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The Croonian Lecture, 1991: Genostasis and the Limits to Evolution

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Phil. Trans. R. Soc. Lond. B 1991 **333**, 289-305
doi: 10.1098/rstb.1991.0079

The Croonian Lecture, 1991

Genostasis and the limits to evolution

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SUMMARY

The Darwinian explanation for evolution is that it is the outcome of the interaction between genetic variation and natural selection. There is now good evidence for both the existence of genetic variation and the occurrence of natural selection, the latter potentially at high intensities. The outcome should be rapid evolutionary change; yet in practice very little change is found. Most species are very stable, and in situations where evolution is observed in one species often none is found in others despite equivalent opportunity. Evolutionary failure is commonplace.

Despite the occurrence of high levels of protein polymorphism, there is good evidence that the supply of variation making a major contribution to fitness is very limited. As a result it is argued that lack of evolution in most species may be due more to lack of appropriate variability than to other causes: a condition for which the term 'genostasis' is proposed. In those situations where appropriate genetic variation is available for one reason or another, evolution is found to be very rapid. There are good theoretical and practical reasons for more attention being paid to the mechanisms of supply of new variation and to those situations where evolution appears not to be taking place.

1. INTRODUCTION

When I look at the scientists who have preceded me in giving this lecture, I cannot but feel apprehensive, especially because it is now a long time since someone has chosen an evolutionary topic, and the person involved, R. A. Fisher (1953), made such an outstanding contribution to the subject. Perhaps the reason why no one has chosen evolution is that the subject has appeared to have been sitting comfortably on its well established principles and therefore did not need a special airing. In part this is true, because Darwinian principles have stood the test of time and recent research. Nevertheless all has not been completely comfortable since the appearance of the 'punctuated equilibrium' hypothesis (Gould 1980). This has been well answered (Stebbins & Ayala 1981; Endler & McLellan 1988). But it does suggest that there are aspects of evolution which are perhaps not quite as straightforward as we once thought.

My own apprehension is because my experience has been broad rather than deep, and practical rather than theoretical. A justification for having taken this approach is that to understand evolution properly we must pay attention to what is actually going on outside, in the field. Only there can we see what actually happens, where life in all its brutality is being lived by organisms for whom the processes of survival and reproduction are the final arbiters. A. J. Cain wisely said (1977) that if you wanted to understand evolution:

> the golden rule is always to ask questions of the animals, not of the pundits.

I agree with him; except that it is just as important to ask questions of the plants also.

The basis of this paper is an argument that, if we examine the animals and plants in nature, we find as much evidence for failure as for success. This implies that there are limits to evolution, for which the controlling agent is the supply of variation. It is a simple, rather obvious, argument. Yet, surprisingly, a survey of current textbooks shows that the processes by which new, useful, variation originates are rarely discussed, even when the techniques available to study it are now so powerful. In particular, mutation is treated as a process which will provide anything required. To make the argument clear, I will therefore present it in a series of steps, in each of which the inference is open to assessment and refutation.

2. THE DARWINIAN VIEWPOINT

Almost all scientists are guilty of enthusiasm for their own ideas. It is difficult to see how science would have made so much progress without this foible to drive them on. Once Darwin had produced so satisfying a theory, it is understandable that his energies and

enthusiasms should have been enveloped by it. His writing shows how complete was his belief in what he proposed.

> What limits can be put to this power, acting during long ages and rigidly scrutinising the whole constitution, structure, and habits of each creature, – favouring the good and rejecting the bad? I can see no limit to this power, in slowly and beautifully adapting each form to the most complex relations of life.

Adaptation is rightly presumed, despite the example of Dr Pangloss, as a proper approach to understanding what evolution has achieved including persistent ancestral plans (Cain 1964). There is justification for assuming that evolution is always leading to better adaptation (where better adaptation means an improved ability to leave descendants compared with what existed previously), even if it does not mean that every character of an organism must have a positive adaptive value and origin (Gould & Lewontin 1979; Harper 1982).

But was Darwin guilty of over-confidence in assuming that he could 'see no limit to this power'? As a young scientist interested in evolution in plants, I was infected with this view. And I was guilty, as have been many others, of looking for the evidence of evolution working at its best. In defence of this, it is not unreasonable that anyone wishing to study a particular phenomenon should analyse those situations where the phenomenon seems to be at work. But with this comes the implication that little or no attention will be paid to those situations where it is not. With a longer perspective, it is not any longer so clear to me that Darwin's confidence is so justifiable. There seem to be many examples of places and situations where evolution has failed. To make such a bold statement is easy; it must be justified. If found correct, an explanation must be found.

To do this, it is necessary to look at individual situations. This is critical because what happens on average is not the same as what happens in individual cases and lineages. No-one doubts that the 'power' is slowly adapting species to the complex relations of life, but is it adapting 'each form'? An army may press forward while individuals stumble. It must be remembered that perhaps 100 times more species have become extinct than exist at present (Simpson 1953).

3. THE MECHANISM

The Darwinian and neo-Darwinian explanation for evolution remains as it always has been: the inevitable outcome of the interaction between the occurrence of (i) genetic variation and (ii) natural selection. For evolution to occur, both of these processes must operate. Essentially, as pointed out by Mayr (1962) and Endler & McLellan (1988), evolution is a two-stage process, consisting of (i) the origin of new variants and (ii) the replacement of older variants by newer. The factors at work in these two stages are inevitably quite different.

Although Darwin had problems in understanding the nature of the variation that was so essential to his theory, the existence of genetic variation, and its nature and origins, are now well understood. We realize, especially from work on proteins and enzymes, that there are considerable amounts, floating in populations, fed by the seemingly random process of mutation, as well as by gene exchange.

Darwin argued convincingly for the occurrence of natural selection, even though he was unable to give any direct examples. There is now plenty of experimental evidence for natural selection, based on careful measurements of survival of genes and genotypes under natural conditions. The observed intensities of selection vary enormously, but the extensive survey by Endler (1986) shows that they can be very high. In any overall survey of the distribution of coefficients of selection it must be remembered that genotypes with large negative values (lethals or near lethals) will be absent in natural populations because they will have been eliminated, and be not available for study. In situations where an existing genotype is being replaced by another with considerably superior fitness, the situation is similar because the latter will be fixed rapidly. If anyone doubts the potential power of natural selection and the existence of high coefficients of selection, they can examine the nature of population dynamics and the rates of turnover of individuals in populations (Bradshaw 1984), or contemplate what can be deduced from the failure of whole species to survive in particular environments.

Given both variation and selection, it is easy to see what can happen in evolution. Simple mathematical models, in which a gene giving a particular level of fitness to the genotypes which contain it is introduced into a population, show that changes in gene frequency can be extremely rapid when realistic levels of fitness are chosen (figure 1) If, for instance, the fitness of the existing gene is less than 50 % of that of the new gene, the new gene effectively replaces the old in 10 generations, assuming intermediate dominance. If the fitness of the old gene is 90 % that of the new one, then the replacement obviously takes longer, but would still be readily observable.

Some people may be surprised at the choice of such apparently substantial differences in fitness, yet they

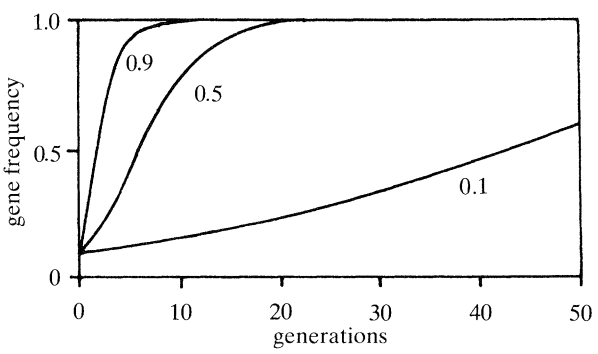

Figure 1. Changes in the frequency of a gene of intermediate dominance subject to selection. The effects of three different levels of selection are shown; coefficients of selection on the unfavoured homozygote of 0.9, 0.5 and 0.1. The higher levels of selection are just as likely in natural situations as the lower level, leading to rapid rates of evolutionary change.

