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Experimental macroevolution†

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The convergence of several disparate research programmes raises the possibility that the long-term evolutionary processes of innovation and radiation may become amenable to laboratory experimentation. Ancestors might be resurrected directly from naturally stored propagules or tissues, or indirectly from the expression of ancestral genes in contemporary genomes. New kinds of organisms might be evolved through artificial selection of major developmental genes. Adaptive radiation can be studied by mimicking major ecological transitions in the laboratory. All of these possibilities are subject to severe quantitative and qualitative limitations. In some cases, however, laboratory experiments may be capable of illuminating the processes responsible for the evolution of new kinds of organisms.

1. Introduction

Evolutionary change takes place only very slowly over vast periods of time, according to the traditional view that dominated biology throughout the first century after the *Origin of species*. This view was challenged by the demonstration of strong natural selection in snails and moths in the 1950s, but the classical view was rescued by creating a distinction between the concepts of 'microevolution' and 'macroevolution'. The earliest use of these terms that I can trace was by Philiptschenko [1], as quoted by Medvedev [2], who used them to distinguish between evolution within species driven by natural selection among 'those mutations with which geneticists mainly deal' and evolution above the species level driven by variation in embryonic development, which mirrors a debate earlier in the century about the roles of gradual and salutatory change in evolution. Microevolution involves shifts in allele frequencies, driven by natural selection, that cause quantitative changes in phenotype within short periods of time. Qualitative change, resulting in the evolution of new kinds of organism, arises from a process of macroevolution, which takes place over much longer periods of time and may involve processes other than straightforward natural selection acting on allelic variation.

The terms were retained by Dobzhansky [3], but purely as a matter of convenience, and without acknowledging any difference in the mechanism of evolutionary change at different phylogenetic levels. The contrary position was advocated by Goldschmidt [4], who argued that 'the facts of microevolution do not suffice for an understanding of macroevolution'. This controversy has continued down to recent times (see [5]), although the terms themselves and the dividing line between them at the level of species are retained in most current textbooks.

According to the conventional view, microevolution is confined to the species boundary, or more precisely within the current range of variation of the population or set of populations. Adaptation by natural selection proceeds through the differential proliferation of lineages with different allelic states, whose frequency and fitness can, in principle, be estimated, and is therefore predictable, parallel and repeatable. Adaptation is predictable, because its outcome is determined by current variation; parallel, because ancestral populations with different allele frequencies will converge; and repeatable, because ancestral populations with identical allele frequencies will not diverge. Macroevolution is not constrained in the same way, and adaptation can transcend the species boundary or current range of variation. Macroevolution involves morphological innovations leading to new kinds of organism and major ecological transitions leading to qualitative changes in global community composition. It may be strongly influenced by

ancestry, history and chance, and consequently may be neither predictable, nor parallel, nor repeatable.

The distinction between microevolution and macroevolution echoes the parallel distinction between ecological time and evolutionary time. Within short periods of time, species fluctuate in abundance, leading to changes in community composition, but the fundamental ecological attributes of species are conserved and are liable to change only over much longer geological stretches of time. This distinction neatly separated ecology from evolution and allowed them to develop independently as different subjects, which they have done until the recent development of ecoevolutionary dynamics [6] and evolutionary rescue [7]. By the same token, an experimental approach to macroevolution was ruled out, because no interesting experiments could be completed within realistic periods of time. It would therefore be impracticable to investigate the mechanisms of macroevolution using the kinds of laboratory experiments that have been so successful in the study of microevolution [8–11], leaving a permanent gap in our understanding of evolutionary processes.

