variation is one of these. But genetic variation alone may not permit reverse evolution, particularly if the genetic variation is maintained by strong selection mechanisms. Among these mechanisms are the maintenance of genetic variation by temporally or spatially heterogeneous environments (e.g., Levene 1953; Rainey et al. 2000) or by antagonistic pleiotropies among fitness characters (e.g., Bohren et al. 1966; Rose 1982). Such mechanisms may establish patterns of balancing selection that will prevent allele frequencies required for reverse evolution. Epistasis may also prevent reverse evolution, if reverse evolution requires the breakup of favorable epistatic gene combinations that have evolved since derivation from the ancestral state (see reviews in Wright 1977; Whitlock et al. 1995). A third type of genetic constraint can be generated by genotype-by-environment interaction. For example, alleles that increased in average frequency during the differentiation of derived populations from the ancestral population might be neutral in some populations (or even favored in others) during reverse evolution. Thus, genotypes of dif-

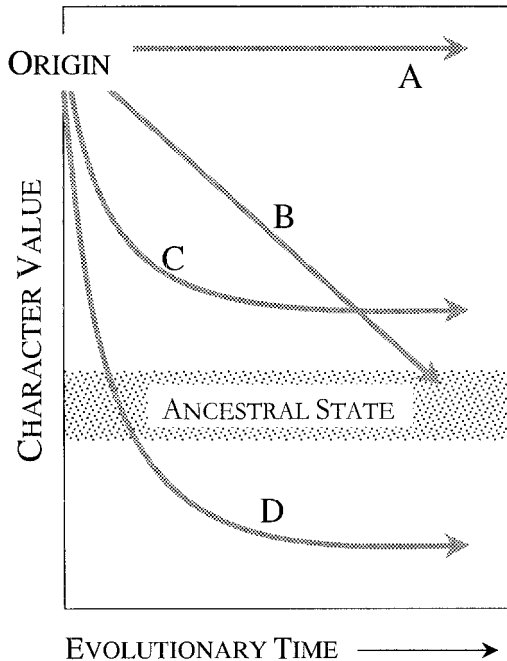


FIG. 2. Possible evolutionary responses of differentiated populations of common origin to the ancestral environment. In trajectory A, either the character is effectively neutral or there is no available genetic variability. Any change in value will be due to new mutations, and possibly some of those will be in the direction of convergence to the ancestral level. Trajectory B depicts a situation where there is response because the character is directly related to fitness, or is fitness itself, with concomitant convergence to the ancestral character state by pleiotropy. The rapidity of this response will depend either on the connectedness between the character and fitness, on the relative frequency of relevant genetic variability, or both. Trajectory C represents a two-phase response. In the first phase, a rapid response through pleiotropy with fitness is accomplished but convergence is only partial. In the second phase, either the character is effectively neutral or all standing genetic variability was exhausted during the first phase. Only the accumulation of mutations will allow full convergence during the second phase. Lastly, trajectory D shows a response that surpasses the ancestral character state, because the genetics of the derived population may allow the attainment of a previously inaccessible character level. This could be a case involving the evolution of epistasis, assuming that any variant with additive gene effects has the same probability of occurrence in both the reverse-evolved populations and the ancestor populations.

ferent history but common ancestry might be subject to different selective pressures when placed in similar environments, forestalling evolutionary return to the ancestral state (cf. Gromko 1995).

As for most of adaptive evolution, two genetic mechanisms facilitate reverse evolution: pleiotropy and mutation. When there is sufficient standing genetic variation, reverse evolution is facilitated by pleiotropy (Fig. 2), which genetically connects characters together, so that effective reverse selection on some of them will drag the other characters back to the ancestral state. When genetic variability is not available, then mutation is the only source of new genetic variation, in the absence of migration (e.g., Fong et al. 1995). The genetic effects of the novel mutations can be independent of the genotypes in which they occur, that is be additive or epistatic. Additive mutations are frequently back-mutations at loci that

have undergone fixation, whereas compensatory epistatic mutations involve alleles at other loci. Recombination, population size, and kind of genetic effect (additive or epistatic) will determine the rapidity and efficiency of fixation of these mutations (e.g., Fisher 1958; Wight 1977; Weber 1990; Burch and Chao 1999; Moore et al. 2000).

The examples reviewed below indicate that most of these genetic mechanisms arise during reverse evolution.