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are entirely justifiable ecologically. In the tough limited conditions of the real world, unless special conditions apply, the penalty suffered by a genotype or species that is less able is to be eliminated (Harper 1977). Elimination in a single generation, a common occurrence, implies a selection coefficient of 1.0. It was unfortunate that in his early calculations of rates of change under the influence of selection, Haldane (1932) used extremely small differences in fitness: a coefficient of selection of only 0.1%. This unwittingly tended to suggest to subsequent investigations that very slow rates of change were the norm.

Whatever might be the predictions of theory, some disbelief that rapid evolutionary change will occur in practice is not unreasonable. Studies of natural situations in which a new selection pressure has been in operation for a known length of time are therefore valuable. In plants we are lucky to have several, but particularly two, good examples. The first is the work of Snaydon and his associates on the grass *Anthoxanthum odoratum* occurring within the different plots of the Park Grass experiment, originally set up by Lawes and Gilbert at Rothamsted in 1856 to test the effects of different fertilizers and manures on the yield and composition of an existing hay meadow. In 1903 the plots were subdivided for a liming treatment which has continued to the present day. In most cases the floras of the plots have diverged almost completely, with individuals species limited to a very few plots. *Anthoxanthum* is one species, however, which has persisted in several different plots. Seventy years after the liming treatment was initiated, it was found that the populations in contrasting limed and unlimed plots had evolved remarkably different responses to soil pH, as well as differences in morphological characters. These responses match what can be found in populations in more natural habitats (Snaydon 1970). The results of a reciprocal transplant experiment reveal average differences in fitness, measured as vegetative growth, between natives and aliens in any given plot, of up to 50% (Davies & Snaydon 1976). So evolutionary differentiation has been very rapid. This is supported by previous experimental studies on evolution in artificial mixtures of different pasture grass genotypes (Charles 1961), as well as by other work.

The second example is that of Wu, who investigated the copper tolerance of populations of the grass *Agrostis stolonifera* growing in the neighbourhood of a copper refinery at Prescot, Merseyside. Tolerance to heavy metals is a well-documented example of evolution (Macnair 1981). It is a highly important character for the plants that possess it, as it allows them to survive and grow in conditions of metal pollution that are lethal to plants not possessing it. It is of particular interest because there are many relatively new mining areas where the evolution must have taken place since mining began, within the last 200–500 years. Copper refining at Prescot, however, began as recently as 1900, in an area where copper had not previously been present, so that any evolution of copper tolerance by the time the populations were examined had therefore to be within 70 years. In fact a series of populations was found, differing from 4 to 70 years in the time that they had been exposed to copper. In all of these, even in the population exposed for only 4 years, marked copper tolerance was found to have evolved (Wu *et al.* 1975). This rate of evolutionary change is supported by experimental studies on the effects of selection on the variability in metal tolerance to be found within normal, non-tolerant populations (Walley *et al.* 1974).

Beyond these examples there is a large number of others in plants where rapid rates of evolutionary change are known (reviewed by Bradshaw 1972; Endler 1986). In many cases it has been possible to determine directly the coefficients of selection pertaining to individual genes, by changes in their frequencies over generations. Notable studies are those by Allard and his co-workers, for instance in bulk hybrid populations of barley (Allard & Jain 1962; Allard *et al.* 1972) and in wild oats (Clegg & Allard 1972). In the barley populations impressive changes have been observed in both quantitative characters and in the frequencies of genes at Mendelian loci, with many going to near fixation (Allard 1988). Resistance to herbicides has become the most recent example of rapid evolutionary change in plants (LeBaron & Gressel 1982), leading, incidentally, to economic and cultural problems.

In animals, too, rapid rates of evolutionary change are known. Perhaps the most interesting is industrial melanism in insects, where the frequencies of melanic forms in different species, built up in response to industrial pollution, now show a decline as pollution has been reduced (reviewed by Lees 1981). This has been especially marked in ladybirds (Creed 1971). Another example of rapid evolution with serious environmental and economic consequences is the evolution of insecticide resistance (reviewed by Wood & Bishop 1981).

Inference

All this, and other, evidence means that evolution by natural selection can now be seen as an everyday affair, easily capable of being caught in action by scientists, and followed by them within their lifetimes. *It leads to a simple inference: because natural habitats can lead to substantial differences in fitness between different individuals and can therefore generate high directional selection pressures, rapid evolutionary change is to be expected as a common occurrence.*

4. LACK OF CHANGE

Yet despite these arguments and examples, in the real world we do not often see rapid evolutionary change of the sort described. The predominant characteristic of plants and animal species is that they do not change. This is born out by the fossil record. Fossils, however, leave only the remains of their structure and morphology; it is impossible to know whether any evolutionary changes have occurred in their physiology, on which natural selection could be expected to have had the greatest effects, although lack of changes in ecological preference, discussed later, gives some indication. For present-day species we can

Table 1. *Examples of success and failure in the evolution of resistance in weed species that have been exposed to herbicides*

(Data from LeBaron & Gressel (1982) and A. M. Mortimer & P. D. Putwain (personal communication).)

have evolved resistance	have not evolved resistance despite exposure
triazines	
Amaranthus retroflexus	*Agropyron repens*
A. powellii	*Anagallis arvensis*
A. hybridus	*Capsella bursapastoris*
Brassica campestris	*Sonchus arvensis*
Chenopodium album	*S. oleracea*
C. strictum	*Stellaria media*
Senecio vulgaris	*Taraxacum officinale*
Solanum nigrum	*Thlaspi arvense*
ureas	
Alopecurus myosuroides	*Bromus sterilis*
acetolactate synthase inhibitors	
Kochia scoparia	*Chenopodium album*
Lactuca serriola	*Polygonum convolvulus*
arylophenoxy propanoates	
Avena fatua	*Setaria viridis*
Avena sterilis	*Aegilops cylindrica*
Lolium rigidum	*Bromus tectorum*

Table 2. *Species to be found in copper contaminated grassland adjacent to the copper refinery at Prescot near Liverpool, compared with those to be found in similar but uncontaminated grassland further away (after Bradshaw 1984)*

copper in soil	species found	
> 2000 p.p.m. (adjacent to refinery)	*Agrostis stolonifera*	*Agropyron repens*
	Agrostis capillaris	*Holcus lanatus*
	Festuca rubra	
< 500 p.p.m. (away from refinery)	*Ranunculus repens*	*Hypochaeris radicata*
	Ranunculus bulbosus	*Leontodon autumnale*
	Cerastium vulgatum	*Luzula campestris*
	Trifolium repens	*Lolium perenne*
	Trifolium pratense	*Poa annua*
	Taraxacum officinale	*Poa pratensis*
	Rumex obtusifolius	*Poa trivialis*
	Prunella vulgaris	*Dactylis glomerata*
	Plantago lanceolata	*Cynosurus cristatus*
	Bellis perennis	*Hordeum murinum*
	Achillea millefolium	

be more definite; their morphological and ecological characteristics, as well as their physiological characteristics, usually remain remarkably constant over successive generations, even in extreme environments.

(*a*) *Evolutionary failure*

In situations where rapid evolutionary change is observed in one or more species, it is significant that there are always other species in which no change can be observed. The evolution of resistance to pesticides is now a familiar story, whether in plants, insects or mammals. What is interesting is that in each case where evolution of resistance has occurred there are many examples of other species which although fully exposed to the pesticide have not evolved resistance. This is known in plants in relation to the use of herbicides (LeBaron & Gressel 1982) (table 1), as well as in insects (such as the tsetse fly) in relation to insecticides (Wood & Bishop 1981). Another example is in the use of sodium monofluoracetate as a vertebrate pesticide. In Australia there are species of possum and kangaroo which have evolved resistance to the compound in their natural food; but so far the introduced rabbit has not (Bishop 1981).