If the quite subjective distinction between microevolution and macroevolution is allowed, then there are two kinds of event that might be grouped under the head of macroevolution. The first comes about when some morphological innovation itself precipitates an adaptive radiation. The focus of interest is the innovation itself, and the experimental problem is to travel backwards in time to recover the ancestral state of modern forms. Feathers are a familiar example of an innovation: by making flapping flight possible, they led to new ways of life that terrestrial archosaurs could never follow. Innovations may lead to adaptive radiation either because they are themselves capable of extensive functional modification (like birds' beaks) or because they enable other features to become extensively modified (like birds' feathers). They are often unique derived characters that define large clades; other examples of innovative structures are cnidocytes, stereom and wood. All are examples of macroevolution because they are thought to have evolved through cumulative change over very long periods of time, rather than arising repeatedly, in more or less their current form, within contemporary populations.

The second kind of event occurs when a new ecological opportunity leads to an adaptive radiation. In this case, the focus of interest is the adaptive radiation itself, or at least the potential for radiation, and the experimental problem is to travel forwards in time by creating a major ecological transition. In the simplest case, a population enters a biologically depauperate locality or habitat and proceeds to diversify, like the gammarid amphipods of Lake Baikal or the moa of New Zealand. Accidental or deliberate introductions of exotic species by human agency are commonplace, and introduced species may evolve in response to their new conditions [12], but none are older than the invention of long-distance sailing ships, and no radiations have yet been observed. Alternatively, any particular way of life is likely to be governed by some suite of characters, and when they are modified a new way of life may evolve. These include the contrasting suites of characters responsible for benthic versus pelagic habit; autotrophic versus heterotrophic; free-living versus parasitic; direct versus indirect development; marine versus freshwater or unicellular versus multicellular. Switching from one to the other, in either direction, constitutes a major ecological transition. This is followed by an episode of expansion and diversification that varies widely among lineages for reasons that are not understood. The evolution of freshwater forms from marine ancestors, for example, may never occur (echinoderms); may yield only rare and local populations (elasmobranchs); may lead to abundant and widespread populations but very few species (hydrozoans) or may result in the radiation of extremely diverse clades exploiting a variety of habitats (gastropods). Although the outcome differs from case to case, the new spectrum of ecological opportunities that becomes available after a major transition has often contributed to macroevolution by provoking extensive adaptive radiations leading to qualitative changes in community composition.

Innovation and radiation have been extensively investigated through comparative methods, which provide a means of describing patterns but cannot conclusively demonstrate processes. Experimental methods uncover processes, but their application to evolution is restricted to short timescales. This dilemma appears to permanently limit our understanding of evolution by creating an unbridgeable gap between macroevolution and microevolution. The purpose of this essay is to explore the outer limits of experimentation in evolutionary biology, and if possible to identify circumstances in which experimentation can be used to illuminate the processes of innovation and radiation.

2. Resurrecting ancestors

The most effective way of studying evolution would be to bring the fossils back to life. This is normally impossible, of course, but there are a few special cases in which it may be feasible, at least in an indirect or partial fashion. There are two possible approaches: the direct resurrection of ancestral lineages, or the physiological manipulation of modern lineages to recreate the ancestral state.

(a) Reviving fossils

Individual cells, or even whole organisms, can enter a state of suspended animation after dehydration by evaporation, osmosis or freezing and remain metabolically inactive but viable for long periods of time. This provides—in principle—access to ancestral types, or even to a complete time series documenting the evolutionary history of a lineage. Adults, embryos, seeds and spores may all survive for considerable periods of time, and might, in principle, provide a living 'fossil record' of evolutionary change.

Bdelloid rotifers and tardigrades inhabit transient water films and survive as encysted anhydrobiotic adults when these dry up; they can subsequently recover full activity when rehydrated. Although there are anecdotal reports of survival for very long periods of time (see [13]), systematic investigations show that very few individuals survive for more than 5 years [14]. Moreover, the age of the animals can be ascertained only in artificial circumstances, such as material from dated herbarium sheets.

Monogonont rotifers and cladocerans produce dormant embryos at the end of the growing season that accumulate in lake sediments. Unlike anhydrobiotic adults, these embryos are provided with thick membranes that protect them from oxidation and can preserve vitality for many years. In lakes with varved sediments, a time series of dormant propagules can be isolated from cores, with some assurance that the sediments have not been mixed by burrowing animals ([15,16]; reviewed

by Orsini *et al*. [17]). Germinating the dormant embryos ('ephippia') of cladocerans has made it possible to trace rapid evolutionary change [18], for example adaptation to historical eutrophication during the past 700 years [19,20].