LABORATORY REVERSE EVOLUTION

Experimental studies of reverse evolution have rarely been done. Among the few that have been reported, most used lines that had been exposed to intense artificial selection for a morphological character. In such studies, reverse artificial selection is usually able to revert character states to levels close to the ancestral levels (reviews in Wright 1977; Falconer and Mackay 1996). This outcome, however, depends on countervailing natural selection and on the particular origin of the lines used (e.g., Robertson 1955), particularly their inbreeding, especially with sexual species. Because the genetics of these lines and their response during reverse evolution may be particular to inbred lines, we focus on examples that are not thought to depend on such problems as inbreeding depression. These examples come primarily from the *Drosophila* and microbial studies. They illustrate the degree to which reverse evolution is a tractable topic for empirical work.

Reverse Experimental Evolution with Drosophila Life Histories

One of the experimental designs for studying reverse evolution imposes the ancestral laboratory environment on populations that have since been subjected to different culture conditions for a number of generations, which requires the use of the ancestral population as a control. This can be achieved if a large sample of the ancestral population has been kept in a state of suspended animation, or if an equivalent population is accessible, such as direct descendants of the ancestral population continuously maintained under the ancestral conditions.

One such experiment was that of Service and colleagues (Service et al. 1988; Graves et al. 1992). In their studies, replicated outbred laboratory populations of *Drosophila melanogaster* that had been selected for more than 5 years for increased late-life reproduction were returned to the same culture regime as that of their common ancestral population, in which selection for early fertility had been imposed (Rose 1984; Service et al. 1988). The populations selected for late fertility were differentiated for various characters, including increased late fecundity, longevity and stress resistance, and depressed early fecundity. During the first 20 generations reverse evolution was rapid for some characters, such as starvation resistance and early fecundity, although convergence to ancestral character states was not complete (Service et al. 1988). For such characters as ethanol and desiccation resistance, the response to selection was negligible over these same 20 generations. After more than 100 generations in the ancestral environment, however, convergence to the ancestral

values became apparent in all assayed characters (Graves et al. 1992).

Partly to investigate which genetic mechanisms were behind the slow response of desiccation resistance to reverse selection, derivatives of the original late-reproducing populations were subjected to direct selection for desiccation resistance (Rose et al. 1992). These new derived populations responded immediately to selection with an increase in both desiccation resistance and ethanol resistance, suggesting that the slow response of these stress resistance characters to reverse selection was not due to an exhaustion of genetic variability, assuming that the loci determining increased resistance are those responsible for decreased resistance during reverse evolution.

If lack of genetic variability is not directly implicated in this experiment, then either epistasis is constraining the reverse evolution of desiccation and ethanol resistance or these characters were neutral when the derived populations were returned to the ancestral conditions. The slow response of these characters and their eventual convergence on ancestral values, after more than 100 generations in the ancestral environment, may have been a result of mutation accumulation, because this process is expected to affect evolution noticeably only after considerable time (Graves et al. 1992; Falconer and Mackay 1996), although there is no data as to the effect (additive or epistatic) of particular novel mutations. The rapid reversion of starvation resistance and early fecundity indicates that they were under the influence of pleiotropic alleles generating a negative genetic correlation between these two characters, a correlation demonstrated in a sibling analysis by Service and Rose (1985). As a result of this correlation, these characters rapidly moved toward their ancestral values during reverse evolution, because selection focused on early fertility. This is represented as case B of Figure 2.

Similar experiments were recently performed by us on a much larger scale, using more populations of diverse selection histories, along with a greater number of fitness-related characters (see Teotónio and Rose 2000). The ancestral environment was reimposed on these differentiated populations over 50 generations. A variety of patterns of reverse evolution were observed: rapid linear response with and without full convergence to the ancestral level, slow linear response, and a response in two phases without full convergence (see Fig. 2; Teotónio and Rose 2000).

To investigate the genetic causes of a slower response to selection and only partial convergence, hybrid populations were obtained by crossing the differentiated populations and reimposing the ancestral environment on these hybrids in parallel with uncrossed populations. This experiment enabled us to test lack of genetic variability and epistasis as mechanisms preventing full reverse evolution. If lack of genetic variability was restricting reverse evolution, randomly mating hybrid populations should be freed of this constraint, because accumulation of identical genetic changes in populations of different evolutionary history is highly unlikely. Also, if epistasis led the derived populations to converge on strong evolutionary attractors, producing stasis under reverse evolution, the large perturbation to gene frequencies caused by hybridization should allow some stalled populations to escape from these attractor states. But the results showed no

difference between uncrossed and hybrid populations (Teotónio and Rose 2000), supporting the action of neither evolutionary constraint. If lack of genetic variation and epistasis are not responsible for the observed reverse evolution patterns, then failure to impose the same selection pressures among reverse populations might be the cause for a lack of reversibility. This might arise if the specific relationship that each character has with fitness in the ancestral environment varies according to the genetic background of each differentiated population. In other words, the lack of complete reverse evolution in this study could be a case of differential genotype-by-environment interaction.