The floras of metal-contaminated areas associated with mining are extremely depauperate (Shaw 1989). Although many species grow in the surrounding areas, only a few species appear to evolve metal tolerance and therefore to survive in places contaminated by metal. This suggests that the others have not been able to colonize and survive because they have not been able to evolve the appropriate tolerance. While this is a reasonable assumption, there is the possibility that these species did not have the opportunity to evolve tolerance. Perhaps they were not present in the area when the pollution first occurred; perhaps other ecological characteristics of the sites exclude them, since the sites are extreme for various soil characteristics apart from the presence of heavy metals.

A critical situation where these two possibilities are excluded is the plant communities in the vicinity of the copper refinery at Prescot. When the refinery was originally established, a large number of species characteristic of normal un-polluted neutral grassland would have been present in the area. After 70 years only five species remain growing on the most polluted soils although the only change has been the aerial fall-out of copper compounds (Bradshaw 1984) (table 2, figure 2*a*, *b*). All of these have been shown to have evolved copper-tolerant populations. What has happened to the other species? They were presumably present beforehand, so they too must have been exposed to copper pollution. All the species present were exposed to the same selection pressures and all would have had the same opportunities for evolution of tolerance. Yet many are not found in the copper contaminated areas of the refinery, but only in uncontaminated, but otherwise similar, areas further away (figure 2*c*), and have not evolved copper tolerance at Prescot or anywhere else. We are forced to conclude that they have not the ability to evolve copper tolerance, at least within the present time span.

A similar conclusion can be drawn about the species occurring in the Park Grass plots. The composition of the control plots – those which received no additions of fertilizer or lime during the 100 years of the original experiment – indicates that a large number of species must have originally occupied the plots. But the species which now occupy more than a few plots in a manner similar to *Anthoxanthum* are restricted in number (Brenchley 1958) (table 3). We have good evidence suggesting that *Anthoxanthum* survives on contrasting plots because of its evolutionary adaptation and differentiation. Evolutionary differentiation has not been studied in the species which have now restricted

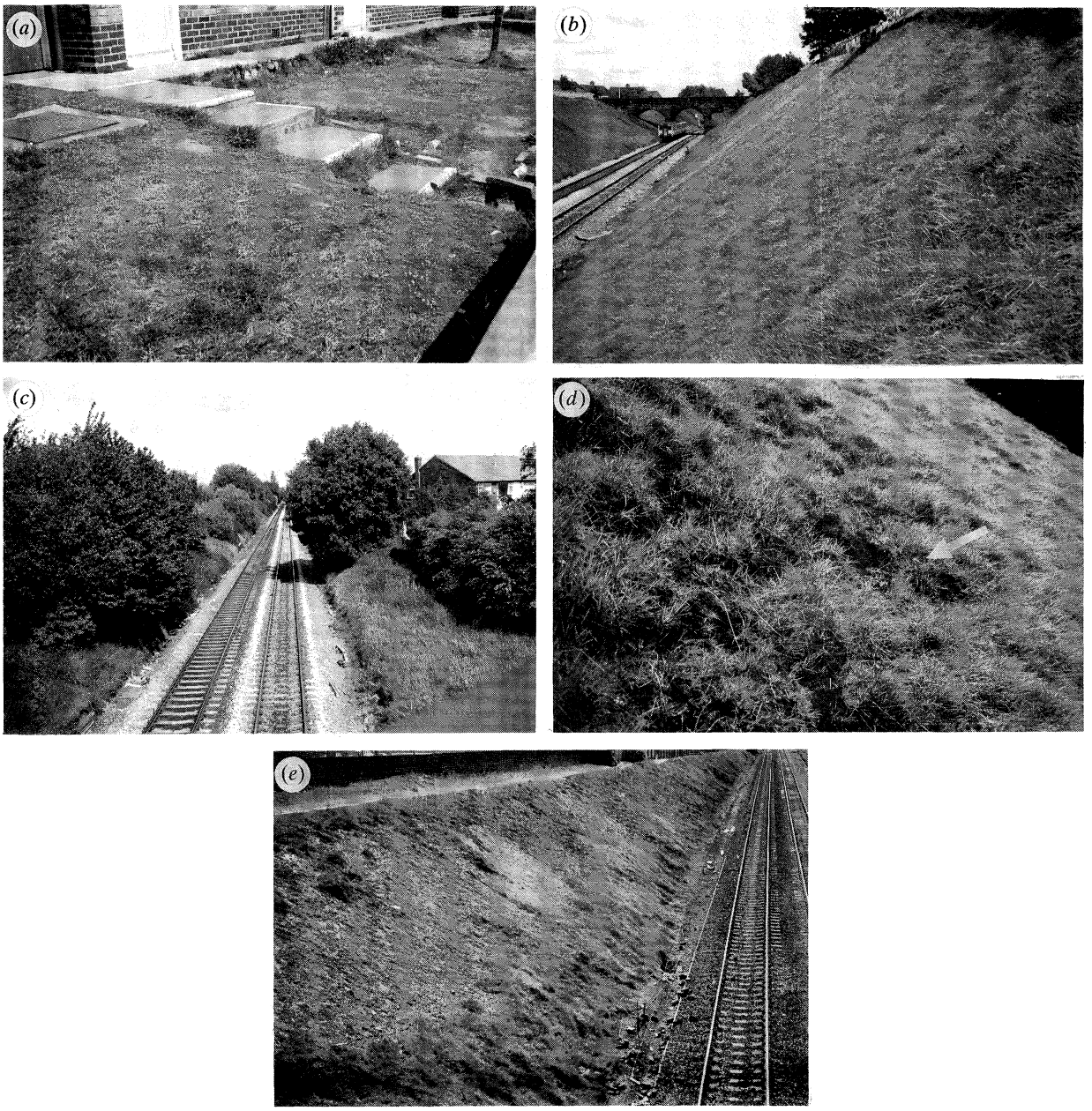

Figure 2. Photographs illustrating the evolutionary situation in the vicinity of the 90-year-old copper refinery at Prescot, Merseyside; (*b–e*) taken in the railway cutting that antedates the refinery and passes through the polluted refinery area. (*a*) A typical front garden; attempts to garden given up and only *Agrostis stolonifera* and *Festuca rubra* (both represented by tolerant populations) surviving; (*b*) typical polluted area; the cutting sides are occupied by only five species, here mainly the grass *Agrostis capillaris* (tolerant population); (*c*) typical unpolluted area further away; the cutting sides are occupied by a much greater variety of species; (*d*) close up of polluted area; pure stand of *Agrostis capillaris* with not more than 50% cover; seedlings from a garden tree of *Acer pseudoplatanus* (arrowed) attempting to invade but without success as they do not possess required variation in tolerance; (*e*) most heavily polluted area; cutting sides with large bare areas because pollution levels are too high for the level of tolerance that has been evolved; some new growth is now occurring because of reduced pollution from improved control measures.

distributions within the plots; but it has to be accepted that they could have followed the evolutionary path of *Anthoxanthum* and yet have not. They are therefore further possible cases of evolutionary failure. Ecologists may be surprised at this deduction because they do not usually expect any such evolution. Yet it has taken place in one species, *Anthoxanthum*, so there is no reason why it should not have taken place in others. If the reason why many species do not colonize metal mine wastes is because they have not been able to evolve metal tolerance, the same arguments should surely be applied to species in more ordinary habitats.