The spores of bacteria and fungi can survive in frozen soil for long periods of time, and when revived provide ancient genotypes and phenotypes dating back at least 5000 years (see [21]). For example, isolates from permafrost cores have been used to document antibiotic resistance in bacteria long before the therapeutic use of antibiotics [22,23]. The bacteria living in permanently frozen soil, however, are capable of low rates of metabolism and growth at temperatures as low as −15° C [24]. These communities are therefore not necessarily perfectly quiescent, although their rate of evolution must be extremely low.

There are records in the literature describing the successful resurrection of much older fossils. Whole living *Silene* plants have been regenerated from tissue 30 000 years old [25]. Very ancient isolates, such as bacteria revived from frozen soils 3.5 Myr old [26] or from amber 40 Myr old [27], are more controversial.

(b) Engineering individuals from fossil genomes

If the fossils are dead, then their genomes may nevertheless survive intact. It would then be possible, in principle, to express this genome in a surrogate enucleated oocyte from a related species, and recover the extinct species as the F1. It has been reported, for example, that nuclei from the tissue of mammoths, about 15 000 years old, survive in the cytoplasm of enucleated mouse oocytes [28]. The ethics of 'de-extinction' have been debated at length [29], somewhat in advance of solutions to the formidable technical problems that remain to be overcome.

(c) Reconstructing ancestral genes

Cladistic methods have long been used in phylogenetic analyses to infer the ancestral states of phenotypes and genotypes. In recent years, it has become feasible to test such predictions by synthesizing a presumed ancestral gene, inserting it into an appropriate model organism and observing the phenotype that it produces. This is an elegant approach that frees experimental evolution from the usual constraints of time, because it is possible, at least in principle, to reconstruct ancestral gene sequences of any antiquity, however remote (see [30–32]).

Opsins are light-absorbing proteins which act as the primary signal generators in the vertebrate visual system. There are many kinds of opsin, each absorbing photons most effectively at a wavelength that is determined by its amino acid sequence. When the inferred ancestral gene of mammals is synthesized and expressed in cultured cells, the opsin absorbs maximally in red light. In contemporary species, red and green opsins differ at five sites. When these mutations are inserted into the ancestral sequence, they produce a fully functional green opsin; moreover, inserting different combinations of these mutations enables their individual effects and interactions to be estimated [33]. This provides a powerful means of discovering how structure affects function and how contemporary proteins have evolved. Comparable studies have elucidated the evolution of steroid receptors in vertebrates [34] and fluorescent proteins in corals [35].

The same approach can be used to reconstruct the evolution of complex molecular machines. The vacuolar ATPase of eukaryotes incorporates a rotatory device, consisting of six subunits linked together in a ring, that pumps protons across the vacuole membrane. Among unikonts, there are two kinds of subunit, arranged in a particular way, that arose from a gene duplication early in eukaryote history; fungi also have a third kind of subunit, which is inferred to have evolved from a second gene duplication after the separation of fungal from animal lineages. Finnegan *et al*. [36] showed that the inferred state of this gene, before duplication, in the last common ancestor of animals and fungi, can rescue a yeast strain in which both the derived kinds of subunit have been deleted. They argued that the derived subunits have evolved through complementary losses of function involving the ability of the subunits to form specific interfaces with one another, and tested this idea by constructing gene fusions that constrained the pattern of interaction between subunits. This confirmed that the ancestral protein could function in any configuration, whereas the derived proteins required particular, and predictable, configurations in order to be active. Knowing the genetic differences between the derived subunits, it was then possible to introduce specific mutations into the ancestral sequence and identify those responsible for the partition of function between the derived subunits. The complete evolutionary trajectory of the contemporary fungal proton pump could then be reconstructed.