Reverse Experimental Evolution with Microbial Morphology

In *Pseudomonas fluorescens*, Rainey and Travisano (1998) have shown that mutation and frequency-dependent selection in spatially heterogeneous environments are the forces responsible for evolutionary diversification. From an original smooth ancestral morph clone, different replicated asexual populations were derived. These were allowed to evolve in a nutritionally heterogeneous environment. After 7 days, diverse colony morphologies evolved: the ancestral smooth morph, along with new wrinkly and fuzzy morphs. Each of these morphological types evolved in association with specific ecological habitats in the culture environment.

When the wrinkly-morph populations were returned to the homogeneous ancestral environment, full evolutionary convergence on the ancestral smooth morph was observed (Rainey and Travisano 1998). Relative fitness data for evolved and reverse-evolved populations reveal multiple evolutionary paths between wrinkly morphs and smooth morphs (P. Rainey, pers. comm.). The wrinkly phenotype appears to be a gain of function, involving the overexpression of a locus encoding a cellulose-like protein. Reverse evolution to smooth may occur by the loss of an allele in the developmental pathway responsible for this overexpression. This pathway is thought to involve at least 18 different genes, which explains the multiple evolutionary outcomes of reverse selection.

Reverse Experimental Evolution with Bacterial Fitness and Resistance

Lenski (1988a,b) studied the genetic basis of reverse evolution in *Escherichia coli*. In his experiments, the ancestral population was sensitive to infection by the T4 virus. Populations that were then selected for T4 resistance initially had lower competitive ability in the absence of the virus, compared to the ancestral population. Not only were these deleterious pleiotropic effects correlated with T4 resistance, but cross-resistance to other viruses evolved in the derived populations (Lenski 1988a). T4 resistant populations that were subsequently allowed to evolve for 400 generations in the absence of T4 reverted to the ancestral fitness values in the absence of T4, while retaining resistance to T4 and another virus (Lenski 1988b). The interpretation of these results was that the original maladaptive effects of resistance were compensated for by subsequent epistatic genetic changes at loci that were not involved in T4 resistance. The cost of resistance was reduced, if not fully overcome. A specific allele that

conferred increased survivorship in the reverted populations was isolated and shown to be beneficial even in the T4 sensitive, ancestral, genetic background. This particular result suggests that the reverse-evolved populations may have achieved new beneficial genetic states, not directly accessible to the ancestor population unless a phase of adaptation to the phage occurs. In terms of the trajectories of Figure 2, this could be an example of a C-type trajectory: reverse evolution occurs until there are compensatory changes that render the character neutral. The epistatic substitutions may have prevented an evolutionary return to the ancestral state.

The evolution of bacterial antibiotic resistance is another example of barriers to reverse evolution when epistasis for fitness is involved (Schrag et al. 1997; Levin et al. 2000; Moore et al. 2000; see review in Ebert 1998; Lenski 1998). In several studies it has been shown that bacterial antibiotic resistance has evolved by the acquisition of resistance plasmids along with other genetic changes. Resistance is usually correlated with a fitness reduction when antibiotics are not present, relative to sensitive bacterial populations. But given enough time in an antibiotic-free environment, resistant bacterial populations evolve genetic changes that compensate for the cost of resistance (Lenski 1998; Moore et al. 2000). Why bacterial populations tend to fix compensatory mutations instead of undergoing reverse evolution by back-mutation is not clear, but the two classes of mutation may have different rates of occurrence (see Levin et al. 2000; next example). The available data suggest that, once resistance is no longer costly, reverse evolution in antibiotic-free environments back to sensitivity is unlikely because the intermediate evolutionary stage is disadvantageous (see Fig. 3).

Reverse Experimental Evolution in Viruses

In the past few years, reverse evolution experiments have been conducted with the objective of better understanding viral adaptation to alternative hosts (see review in Ebert 1998). We discuss two such experiments.