(*b*) *No room?*

It has already been suggested that there might be ecological reasons why such evolution has not taken place. One explanation for the absence of species from particular habits is the 'no room' argument: that they are excluded by the other species already present. This

Table 3. *Examples of species persisting in both the limed and unlimed halves of plots given ammonium fertilizer in the Park Grass experiment at Rothamsted, compared with those to be found predominantly in either the limed or the unlimed halves. Only some species have a capacity similar to* Anthoxanthum odoratum *to grow in both halves (from Brenchley 1958)*

species common in both limed and unlimed halves
Anthoxanthum odoratum
Arrhenatherum elatius
Festuca rubra
Plantago lanceolata
Rumex acetosa
species common only in limed halves
Alopecurus pratensis
Helictotrichon pubescens
Lathyrus pratensis
Lotus corniculatus
Poa pratensis
species common in only unlimed halves
Agrostis capillaris
Holcus lanatus

must apply in some cases and would seem particularly likely in the Park Grass situation where nearly all the plots are occupied by dense swards. As a result, even if the species had the potential to evolve in adaptation to the habitat, it would not be realized, because it would not have had the opportunity to get into the habitat and be selected. In such a case it would be difficult to discover that the potential existed, unless specific steps are taken to investigate it experimentally, as will be discussed later. The 'no room' explanation does, however, take for granted that the species concerned could not have evolved to compete with the species already present, and therefore implies that there is an evolutionary constraint operating.

Whatever the ultimate explanation for species distribution in the Park Grass situation, the 'no room' hypothesis certainly does not apply at Prescot or in most metal mining areas. A conspicuous feature of these habitats is the large amount of bare ground: plant cover is usually below 50% (figure 2*d*). It also does not apply in other extreme habitats, such as salt marshes and mountain tops, where plant cover is equally low. The floras are restricted to very few species which can be shown to have evolved tolerances appropriate to the conditions occurring (Crawford 1989). This evolution is often at the level of the population, for example in the *Achillea* and *Potentilla* populations occupying habitats at high altitudes in California, studied in such detail by Clausen *et al.* (1940).

But we must not take these species as the norm. There are many other species that have not colonized these habitats, although there is no lack of space for them. It is possible to argue that the sites on a mountain top are inaccessible to species with poor mechanisms for distribution; but this does not apply to all species. It certainly does not apply in salt marshes which are usually so close to normal habitats that they receive a constant rain of propagules of many different species. These do not survive, as do not potential colonists of metal-contaminated habitats (figure 2*d*). We are forced to conclude that these species, despite more than adequate opportunities, do not have the ability to evolve the tolerances required.

(*c*) *No time?*

Another explanation for such evolutionary failure is that there has not been enough time for evolution to have occurred. Many environments, indeed most present-day environments, have been either produced, or grossly modified, by human activity in the last 100–5000 years. Most metal-contaminated areas have been produced by mining within the last 200 years. If a species has a long generation time, such as 25 years, only a few generations will have occurred. In northern latitudes many habitats have only been available for about 10000 years since the retreat of the ice sheets, which could be little time for some species. But in extreme habitats high selection pressures are operating. In which case theory makes it clear that at least some evolutionary change would be expected. For this there has been plenty of opportunity.

For salt marshes and many mountain environments this 'no time' argument clearly does not hold. The exact climatic and soil conditions may have moved slowly up or down in altitude or latitude owing to global climatic changes, but the environments have been in continuous existence for hundreds of millennia. Yet it is in these that lack of evolutionary success is most evident.

(*d*) *Migration rather than change*

It is not the intention of this paper to consider the problems of the fossil record and its evidence of lack of change, or stasis. Such material is too inaccessible, especially because in most cases its representatives no longer exist. The problems it raises have, anyway, been well discussed (Eldredge & Gould 1972; Gould 1980; Charlesworth *et al.* 1982). There is, however, material of the present day and recent past which we can consider.

During the quaternary period there have been major climatic fluctuations. As a result there have been considerable changes in the floras of most areas of the world, related to the migration of whole associations of species. These are both very obvious and well studied in northern areas; the migrations northward, in particular, of individual species in the post-glacial period have been carefully mapped and dated (Webb 1987; Huntley & Webb 1989). It is customary to take these migrations for granted as a reflection of the ecological preferences of the species concerned. Yet is this acceptable? There is a valid alternative scenario that the species concerned, instead of migrating so regularly, could have remained *in situ* and coped with the environmental improvement by evolutionary change. There is no sign of this; the stability of the ecological preferences of the species in the face of such major environmental alterations is impressive.

We do not know the reasons for this stability. Certainly the 'no room' argument cannot apply

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because the species were already in occupation. The argument of 'no time' is more possible, but, as has already been argued, in relation to the severity of selection there has certainly been time for substantial evolutionary change.

Inference

We are all familiar with the limits to the ecological capabilities of species and their inability to colonize particular environments. Indeed, this is accepted as a normal and often diagnostic character of individual species. But it represents evolutionary failure. *Because in the situations in which this failure occurs selection pressures must be high, we must infer that the explanation for failure must lie in the other half of the mechanism of evolution, that there is a lack of appropriate variation.*

5. LACK OF VARIATION

To suggest that a lack of variation is causing evolutionary failure may seem surprising. One of the major discoveries of the last two decades has been the large amount of variation to be found, particularly at the molecular level, within populations of plants and animals. The wealth of this variation raises questions about the reasons for its existence. Either is is there because it is selectively neutral or because selection is active and has a balancing effect (Lewontin 1974). Both explanations have to take into account a wide range of conflicting evidence, and almost certainly both mechanisms are operating; neutrality is probably the commoner (Wilson 1985), but there is certainly evidence for the operation of selection (see, for example, Nevo (1988)).

But we concerned here with the way in which evolution succeeds, or fails, in increasing the adaptation of species and populations to their environment. For this to occur, genes conferring increased adaptation must be incorporated in the genotype. Although there are minor mechanisms such as genetic drift, incorporation can effectively only be achieved by directional selection acting on genes with positive effects. In this way a phenotype is created whose characteristics are heritable, i.e. able to be passed on to subsequent generations (Falconer 1981).

If the required genes are present in a population and are subject to selection, their frequency will change in a positive manner, as we have already seen. In a simple directional selective system they will ultimately go to fixation, i.e. become homozygous. This can be seen in figure 1. There is no doubt that as a result of the cumulative effects of directional selection, most of the genes possessed by individuals are in a homozygous state. It is not without significance that roughly two thirds of observable loci in *Drosophila* do not show electrophoretic variation and appear to be fixed (Lewontin 1985). The occurrence of fixed genes, the presence of which can otherwise be only surmised, determining differences between species and populations, is readily shown when different species or populations are intercrossed. Any genes, therefore, that remain floating in a heterozygous state are unlikely to be under the influence of directional selection. The exceptions are those showing overdominance or other forms of interaction. This is merely restating the reasons already given for the existence of variation floating in populations.

The crucial point of this discussion is that variation that can confer permament advantage to the individuals that carry it, in the environments we are considering, will be rapidly selected for and become fixed. Any variation that remains floating must, by comparison, be of little or no value to stable adaptive advance. Evolution cannot proceed far by the incorporation of genes with non-additive effects such as heterozygote advantage, because of the impossibility of maintaining a stable set of genotypes in a population. By using this ruthless selectionist standpoint we are forced to the view that this floating variation is 'genetic junk' (Lewontin 1974), of little or no value in the adaptive process. This does not, however, imply that it might not be of value at some other time or in another environment.

The presence, therefore, of apparently large amounts of genetic variation in a population does not necessarily mean that the population has large amounts of variation available to increase the adaptation to the environmental conditions that it faces. Such variation can be largely irrelevant. For the evolutionary change that the environment is demanding, the population may have run out of variation altogether. Unfortunately, many investigators have used the levels of molecular variation in populations to provide information about the evolutionary state and potential of organisms. Although such information can give useful understanding of the mechanisms and rates of generation of variation, it is likely to be misleading on the matter of evolutionary potential. The variation that is critical to study is that which is appropriate to the environmental conditions pertaining, in other words the variation that has positive and additive effects on the survival and fecundity, the reproductive success, of the species being studied.

(*a*) *Direct evidence for lack of variability*

It is surprising that, despite its critical role in evolution, very little work has been done on the supply of genetic variation, a point made by Endler & McLellan (1988). The important exceptions are the selection experiments done particularly on *Drosophila*, and, for obvious reasons, the very extensive and excellent work carried out by plant breeders. The latter is in many ways the most informative; but because of its immediate connection to previous arguments, some simple work on metal tolerance will be used as a starting point.