(d) Homeotic mutations

Even the most fundamental features of body plans can be altered by inducing mutations or intervening in development, as geneticists and experimental embryologists have demonstrated over the last century.

There is a remarkable class of 'homeotic' mutations in which the features that define an extensive clade are modified so as to recapitulate the ancestral state, or to produce a novel conformation. In the snapdragon *Antirrhinum*, for example, the flowers are borne on an indeterminate inflorescence, and each consists of four whorls, bearing, from outside in, the sepals, petals (partly fused to form a corolla tube), stamens and carpels. This design can be dramatically modified by mutation, however, such that the normal contents of one whorl are repeated in another (for example, by substituting separated sepals for fused petals in the second whorl); or the form of the flower is altered (for example, towards a more symmetrical shape by making the three lower lobes of the corolla similar) or the pattern of flowering is changed (for example, by switching to a determinate inflorescence terminated by a flower of unusual shape). There is a well-known series of homeotic mutations in the fruit fly *Drosophila* which modify the characteristic pattern of appendages borne on the thoracic segments. The normal dipteran condition of a single pair of wings followed by a pair of halteres, for example, can be replaced by two pairs of functional wings, recapitulating the ancestral condition of pterygote insects.

(e) Physiological modification

Bodies can also be altered by intervention later in development. The wingless worker caste of ants is differentiated in many species into the minor workers, which forage for food, and soldiers, which defend the nest. In some species of *Pheidole*, there is a third caste of supersoldiers, which have markedly larger bodies and hypertrophied jaws. The production of supersoldiers is likely to be the ancestral state, because it occurs in the

most deeply branching species that is the sister taxon to the rest of the genus. Other supersoldier-producing species are only distantly related to one another, suggesting that this capacity was lost early in the radiation of the genus but then re-evolved in a few lineages. Supersoldiers can be produced, however, even by species that normally lack them, by hormonal treatment applied after the developmental switch separating queen from worker, but before the switch separating minor worker from soldier [37]. This developmental pathway had not been lost, therefore, but rather retained as a cryptic phenotype that could still be expressed by exposure to particular conditions during development.

3. Anticipating descendants

(a) Artificial selection

The innovations that characterize major groups and evolve over long periods of time seem to lie permanently beyond the limit of experimentation because they lie beyond the limit of variation expected to exist in any ancestral population. This may not be a conclusive objection, however, because the potential range of variation may greatly exceed the standing variation actually expressed at any given time. Artificial selection can readily shift populations far beyond the limit of variation expressed by their ancestors—the 'sorting horizon'. A classic experiment by Clayton & Robertson [38] showed that selection for the number of sternopleural chaetae in *Drosophila* resulted in an advance of about 20 phenotypic standard deviations within 35 generations. Similar advances have been made in economically desirable characters of crop plants and livestock as the result of the systematic application of artificial selection. The evolved lines are, to be sure, little more than exaggerated versions of their ancestor. Many domesticated plants and animals differ radically from their wild ancestors, however, to the extent that they would be unhesitatingly classified as distinct species if their history were not known. In these cases, artificial selection, or unconscious selection in a humanized environment, has produced extreme modification of body form.

More ambitious experiments using artificial selection to alter more fundamental features of bodies do not seem to have been attempted yet. Homeotic mutations have been very extensively used to investigate the genetic basis of development, but have not yet been fully exploited to study the experimental evolution of new kinds of organism, although many are viable and fertile.

(b) Natural selection

Natural selection does not often produce such rapid and extensive transformations as artificial selection, because it is neither as strong nor as specific. Microbial systems allow us to circumvent both difficulties. Large populations of short-lived organisms often respond to natural selection within a short period of time; for this reason, most experiments concern microbes—bacteria, algae and yeast—although small animals and plants can also be used. The evolutionary processes that produce adaptation, such as the appearance, passage and interaction of beneficial mutations, can then be isolated, manipulated and evaluated more reliably, more precisely and more accurately than would be possible in the field, or by using comparative data. The usefulness of these simple model systems depends on the existence of general mechanisms of evolution that can be analysed in microbial populations and then used to interpret events that have taken place in larger organisms over much longer periods of time.