Burch and Chao (1999) have used the RNA virus $\phi 6$ as their experimental system. A single ancestral clone was cultured through a series of severe population bottlenecks. As a result of this process a deleterious mutation was fixed in one of the experimental lineages, thus reducing fitness. Populations were derived from this low-fitness lineage and allowed to evolve for 100 generations at various population sizes. At higher population sizes, recovery of ancestral fitness levels was rapid and complete. At lower population sizes, only partial reversion to ancestral fitness was observed. Furthermore, at higher population sizes, only one genetic substitution appeared to be required for a full recovery of fitness. At smaller population sizes, several substitutions were needed to recover the ancestral fitness. Taken together, these results suggests that fully revertant back-mutations appeared at higher population sizes, but apparently not often enough to undergo substitution at lower population sizes. Compensatory epistatic mutations of less benefit appear to occur in smaller sized populations. This also supports the hypothesis that epistatic modifiers are more common than back-mutations. Isolation of these mutants, their functional characterization, and analysis of their nucleotide sequences might con-

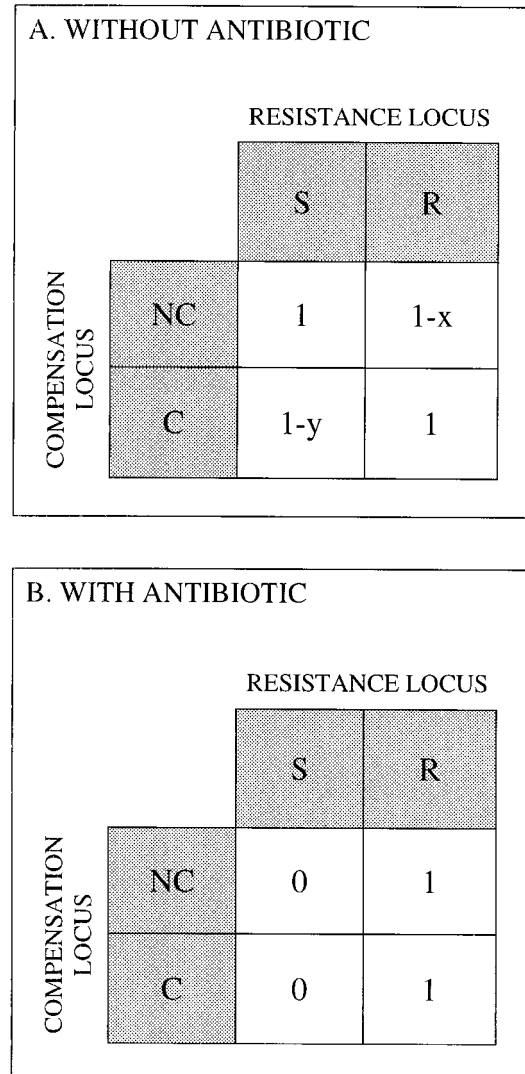


FIG. 3. A possible genetic model for the evolution of bacterial resistance to antibiotics. Gray cells in the panels specify genotypic states and white cells indicate relative fitnesses. In a haploid genotype, the resistance locus either has an R allele conferring resistance to antibiotics or an S allele conferring sensitivity; the other locus will determine compensation of fitness costs (y or x) in the antibiotic-free environment with a C allele conferring fitness increases while NC with no benefit. Epistasis for fitness occurs when the selective value of the R allele and the S allele depends on the compensatory locus allelic state, only when in the ancestral environment. Evolution takes place with a first phase of adaptation to antibiotics, where only resistant clones (with R) will be able to survive, followed by a second phase of adaptation to an antibiotic-free environment. When this type of selection sequence is used, fitness-compensatory mutations usually undergo substitution more readily than back-mutations to sensitivity (Lenski 1998; Levin et al. 2000; Moore et al. 2000). Because there is the assumption of epistasis for fitness, an evolved R/C population will be surrounded by genetic states of lower fitness. Reverse evolution to sensitivity to antibiotics will be dependent on the magnitude of the y and x cost, whereas reverse evolution of fitness itself easily occurs. The most likely evolutionary pathway thus goes from S/NC to R/NC to R/C, then being stalled. In all these transitions it is assumed that only one genotype survives.

firm that the smaller-effect mutations are compensatory-epistatic and larger-effect mutants are back-mutations (see Burch and Chao 1999, 2000). Similarly to what is observed in sexual populations, it appears that higher microbial population sizes lead to more new beneficial mutations, which selection can then exploit more efficiently (cf. Fisher 1958; Weber 1990). The reason epistasis for fitness is more important at smaller population sizes might be that more frequent epistatic mutations arise often enough for selection to act on them, even though they are not as beneficial as back-mutations, which are presumably too rare to arise in most small cultures within the period of the experiment. Similar mechanisms might be involved in the reverse evolution of phage and antibiotic resistance discussed above (for a review, see Levin et al. 2000).