The origins of metal tolerance appear to lie in the occurrence at low frequency of highly heritable variation for tolerance in normal populations (Gartside & McNeilly 1974; Walley *et al.* 1974). By using an improved technique for screening this variation, Ingram (1984) showed that it was not found in all species. Although it could always be found in the normal populations of species that evolved tolerance,

Table 4. *The percentage of copper-tolerant individuals in normal populations of various grass species, related to the occurrence of the sames species on copper-contaminated wastes and its possession of copper-tolerant populations. The evolution of metal tolerance is not found in species that do not have variation for metal tolerance occurring in their normal populations (from Bradshaw 1984; data of C. Ingram)*

species	percentage of tolerant individuals in normal populations	occurrence of species on mine waste	possession of copper-tolerant populations
Holcus lanatus	0.16	+	+
Agrostis capillaris	0.13	+	+
Festuca ovina	0.07	−	−
Dactylis glomerata	0.05	+	+
Deschampsia flexuosa	0.03	+	+
Anthoxanthum odoratum	0.02	−	−
Festuca rubra	0.01	+	+
Lolium perenne	0.005	−	−
Poa pratensis	0.0	−	−
Poa trivialis	0.0	−	−
Phleum pratense	0.0	−	−
Cynosurus cristatus	0.0	−	−
Alopecurus pratensis	0.0	−	−
Bromus mollis	0.0	−	−
Arrhenatherum elatius	0.0	−	−

Table 5. *Examples of the different sources that have had to be used by plant breeders to obtain required characteristics. Often the variation can only be found outside the original population that forms the starting point (from Bradshaw 1984)*

source	character
from original population	
Potato	blight resistance within *Solanum tuberosum*
Alfalfa	spotted aphid resistance
Sugar beet	sugar content
Rye	reduced height
from other cultivars	
Barley	yellow dwarf resistance from Abyssinian cultivars
Wheat	dwarfing genes from Japanese cultivars
Grapes	root aphis resistance from American material
Cotton	blackarm resistance from African cultivars
from other species	
Oats	mildew resistance from *Avena ludoviciana*
Bread wheat	stem rust resistance from *Triticum dicoccum*
Bread wheat	eye spot resistance from *Aegilops ventricosa*
Rice	grassy stunt resistance from *Oryza nivara*
Delphinium	red flower colour from *Delphinium cardinale*
Potato	blight resistance from *Solanum demissum*

tolerance was never found to have evolved in species that did not possess this variability (table 4). The level of general genetic variation in these latter species has not been investigated, but they are outbreeders with normal powers of local evolutionary differentiation and are likely to have high variability. Yet in the critical variability in metal tolerance they are deficient. The fact is clear, although why it should be so is a separate matter to which we will return later.

In their everyday work, plant breeders are constantly seeking variability appropriate to their needs. In many cases they can find selectable variation within existing material. One of the most spectacular examples is provided by the Illinois corn experiment. Fifty years of selection for oil and for protein in both upward and downward directions has produced lines that have diverged completely from one another, from the original population means by a factor of three, and are now completely outside the ranges of variability for these characters in the original populations (Woodworth *et al.* 1952).

In many cases, however, the variation that plant breeders seek, despite considerable search, is not to be found within existing material, so that they have to look for it elsewhere. There are innumerable examples of this (table 5). In some cases the necessary genes can be found in other populations of the same species. But in many cases it may be necessary to go to other related species, a procedure that can bring concomitant problems of gene transfer. That the necessary gene or genes may not be present within a species is, of course, the fundamental justification for the modern genetic engineering industry.

(*b*) *Evidence for exhaustion of variability under selection*

Many long-term selection experiments have been carried out on *Drosophila* and other animals (reviewed by Falconer 1981). In some cases continuous progress has been achieved throughout an experiment, even for 50 generations as in the Illinois corn experiment, for example in the replicated selection experiments of Yoo (1980) and Weber (1990). But there are other examples

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where although progress has been achieved in early generations, it has come to a halt later. A good example is in selection for thorax length in *Drosophila* (Robertson 1955). In *Drosophila* cessation of progress can be attributed to the small size of the genome with consequent limited store of variability. But the same can occur in other species such as mice and chickens with larger genomes. Inbreeding and population size can obviously play a part. Nevertheless the overall evidence is that termination of response to selection is because all appropriate genes have been fixed, with a consequent loss of genetic variance. A loss of variance does not, however, always occur. Once genes with additive effects have been fixed, selection may favour genes showing heterozygote advantage, maintaining variation that is unfixable. But the critical point is that additive variation has been exhausted. There can be other causes, which will be discussed later.

That progress under selection is limited by the supply of variation finds support from observations on the heritability of different characters in the same organism. In chickens, for instance, Lerner (1958) showed that characters most directly connected with fitness have the lowest heritability. The same is found in other species (Falconer 1981). This implies that additive variation has been more depleted in these characters.

Evidence for the depletion of available variation by selection is also provided by the situation commonly to be found in metal contaminated areas. In sites where the evolution of metal tolerance has been shown, there are, even so, areas where metal-tolerant plants do not grow. These can be due to causes unconnected with metal contamination, but in many sites, such as at Prescot, plants are clearly absence from the areas of highest metal contamination (figure 2*e*). This suggests that there are limits in the degree of metal tolerance that can be evolved. Despite the presence of variability enabling a certain level of tolerance to be produced, variability to enable tolerances to higher metal concentrations to be achieved does not appear to exist.

(*c*) *Rapid change in new environments*

A corollary of the fact that selection rapidly exhausts the supply of appropriate variation is that evolutionary change should be most obvious in material exposed to a new environment, because it will not have been subjected to the selection imposed by the new environment, and it is unlikely, in consequence, that the relevant variability will have been exhausted. The existence of such unselected, hidden variation, has been most clearly shown by Cooper (1954). This principle may lie behind some of Darwin's observations on the increase of variability under domestication.

It is difficult to provide critical evidence, but it is noteworthy that almost all the cases in which rapid evolution has been reported are in new environments, particularly those associated with human activity. It is possible that this is because most situations now available for study have been influenced by man to varying degrees. It is noteworthy, however, that it is the occurrence of distinctly new environmental conditions, brought about by factors such as air pollution, metal contamination, insecticide use, herbicide use, control of mammals by pesticides, and monoculture of new crops, that have provided our best examples of evolution in progress (see Bishop & Cook 1981). Ordinary environments have not provided us with anything like these examples.

(*d*) *Rapid change with new genes*

An alternative situation where rapid change can be found is where the available genetic material changes. For many years there has been the suggestion that the distributions and success of many angiosperm species have been substantially favoured by the acquisition of new genetic material. Much of the evidence has centred round the success of polyploid species. But in these there has always been the possibility that their success was due to the increase in their chromosome number. Critical evidence now suggests that this is not so, and that success is related to hybridization in which the species concerned pick up new gene combinations (Dobzhansky *et al.* 1977; Stebbins 1985).

The power of new genes to change the capability of species completely is no more evident than in the grass *Spartina anglica*. This has a remarkable ability to grow in the lower levels of salt marshes in conditions which no other angiosperm is able to exploit. The critical point is that this habitat has been available to angiosperms since they first evolved in the Cretaceous, but *Spartina anglica* only appeared at the end of the last century, the product of hybridization (followed by polyploidy) of *S. maritima*, a long-standing native restricted to the upper zones of salt marshes, with *S. alterniflora*, an alien introduced in ballast from N. America, no more capable than *S. maritima* of growing in the lower parts of the marsh (Gray *et al.* 1991). *S. maritima* has had unlimited opportunity to evolve and invade the lower marsh but failed to do so until the advent of the genes from *S. alterniflora*. The original undoubled hybrid, known as *S.* × *townsendii*, which is sterile, has a similar capability to *S. anglica* (A. J. Gray personal communication). Despite its immediate success owing to the possession of a new gene combination, it is interesting that *S. anglica* is perhaps now in an evolutionary straight-jacket, because its allopolyploid origin means that it has no further variability immediately available. This is affecting its ability to cope with new evolutionary situations, such as infection by the ergot fungus *Claviceps purpurea* (Gray *et al.* 1990).