(c) Capturing very rare or unexpected events

The use of microbes with large population sizes and short generation times makes it possible to observe even very rare events. *Escherichia coli* cannot use citrate aerobically, and indeed, this inability helps to diagnose the species. Citrate utilization eventually evolved nevertheless in long-term experimental lines of *E. coli* grown in glucose-minimal medium to which citrate is added as a buffer [39,40]. This ability evolved in one of 12 experimental lines after about 30 000 generations of culture. Given the growth of the lines from inoculation (about 10^7 cells) to transfer (about 10^9 cells), and a genomic mutation rate of 0.01 per division, about 3×10^{10} mutations would have appeared in the lines before the appearance of citrate metabolism. Experiments that screen such large numbers of mutations are able to isolate and amplify phenotypes that in larger organisms would evolve only after much longer periods amounting to tens of thousands of years or more in calendar time.

Bacteria that enter the body through cuts and grazes are usually killed by the antimicrobial peptides of the innate immune system. These have been regarded as good candidates for using therapeutically as antibiotics, because they target a basic attribute of the bacterial cell membrane, its net negative charge. This is a property of the phospholipids on the outer surface of the membrane, and because this is a highly conserved feature, it is unlikely to evolve in response to exposure to artificial peptides [41]. Nevertheless, resistance evolved rather rapidly and repeatably in laboratory populations of bacteria [42], probably through modification of membrane phospholipids (Schwarz-Linek, Perron & Bell 2014, unpublished data). Even highly conserved features of organisms, for which variation might be thought minimal, may respond to natural selection with unexpected ease in laboratory experiments of modest scale.

Experiments like this show that qualitative changes in metabolic and structural characters evolve quite readily in experimental populations of bacteria. The main features of evolutionary change are the same in the laboratory and the field. A minimal capacity to exploit a novel substrate must exist in the ancestor; evolution proceeds through modification, not invention. This minimal capacity may simply arise from the fact that enzymes are not completely specific. Intermediate stages in adaptation leave their footprint in evolved lines, emphasizing the cumulative, historical nature of evolutionary change. Finally, evolved lines are often impaired or inviable in the ancestral environment, so that adaptation results in the complete ecological segregation of two divergently specialized populations. However, these adaptations, however unexpected, do not necessarily lead to a broad range of new ecological capabilities. Lineages that are able to use citrate or resist antimicrobial peptides have acquired a new specialization but do not necessarily have any greater potential for diversification.

(d) Evolution of heterotrophy in autotrophic algae

The evolution of a novel metabolic capacity may create novel ecological opportunities and thereby lead to the subsequent

radiation of new kinds of organism. Heterotrophy and autotrophy are strongly contrasted ways of life normally associated with different kinds of organism that play different roles in ecosystems. They are driven by different metabolic pathways, involving the Krebs cycle in heterotrophic microbes and the Calvin cycle in photoautotrophs. These are located primarily in different cellular compartments, the mitochondrion and chloroplast, respectively. Some unicellular eukaryotes, such as dinoflagellates, are mixotrophs that use both systems (the Krebs cycle is, of course, a universal feature of aerobic metabolism). The unicellular green alga *Chlamydomonas* is mixotrophic by virtue of its ability to take up and metabolize acetate, even in the dark. Dark growth is normally much slower than growth in the light driven by photosynthesis; many cells in laboratory cultures long maintained in the light are incapable of growing at all in the dark. When experimental populations are kept permanently in the dark, however, they may evolve efficient heterotrophic growth within a few hundred generations. They grow many times faster than their ancestors, and rapidly overwhelm them when competing in mixtures [43]. Some of the evolved lines require acetate for growth and are unable to grow in the light unless acetate is supplied. Others actually shun the light: they die when exposed to light, whether or not they are provided with acetate. Hence, experimental lines descending from a primarily photoautotrophic ancestor can evolve rapidly into obligate heterotrophs. Under natural conditions, ancestral and evolved lineages would live in different habitats, would occupy different trophic compartments, and would evolve independently as different species, because they would never meet.