Recently, Bull and colleagues (Bull et al. 1997; Crill et al. 2000) reported that the single-stranded DNA virus ϕ X174 was capable of adaptation to alternative bacterial hosts, with multiple adaptive reversals occurring when there is switching between the two hosts. An ancestral clone was first selected on a *Salmonella enterica* host, after which replicate clonal lines were selected in *Escherichia coli* (Bull et al. 1997). Serial transfer was conducted by picking from individual clonal plaques. The next step in the experiment was to propagate the resulting populations back in the first host, the *Salmonella*, and then again in the *E. coli* host (Crill et al. 2000). Adaptation to the two hosts was measured as population growth rate at low host density. Growth rates rapidly responded to selection on both hosts, regardless of previous history. This occurred independently of the number of host-switching events, that is, adaptive evolution was repeatedly reversible. Interestingly, populations whose most recent history was in *E. coli* did not lose their ability to grow efficiently in *Salmonella*, whereas survivorship in *E. coli* was reduced in clones whose most recent host had been *Salmonella*. This was also an evolutionary pattern that occurred repeatedly, with successive adaptation to alternative hosts. Complete nucleotide sequence analysis of both the ancestral and derived clones showed that, among the 55 sites that underwent substitutions, 11 were back-substitutions, a few of these repeatedly changing with adaptation (Crill et al. 2000). This illustrates the point that reverse evolution may proceed by substitutions that return genotypes to their ancestral phenotypic and (molecular) genetic condition.

Ultimately, adaptation in *P. fluorescens*, *E. coli*, ϕ 6, and ϕ X174 depends on the accumulation of novel mutations. In these organisms, the response to selection in most laboratory experiments is not dependent on recombination events because there is no standing genetic variability, because all experimental populations are usually derived from just one clone. As such, the influence of standing genetic variation on reverse evolution was not investigated. Furthermore, once beneficial mutations arise they can only be selected to fixation because the entire microbial genotype in which a particular favorable mutation occurs is selected *in toto*, because there is complete linkage disequilibrium. This situation will favor the long-term evolution of epistatic interactions because selection will act on all components of V_G (additive, dominant, and epistatic variance components), which in turn might generate absorbing boundaries for adaptive evolution (Bull and

Charnov 1985; Kondrashov 1988; Moore et al. 2000). We predict that when such epistasis is involved, reverse evolution becomes less likely.

NATURAL REVERSE EVOLUTION

There are few instances where reverse evolution has been observed outside the laboratory. Perhaps the best characterized example is the adaptation of the peppered moth, *Biston betularia*, to increased and decreased levels of air pollution in industrialized areas (see reviews in Kettlewell 1973; Majerus 1998).

As is common among many moth species, the peppered moth has several color morphs that grade from a pale (typica) form to a dark (carbonaria) form, with several intermediate (insularia) forms. This melanistic polymorphism involves one locus with five alleles, the darkening alleles generally dominant (Majerus 1998). Although several other loci are known to affect melanism, most of the variation is explained by this one locus, which gives this character a fairly simple genetic basis.

As the levels of air pollution rose during the second half of the 19th century, British populations of the peppered moth changed from being mainly pale typica forms to primarily dark carbonaria forms (Kettlewell 1973; Majerus 1998). This response to pollution in the frequency of melanistic forms has also been observed in the Netherlands (Kettlewell 1973), central Europe (Majerus 1998), and the United States (Grant et al. 1996). With the enactment of laws to reduce air pollution during the 1950s, the British environment gradually returned to ancestral conditions, that is, to lower levels of air pollution. This was followed by a decline in the frequency of darker melanistic forms with a concomitant rise in the frequency of paler forms (Clarke et al. 1985, 1994; Majerus 1998; Cook et al. 1999). Again, this reverse evolution of melanism has been observed in other geographic areas as well (Grant et al. 1996; Brakefield and Liebert 2000).