Genes can obviously be acquired by hybridization without polyploidy. It is, unfortunately, difficult to be sure that genes found in a species that has experienced hybridization were not already present, so there is little critical evidence for hybridization itself overcoming an earlier genetic constraint. The possible expansion of the range of the Queensland fruit fly, *Dacus tryoni*, by introgression of genes from *D. neohumeralis* (Lewontin & Birch 1966) is, however, very persuasive.

Inference

Although there is a need for further evidence, it is difficult not to extend the previous arguments. *We can*

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infer that, despite the occurrence of a wide range of variability particularly at the molecular level, in most situations the supply of appropriate variability, of value in the specific selective conditions occurring, has been exhausted. In view of the potential importance of this condition, in which evolution is limited by lack of appropriate genetic variation, it should be recognized by a specific term: 'genostasis' is suggested (Bradshaw 1984).

6. OTHER EXPLANATIONS

It must not be forgotten that there can be other reasons for a lack of evolution. There are, ultimately, constraints owing to the laws of physics and to the properties of the physical environment (Alexander 1985). Within this framework a number of different reasons have been suggested for lack of evolution, which must be considered.

(*a*) *Ancestry, sow's ears and tinkering*

The most obvious reason is that there may be constraints arising from phylogeny, which have been built into the species by its previous evolution. Allied to this are the constraints from development, related to the specific pathways that determine the growth and maturation of the individual. These can be highly complex and can provide major constraints, perhaps best understood in terms of networks (Kauffman 1985; Endler & McLellan 1988). Although the outcome of the structure and functioning of the individual, these must be phylogenetic in origin, related to the evolution which has taken place in the past.

To an uncertain degree, some aspects of long-term evolution may be determined by stochastic events, particularly those associated with speciation. It follows that some of these constraints may be due to chance. But whatever their detailed origin, we must accept that ancestry can impose restrictions on present-day evolution and adaptation, a point emphasized by Gould & Lewontin (1979). This can be termed the 'sow's ear' argument because the inability, for instance, of a fish to evolve immediately into an animal inhabiting a desert, because of the absence of a number of important characteristics, even if in the fullness of time this evolution has been achieved, is analogous to our inability to make a silk purse out of a sow's ear. Ancestry must lead to constraints, because the evolutionary process can only work on what already exists, and modify it. Evolution is not a process of new construction, but as Jacob (1977) has eloquently described it, a process of tinkering, of making use of what is already available. A tinker 'uses everything at his disposal to produce some sort of workable object'. This must lead to constraints not only at the level of organism, organ and process, but also, very clearly, at the level of the gene.

Similarly, the particular advantageous features of major taxonomic groups, whether in plants or animals, provide bias, and even problems, for evolution in those groups. Nevertheless we must heed the warnings of Cain (1964), and not believe that this necessarily leads to poor adaptation.

(*b*) *Limitations in other characters*

In parallel with constraints arising from development, there are constraints that arise from characters other than the one being considered. It is possible that a species or population has the variability within a character obviously necessary for survival in a particular habitat, but not the variability in a second character of equal necessity but not apparent on first study. Despite the simplicity and likelihood of this, it is difficult to find clear evidence. But in studies of metal tolerance an interesting pointer was the discovery of clear, heritable, variation for copper tolerance in normal populations of the grass *Dactylis glomerata*, but no sign of the species growing in copper contaminated areas normally associated with the evolution of copper tolerance (Gartside & McNeilly 1974). Eventually a tolerant population was found in an area where copper-rich material had spread over a normal soil. It appears that, although the species has the ability to evolve coper tolerance, it does not have the ability to evolve tolerance to poor soil nutrient conditions typical of copper mine wastes.

Tolerance of saline conditions is a character somewhat similar to metal tolerance (see, for example, Venables & Wilkins (1978)). It could be supposed that species which do not colonize saline habitats are prevented by an inability to evolve salt tolerance. Screening experiments on normal populations, however, show that appropriate heritable variation in tolerance to sodium chloride occurs in many such species (Ashraf *et al.* 1986). Further analysis suggests that they do not have the variability necessary in other characters that are important for growth in saline conditions (Wu 1981; Ashraf *et al.* 1989).

All that this argument does is to transfer the problem of lack of genetic variation from one character to another. The essential point remains that the species or population lacks the appropriate variability.

(*c*) *The effects of genetic systems*

A number of properties of the genetic system can also provide constraints. They have been dealt with extensively by other authors and will therefore be only considered briefly here. The restrictive effects of inbreeding on the supply of variation are well documented and understood. It is possible for almost complete homozygosity to occur, preventing all possibilities of recombination, although this is counteracted by quite small amounts of outbreeding (Imam & Allard 1965).

Epistasis, departures from additivity of the effects of genes at different loci, can cause problems because in extreme cases selection may have to get hold of two or more genes at once, perhaps in a particular order, to develop what can be termed a coadapted complex. This can reduce the possibility of success, but in the longer term it will not totally preclude the possibility of evolutionary change (Barton & Charlesworth 1984).

Genes can have more than one phenotypic effect, a condition known as pleiotropy. This can produce constraints if the secondary effects are deleterious,

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thereby negating any positive influence that the gene might have on fitness; the costs associated with the benefits may be too great. This is another way of looking at developmental constraints, which are often suggested as causes of lack of evolution (for examples, see Gould & Lewontin (1979); Maynard Smith (1983)). The same effect can arise by linkage, if the gene is tightly linked to one or more other genes having negative effects. In both cases selection favouring the primary gene will give rise to disadvantageous correlated response in another aspect of the phenotype.

These effects are real, but again they do not alter the original argument, as they apply to specific genes. There is no reason why other genes should not arise that do not show pleiotropic effects. Equally there is no reason why other genes should not arise that are not linked to disadvantageous genes. That these possibilities exist is well demonstrated by Scharloo (1987). Therefore, when the constraint is found, its ultimate cause must be a lack of appropriate variation. In this statement the word 'appropriate' implies a favourable cost–benefit relation.

(*d*) *Stabilizing selection*

Another commonly suggested 'constraint' is the occurrence of stabilizing selection. There is no doubt that this is an important type of selection. It was the first type of selection to be shown experimentally (Bumpus 1899), and there are now many examples (reviewed by Johnson 1976; Endler 1986). It can be a cause of stability or stasis in evolution because selection favours an intermediate phenotype. But it is difficult to see this as a total constraint; there is always the possibility that a new variant could appear and take the species out of its constraining environment, allowing the species to exploit a new niche, a new resource, or new environment. The observation that this does not happen and that the species remains subject to the stabilizing selection argues once more that it does not possess the appropriate variability. It must be admitted that the size of the adaptive valley which has to be crossed may in some cases make it unlikely that such variability would ever be forthcoming.

Inference

A number of reasons are put forward for lack of evolutionary progress. These have recently been reviewed in relation to the arguments for and against neo-Darwinian explanations for long-term stasis in evolution (for example Charlesworth *et al.* (1982)). It is clear that, because evolution is a process of tinkering, ancestry sets a species on a particular evolutionary pathway within which it is constrained thereafter. But in relation to other constraints we must not forget the essential 'mouse trap' argument of R. W. Emerson that:

> if a man can write a better book, preach a better sermon or make a better mouse trap than his neighbour, though he build his house in the woods, the world will make a beaten path to his door.