(e) Evolution of novel endosymbionts

Eukaryotes harbour a variety of endosymbionts whose ancestors were free-living bacteria or algae. Most of them, such as mitochondria or the chloroplasts of land plants, are highly derived structures with reduced genomes. At the other extreme are the intact algal endosymbionts of organisms such as ciliates, corals and acoels. This range of variation suggests that it might be possible to reconstruct the initial stages of endosymbiosis in the laboratory. This is illustrated by the accidental infection of a laboratory population of *Amoeba* with a pathogenic bacterium which multiplied inside its host, eventually killing it and lysing the cell to release bacteria which proceeded to infect new hosts. A few host individuals were tolerant, maintaining populations of bacteria in their cytoplasm without succumbing to the infection. Tolerant individuals passed on their resident bacteria to their progeny when they divided. The vertical transmission of these bacteria should favour the evolution of mutualism, because the interests of symbiont and host are linked. After about 100 host generations, some lineages of *Amoeba* were unable to grow successfully if their resident bacteria were removed by treatment with antibiotics [44]. At this point, the association had become obligate, or nearly so, opening the way to a close integration of metabolism between the partners.

(f) Evolution of marine tolerance in freshwater algae

Chlamydomonas is a freshwater alga with low tolerance for salt. The growth in laboratory populations is halved at 5 g l^{-1} NaCl and almost completely suppressed above about 15 g l^{-1} [45]. When populations are transferred to a medium of gradually increasing salt concentration over many growth cycles, however, these limits can be transcended [46]. A very small fraction of lines even become capable of continued growth in medium with the salt concentration of seawater, as the result of both epigenetic and genetic modifications [47]. These modifications may be no more radical than those which confer a high level of tolerance to some particular stress, such as antibiotics or herbicides, which merely permits a population to resume a normal level of activity in its accustomed way of life. The tolerance of marine conditions, on the other hand, would open up an entirely new range of ecological opportunities were it to evolve in a natural population. The saltmarsh grass *Spartina*, for example, has recently evolved the ability to occupy the lower, fully marine, section of the marsh following hybridization between European and North American species [48].

(g) Evolution of multicellularity in unicellular eukaryotes

Chlamydomonas belongs to a group, the Volvocales, in which multicellularity, with division of labour between germ line and soma, has evolved independently in several lineages, giving rise to relatively large organisms such as *Volvox*. This suggests that even the dramatic transition between unicellular and multicellular form may occur readily enough to be elicited by experimental evolution. One of the potential benefits to large size is protection from ciliary-stream predators such as rotifers and ciliates. Unicellular eukaryotes such as *Chlamydomonas* will often adopt a palmelloid form consisting of large unorganized clumps or sheets of cells when they detect the effluent of predators. In the longer term, they may evolve regular multicellular bodies. When the unicellular chlorophyte *Chlorella* was cultured in the presence of *Ochromonas*, a predatory chrysophyte, regular colonies of eight cells evolved within about a month [49]. These colonies were almost completely invulnerable to predation. These were truly multicellular, because each cell itself reproduced by developing into a new colony.

Ratcliffe *et al*. [50] simply selected for rapid sedimentation in yeast populations and very soon obtained large clusters of cells that reproduced by fragmentation. Colony fission is accomplished the apoptosis of cells inside the cluster, which can be viewed as an unusual kind of reproductive specialization.