The selective mechanisms behind the rise and then fall in the frequency of melanistic forms with air pollution have not been fully understood, although it is known that melanism affects moth crypsis and thus escape from visual predation by birds. Other selective mechanisms that might be involved include differential preadult viability and migratory rate differences between the several melanistic forms. There is some evidence that the genetics of melanistic reverse evolution may differ between Britain and the Netherlands. In the latter case, several intermediate insularia forms have increased in frequency, the paler forms gradually replacing darker ones, with maintenance of considerable polymorphism (Brakefield and Liebert 2000). This is unlike the reverse-evolved British moth populations, which appear to have replaced the carbonaria dark form with the typica, effectively replacing the carbonaria allele by the typica allele, the insularia intermediates never occurring at significant frequencies. These differences might not reflect different patterns of selection. A possibility is that these different outcomes of reverse evolution are merely a reflection of differences in initial standing genetic variability. For example, in the British populations only the two extreme alleles carbonaria and typica may have been common, where-

as in the Netherlands all five alleles could have been relatively common.

The example of melanism in *B. betularia* is the best natural example of reverse evolution in which an environmental change has been followed closely by a phenotypic change and that has been naturally replicated several times. Reverse evolution has occurred because sufficient genetic variability was maintained, adaptive evolution being merely a reflection of a change in the frequency of relevant alleles.

FINAL COMMENTS

Although the occurrence of reverse evolution in the short term might seem obvious to some, its empirical features remain somewhat equivocal. Only a few generalizations can be made. First, reverse evolution of fitness and characters of varying relatedness to fitness does occur on small evolutionary time scales. Second, although natural selection can lessen the effects of past history by promoting adaptation, these effects are not always obliterated. Reverse evolution is contingent on previous history. Third, the genetic mechanisms involved during reverse evolution are pleiotropy of gene effects between the diversifying environments and the ancestral environment and mutation accumulation. In asexual populations, this latter mechanism is the only one that has been observed because no experimental tests on standing genetic variation have been done. Fourth, in asexual populations, reverse evolution involved, in part, the evolution of new epistatic interactions, mitigating the phenotypic effects of previous evolution. In sexual populations standing genetic variation allows reverse evolution to occur, while differential genotype-by-environment interactions evolved during previous evolution can prevent it. Several problems remain in need of proper theoretical treatment and empirical evaluation, among them the following.

What is the role of sex and sexual reproduction?—This question not only bears on reverse evolution but on any kind of evolutionary process. It is expected that sexual reproduction will accelerate reverse evolution if independent lineages have accumulated different genetic changes and are able to intercross (see Teotónio and Rose 2000). An experimental comparison between asexual and sexual lineages is lacking, however. Are the observed conditions for irreversibility in sexual populations (e.g., genotype-by-environment interaction) or reversibility (maintenance of genetic variation) similar in asexual populations? What is the role of standing genetic variation in asexual populations? Is epistasis an important factor in the reverse evolution of sexual lineages (cf. Wright 1977; Whitlock et al. 1995)?

At what level should reversibility be studied?—Reversion may be complete at one level of biological organization, while at another level it may not follow the same pattern. The studies of Bull and colleagues (Bull et al. 1997; Crill et al. 2000) are remarkable because some phenotypic reversions coincide with nucleotide reversions. But how general will these patterns be? There is, of course, no one level at which to study the reversibility of evolution; it will depend on one's interest.

How long before evolution becomes irreversible?—To our knowledge there have been few attempts to experimentally

address this problem. Lande (1978) estimated that the capacity to reevolve atavistic hind limbs in cetaceans is retained for 10^6 to 10^7 generations. But irreversibility can occur over very short time spans, especially when it involves the dramatic reorganization of the genetic system, such as the evolution of polyploidy in just one generation (cf. Simpson 1953; Bull and Charnov 1985). The experiments discussed here also suggest that reverse evolution might be impeded even over short time spans.

Is future evolution constrained?—Even if reverse evolution back to the ancestral character states can be achieved, the genetics of the reverse-evolved populations may be different from that of their ancestor population. In this case, the future evolution of reverse-evolved populations and the contemporaneous equivalents to their ancestors might differ, even under identical environmental conditions (cf. Cohan 1984). In some of the examples discussed here, especially adaptation of *Pseudomonas* and ϕ X174 to alternative environments, reverse adaptive evolution does not appear to be limited by history, at least for the characters studied.

There are undoubtedly more questions that will arise. Reverse evolution is both a research tool and a research problem in itself. Complementing the study of standing variation and forward selection, reverse experimental evolution provides a useful third avenue for evolutionary research.

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