If better genes could appear they would be used. *We can therefore infer that although these constraints are important, they are not necessarily absolute. The ultimate cause must be a lack of appropriate variation by which the constraint could be overcome or obviated.*

7. SOURCES OF VARIATION

The emphasis in this argument is that the key to evolution is the supply of variation. Although a major component in the supply of variation is the complex process of recombination, this is of no value without the underlying process of mutation by which the ultimate units of new variation are produced. Mutation is the only mechanism that feeds new variation into the stock of variation floating in populations and can provide new material upon which selection can act.

(*a*) *Mutation*

Mutation is a peculiar process to which evolutionists have given too little thought. We understand well, now, that its fundamental origin is the replacement of DNA bases, although such changes are supported by a number of processes apart from simple substitution, including sequence rearrangements, slippage, gene conversions, deletions, transpositions and duplications. It is not necessary at this stage to go into details of these processes, but to ask what is the outcome for the supply of variation. It is curious how little attention is paid to the process of mutation in textbooks on evolution. Perhaps it is thought of as a random process which can really, in the end, provide more or less everything required.

Any assumption that it is a random process must be questioned. There is no doubt that it is random in time of occurrence. There is now good evidence that the process occurs at a steady rate over time, resulting in a steady replacement of amino acids. This has produced the concept of molecular clocks with implications of regularity, and suggestions of neutrality in what is produced, despite the contrary evidence (review by Gillespie 1986).

In what it produces, however, the process cannot be random. What is produced is determined by what was there before, because mutation is a form of tinkering. This must cause substantial limitations to what appears, although it is of major value in reducing excessive effects of chance and the production of nonsense. An aspect of this is that the order of appearance of mutants, itself random, can have important effects (Clark *et al.* 1988). At the same time we are now beginning to see that there are constraints in the mutation process itself (Golding 1987). Eventually we should be able to understand, from our knowledge of gene structure and function, what particular mutations can be produced in a specific gene and at what frequency, and also what cannot be produced. The latter constraint must exist, but has so far received rather little attention.

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The mutation process cannot escape from the constraints of metabolic pathways in translating the effects of a small change in an amino acid to the final product in the phenotype of the organism. Selection has immediate effects. Most mutations are likely to be inviable, but some are less likely to be inviable than others, for instance those in the third position of codons as these are less likely to cause disruption of protein function (Wilson 1985). Selection can have similar effects further along the development pathway. The result is that when we look at what mutations cause at the level of the phenotype, the products are by no means random in nature.

This is clearest when the range of visible mutations occurring in any given species is examined, whether in maize, *Drosophila* or man. They are only a very small part of what might be produced, even if it is difficult to know exactly what this would be. Some idea of the limits to the mutation process can be gleaned by an examination of the non-lethal mutations affecting a single organ, such as the mutations found and collected in the breeding of new varieties of sweet peas. Here novelty is everything, so almost any mutation will be valued and saved. Yet the history of the sweet pea (Crane & Lawrence 1947) shows that only certain mutations have occurred in either flower shape or colour. Flower colour is a character in which the limitations of the mutation process are all too apparent. Mutation to a red flower colour has never been found in a garden delphinium; it has only been achieved by patient crossing with a distantly related species (Legro 1965). The justification for a genetic engineering industry has already been mentioned.

The problem in this discussion is that although a great deal is now known about the mutation process from a molecular standpoint, much less is known about it from an organismal standpoint, a point made clear by Wilson (1985) and Endler & McLellan (1988) who call it a 'black box' relative to evolution. Yet in the end the fates of mutations are determined at the level of the organism.

In particular we know very little about the processes by which mutation leads to new functions. By contrast the degree to which mutations lead to lethal effects is very evident. It is crucial to know how mutations, by duplication and exon shuffling, as well as by simple base changes, can contribute new characteristics to a species, and increase its fitness. Only in this way can the careful arguments of population geneticists about processes of innovation, and the importance of mutations with large or small effects, be resolved (for example Charlesworth (1990)).

(*b*) *Effects of chance*

One attribute of the mutation process requires further attention. It is that, in general, mutations occur at very low frequencies. Base substitution rates are of the order of 1 in 10^9 per cell division in both prokaryotes and eukaryotes, equivalent to about 1 in 10^6 per generation (Maynard Smith 1989). This means that their appearance in any population is highly subjective to chance. Their persistence in that population even in the presence of favourable selection is then again subject to major stochastic sampling effects. These problems are well known (see, for example, Fisher (1930); Futuyma (1979)), but this does not diminish their importance. They put restrictions onto the contributions to be made by mutation to the process of adaptation, even though large population size can have mitigating effects (Weber & Diggins 1990).

This restriction is compounded by the limitations, set by gene flow, to the migration of a given gene from one population to another. By using Fisher's (1937) formulation applied to eight species, Levin (1988) calculates that the mean spread of an advantageous mutant ($s = 0.50$) would be in the order of 1.5 metres per generation. This low figure is affected by the model of dispersion used. In practice dispersal of propagules is usually leptokuritic, with a very few moving relatively large distances. But restriction will still be present.

This means that the occurrence of variation in populations within a species has a strong stochastic element, a finding of many authors in both plants and animals (see, for example, Schaal & Leverich (1987); Levin (1988)). In a situation where a new selection pressure is operating, the process of evolution can be substantially affected. Tolerance to zinc can be readily evolved in *Agrostis capillaris* (*tenuis*). It originates in the same manner as copper tolerance, by the selection of tolerant individuals occurring at very low frequency in normal populations. Zinc tolerance has recently been shown to evolve in this species in the zinc contaminated areas underneath electricity transmission pylons, caused by the corrosion of their zinc coating, despite the towers having been in place for less than thirty years and the areas of zinc contamination being less than 10 m × 10 m (Al-Hiyaly *et al.* 1988).

Because electricity pylons occur in lines they provide replicated environments for evolution. The distance between pylons, 300 m, means that the populations beneath different pylons are effectively isolated from one another. It has been found that zinc-tolerant populations do not occur under some pylons, suggesting a failure of evolution to take place. Single-generation screening experiments, and attempts to select, over three generations, for zinc tolerance in the normal populations of *Agrostis* in the neighbourhood of such pylons has shown that variation for zinc tolerance does not exist in these populations (Al-Hiyaly *et al.* 1991). This absence of variability for zinc tolerance in some populations is supported by laboratory screening experiments (Symeonidis *et al.* 1985). Whatever the precise cause of this patchy distribution, the stochastic occurrence of rare variants having important effects on fitness is supported.

Inference

It is easy to believe that because it seems to be a random process, mutation is always occurring, feeding in the necessary variation on which selection can act. Our knowledge of gene structure and the mutation process emphasizes that, even if it is random in occurrence, because of the processes involved it is not

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random in what it provides. We still await good information about the generation of new functions. *For the present we can infer that with respect to the provision of specific (evolutionary useful) characters mutation is a very capricious process, capable of setting distinct but at present unpredictable limits to the supply of variation so crucial to evolution.*

8. DIFFICULTIES

(*a*) *Problems of proof*

There is a fundamental problem in the arguments that have been put forward, connected with the asymmetry of proof. It is not possible to provide direct proof of limits in the mutational, variation supplying, process for the simple reason that it is impossible to prove that something never happens. This is particularly true because some mutations do occur and are clearly observable. The argument is not that mutation, and the general process of supply of variation, does not occur, but that it has limits.

We should, however, be able to make progress if we could have more detail of the consequences of changes in DNA to effects at the level of the organism. At the moment there is a gulf between studies at the molecular level and studies at the level of the survival and fitness of whole organisms. Although great progress is being made in understanding the nature of mutations, too little is yet known about the way in which mutations can lead to completely new functions, either in respect of the way they might occur or in respect of their actual occurrence.

The way in which new functions can be created by mutation is a problem for molecular and developmental geneticists. Such studies should be able to provide evidence showing why a new function appearing in one species could not occur in another because of limitations at the level of the DNA and gene architecture. However, the complexities of the mutation process may make such predictions difficult. Whether it could show limitations arising within development seems less likely because of the even greater complexities of developmental pathways.