(h) The experimental origin of species

Divergent lineages that are morphologically or ecologically distinct and (if eukaryotes) sexually isolated may be identified as separate species and given different names. Species formation seems to be a common process in nature, because its early stages have been repeatedly observed in birds, fishes, insects and other organisms. The extent of phenotypic differentiation arising in the laboratory, for example the citrate-using strains of *E. coli*, may approach or exceed the threshold at which a new binomial is usually conferred. The sexual isolation that is the hallmark of eukaryote species has seldom been the target of experimental evolution. It can readily be produced as a consequence of selection for habitat choice. *Drosophila* populations were offered a series of binary choices between environmental cues, such as odours and light [51]. Each sequence of preferences led to a different oviposition chamber, but the progeny was discarded from all except two. The population quickly evolved a strong preference for these two chambers alone, with offspring returning to the

parental site. This leads to almost complete sexual isolation between the two subpopulations, although the flies mate randomly if they are deliberately mixed. Another approach is to apply divergent natural selection to isolated populations and evaluate sexual isolation when they are mixed. Some degree of partial isolation, usually through hybrid inferiority, was reported from *Drosophila* experiments in the 1960s and 1970s [52,53]. More recently, some degree of isolation was reported from experimental yeast populations as a by-product of divergent adaptation to high-salt and glucose-minimal medium [54]. Hybrids between lines selected in different environments had reduced rates of vegetative growth and sporulation. This creates partial isolation between the divergent lines, creating the potential for sexual selection favouring assortative mating and thereby leading to complete isolation.

Saccharomyces cerevisiae and *S. paradoxus* are two closely related yeasts that are found together and mate readily, but remain separate, because almost all the hybrids are inviable. The small minority of viable hybrids can readily be isolated in the laboratory, because of the very large population sizes that can be maintained, and quickly give rise to new hybrid species [55]. First, the hybrid haploid spores were selfed to give diploid cells. These sporulated readily and yielded a high frequency of viable spores, showing that they were proficient in both meiosis and mitosis. When backcrossed to either parent, however, the great majority of spores were inviable. Crossing these fertile diploid lines and selfing the resulting spores gave a second diploid generation whose fertility was further enhanced. These hybrid strains grow normally at low and high temperature and are not obviously impaired in any way. Their success seems to depend on particular combinations of chromosomes from the two ancestral species which express severe genetic incompatibilities when backcrossed to either species. In this way, almost complete sexual isolation evolved rapidly through the selection of a very small minority of viable hybrids.

4. Limitations of experimental macroevolution

There are now several cases in which experimental populations have evolved the distinctive ecological attributes, and sometimes, a degree of sexual isolation normally associated with species formation, and has thus passed the fringe, at least, of macroevolution. This may not satisfy everyone that experimental macroevolution is either an accomplished reality or even an interesting possibility. There are both quantitative and qualitative limitations to the evolutionary changes that can be observed in the laboratory. The quantitative limitations involve time: the length of time over which ancestors can survive or descendants can be propagated. The qualitative limitations involve characters: the attributes that interest most evolutionary biologists—chiefly morphological attributes of animals and plants—are difficult to study in most kinds of experiment, especially if microbial systems are used.

(a) Quantitative limits in time

The survival of ancestors, even under the most favourable conditions, is inexorably limited by the physical and chemical stability of biological molecules, especially DNA and proteins. Despite ambitious claims, it may never be possible to revive dormant organisms more than a few tens of thousands of years old.

It may also be difficult to project lineages more than a few tens of thousands of generations into the future. All products of replication vary and are potentially exposed to selection, so the number of replications sets one kind of limit to the rate of evolutionary change. The annual number of replications for any given species will be the product of its total abundance, the number of litters produced per year and the number of offspring per litter. This is unexpectedly difficult to calculate: the last two quantities can usually be estimated quite precisely, but the overall abundance of a species, as opposed to its population density, is scarcely ever estimated (unless it is on the verge of extinction). There appears to be no sound estimate, for example, of the overall abundance of as familiar a species as *Mus musculus*. Suppose this to be 10^{10}, on the dubious assumption that there is about one mouse per person; with five litters of 20 offspring each per year this will yield 10^{12} replications per year. Taking the average longevity of a mammal species to be 5 Myr, the total number of replications in the *M. musculus* lineage will be 5×10^{18}. The allometric relation between body size and maximum population density leads to corresponding estimates for *Arabidopsis thaliana* of 2×10^{21}; *Daphnia pulex* of 2×10^{22} and *Drosophila melanogaster* of 10^{24}, bearing in mind that these figures are all based on an untested guess of the world population of mice. Much more reliable estimates can be made for experimental populations: for the long-term Lenski lines of *E. coli*, up to the appearance of citrate metabolism, the overall number of replications is about 5×10^{14}, and for my heterotrophic lines of *Chlamydomonas*, about two orders of magnitude fewer. Hence, unless the overall numbers of very abundant species are much less (many orders of magnitude less) than I have supposed, there is a wide gap between the extent of laboratory experiments and the evolutionary potential of common species. It is difficult to see how this gap can be substantially narrowed for experiments on cellular organisms.

(b) Qualitative limits

'Macroevolution' usually refers to the evolution of morphological innovations in multicellular organisms. Microbial experiments may certainly illuminate the evolution of such basic features as metabolism, photosynthesis, phagocytosis, flagellar locomotion and endosymbiosis. They may also establish whether macroevolution requires mechanisms distinct from those involved in microevolution, because this can be legitimately investigated in microbial model systems. Nevertheless, the reservation will remain, and will be overcome only if suitable plant or animal model systems can be found. Even if these systems can be developed, then they will inevitably face even more restrictive quantitative limitations than microbial systems.

(c) Overcoming limitations

Some of these limitations represent physical limits to possible experiments. Others may eventually be overcome, however, using existing or future technologies.

The reconstruction of ancestral genes is less affected by quantitative or qualitative limitations than other techniques, and provides a powerful means of analysing the evolution of animals and plants over very long periods of time. It might be extended, moreover, to the reconstruction of the entire genome of the last common ancestor of a given set of contemporary taxa. This would be a very laborious

undertaking, but no more laborious, perhaps, than the sequencing of the entire genome of a eukaryote appeared to be 25 years ago.

The extent of evolution experiments can be increased by automation. Most experiments have until now comprised only a few (of order 10) replicate lines, but liquid-transfer robots make it feasible to employ many more (of the order of 10 000 at least). This does not directly overcome the time constraint, but increases the range of variation and the number of independent lineages that can be screened.

The potential for far-reaching modification is revealed by the unexpected phenotypes found in extensive screens. These have been exploited by practical breeders to produce distinctively divergent forms, such as modern maize from teosinte or Yorkshire terriers from grey wolves, but have not so far been used for evolution experiments. There may be an unappreciated opportunity to use cryptic 'macrovariation' in structure and development to evolve new kinds of organism in the laboratory. This could readily be harvested by artificial selection, or even through natural selection if conditions of growth could be manipulated to ensure the rapid magnification of rare variants.

The limitations of cellular organisms can be overcome by using non-cellular replicators, if the hypothesis to be tested concerns general evolutionary mechanisms rather than particular attributes of organisms. Viruses have been extensively used to investigate evolutionary processes [56–58] and could increase the rate of transfer by about an order of magnitude. It is possible to imagine a model DNA molecule specifically engineered to optimize its potential for investigating evolution, but so far as I know this has not yet been done. A more radical approach is to use self-replicating algorithms to investigate macroevolutionary processes [59,60]. This completely circumvents any time limit, at the cost of restricting investigations to the most general features of evolution.

The major events in the history of life happened long ago; vastly longer than the human lifespan or human history. We may never be able to recreate them, and they are certainly beyond the reach of current technology. There appears, however, to be some limited opportunity to illuminate some aspects of innovation and radiation through 'referred experimentation', by which events in simple and tractable model systems usefully represent comparable events that take place over much longer periods of time. The reconstruction of ancient proteins and the experimental study of major ecological transitions seem to be particularly promising techniques for elucidating macroevolutionary mechanisms. I have indicated some of the severe limitations that must apply to such experiments. Nevertheless, the basis of a research agenda has been assembled over the last decade, and there are good reasons to hope that this will give rise to a systematic experimental approach to macroevolution by the next generation of researchers.

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