This points to the importance of comparison. Because the whole emphasis of this paper is that what may occur in one organism, population, or species, may not occur in another, the approach most likely to be productive is to compare evolutionary potential in different, but related, types of material, whether population, species, or higher group. The study of differences has for long been a valuable technique in biology (Bradshaw 1987).

(*b*) *Difficulties in finding the new*

To capture the actual occurrence of mutations leading to new functions increasing fitness is a much more difficult task. If a mutation leading to increased fitness occurs within a population, it will be selected rapidly. Its spread and incorporation will only be held up by the problems of chance elimination and limitations to migration. Its spread through a population, although dependent on the intensity of selection, will take very little time indeed, in the order of 10–20 generations, which is a twinkling of an eye on a geological timescale.

The consequence is that the arrival of any such new advantageous mutants will be very difficult to observe. They will have appeared and been incorporated before they can be noticed. The only place where we might see them is where a new ecological factor begins to operate, such as in the man-made environments we have already discussed. But we will have to look out for them from the beginning, or we may miss their appearance. An excellent example of what observations may be possible on the occurrence of an advantageous mutation in a new environment is provided by warfarin resistance in rats (Bishop 1981). The origin of resistance is clearly a mutation that has spread from only very few centres of origin, under the influence of conflicting selectional forces. It is interesting that the mutation is not new, but recurrent, having been observed before warfarin began to be used. A molecular analysis would be valuable in indicating how the mutant has originated and whether it could occur in other species.

Understanding may be forthcoming from an examination of the structure of the equivalent gene in rather different organisms. Such is the progress being made in comparative studies of molecular architecture, for instance of the serine protease gene (Rogers 1985), that a new discipline of molecular archaeology seems to be becoming possible. From this a clear picture of what has and has not been achieved in evolution should be forthcoming. However, whether it could indicate what can and cannot be evolved in the future is another matter.

The fact that new advantageous mutations, or other variants, will spread through populations rapidly and become fixed, has the important corollary, already mentioned, that what is not fixed in populations is unlikely to be as important in the adaptive process. It is not unrealistic to say that any variation that is not fixed is likely to be of rather little importance at the present time, even though it cannot be denied any effect on fitness and its importance at some future time precluded. In which case it appears that our present interest in the variation floating in populations, although providing academic challenges, could be misdirected, as it is unlikely to provide us with information on the basic evolutionary processes leading to increased fitness, by which organisms have been constructed. We seem to be falling into the trap of putting our energies into a peripheral feature just because it is there, when serious gaps exist, as Endler and McLellan (1988) point out very clearly, in our knowledge.

Inference

It is clear that there are major problems of observation and proof to be overcome. It is all too simple to examine what we see and believe that this is the essence of evolution. *We can infer that to look at what we can see is likely to be misleading, because what is important occurs rarely and rapidly, particularly in relation to our own timescale. In the end the most satisfactory approach may be to*

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test the concept of genostasis by comparative work, on what the genome has and has not produced, at the molecular level, to parallel what it actually can produce at the present.

9. CONCLUSIONS

Despite the obviously great successes of long-term evolution, and recent examples of rapid evolutionary adaptation, we have to accept the fact that most of the time little or no evolution is occurring. The stability of most species and populations in both the short and long term is a dominant characteristic of the living world. At the same time we have clear evidence, in many different situations, of evolutionary failure in some species but not in others. Both this stability and this failure must be fitted into a Darwinian view of the world, unless this view is incorrect, which seems unlikely.

Application of the Darwinian paradigm requires an explanation in terms of both selection and variation, as these are the two components of the evolutionary mechanism. Up to now many authors have suggested that the most likely cause of changelessness is some sort of stabilizing or balancing selection, an argument prompted perhaps by a Victorian view of society in which everyone knows their place. This seems erroneous because in every habitat there is room for organisms that, by one means or another, can do better than those that are there already. In other words directional selection is always present, even if there is concomitant stabilizing selection for some organisms to fit particular habitats or niches. From the ecological evidence, the power of this directional selection can be considerable.

This being so, it does not seem that the explanation for a lack of all evolutionary change can lie only in selection. It is certainly a most unlikely explanation for evolutionary failure, especially in those cases where other species or populations have succeeded.

We are forced to the conclusion that an explanation must lie in the supply of genetic variation. There seems no reason at all why we should assume that the processes of supply of variation are omnipotent and capable of providing whatever is needed. Restriction of supply seems much more likely. This is certainly supported by a wide variety of evidence from both natural and artificial populations.

The condition in which evolution is limited by the supply of variation, which can be described as genostasis, seems likely to be commonplace. But it requires critical tests for confirmation, at both the population and molecular level.

The common view of most evolutionists is that evolution is a net change in the genetic makeup of a population or species. From the preceding discussions it would appear that the process of evolution would be clarified if we were to recognise that it is a two-step process as suggested by Endler & McLellan (1988), and that there are in effect two types of evolution:

(i) that where existing variation is exploited: usually characterized by being immediate, fast and predictable;

(ii) that dependent on new variation: usually characterized by being long-term, slow and unpredictable.

There is very little in common between these two types, although they are different aspects of the same process. It would seem that at the moment we know much more about the processes involved in immediate evolution than we do about the processes involved in long-term evolution, particularly over the matter of the supply of variation.

Interaction of these types of evolution could produce, by entirely Darwinian processes, a pattern of punctuated equilibrium, without any need to resort to any new concepts. There is no obvious reason why much of the stasis over geological time so much discussed recently could not be explained by genostasis, and the periods of rapid change explained by situations where new variation has allowed a species to get into a new environment in which evolutionary opportunities abound. However, the intention of this paper is to suggest that genostasis is a widespread and important phenomenon of the present, even if it is also significant in geological time.

Genetic constraints on variation would appear to be very important in determining what direction evolution actually takes. Despite the overall regularities brought about by environmental (and therefore selective) pressures, we should expect evolution to be unpredictable in detail, but not overall when there will be an averaging process. In evolutionary situations where some species succeed we pay too little attention to the others that fail. Whereas it is reasonable and proper to look for an explanation of evolutionary anomalies in terms of unexpected environmental circumstances, there is no reason to dismiss the alternative possibility of genetic constraints, even if these may be difficult to prove.

This lecture is given during a symposium on the evolutionary interactions of plants and animals. Nowhere is there better circumstantial evidence for the limitations set by the availability of appropriate variability than in the coevolution of butterflies and plants so carefully analysed by Ehrlich & Raven (1964). They argue that the occurrence of a new mutation allows the organism which carries it to 'enter a new adaptive zone'. Whether we take the success of the Pierinae related to their evolution of an ability to cope with the thioglucoside armoury of the Cruciferae and Capparidaceae, or the new steps represented by *Stalachtis* on Asclepiadaceae or by *Neophasia* on pines, the control on evolution set by variability is apparent. It cannot be a control set by selection because the hosts have been present for a very long time. This picture is supported by more recent work (Gottlieb 1980; Edwards 1989), that gives even evidence of a geographical pattern to the occurrences of particular compounds in taxa. The control also operates on the plant hosts. Those like the Rubiaceae, which have successfully evolved appropriate defences, have been rewarded. The path of this coevolution is dominated by what appears to be the chance occurrence of new variants. The same conclusion has been reached over the evolution of plant–vertebrate seed dispersal interactions (Herrera 1986).

The importance of genetic variability in controlling evolution is not a new idea. It was perhaps most elegantly formulated by the last evolutionist to give this Croonian lecture, R. A. Fisher. In his *Fundamental theorem of natural selection* (1930) he showed that the rate of increase in fitness of any organism is equal to its additive genetic variance of fitness at that time.

The idea of specific genetic constraints to evolution is also not new. It was first put most clearly by J. B. S. Haldane, also a Croonian lecturer, in 1932:

> Neither of these processes alone can furnish a basis for prolonged evolution. Selection alone may produce considerable changes in a highly mixed population. A selector of sufficient knowledge and power might perhaps obtain from the genes at present available in the human species a race combining an average intellect equal to that of Shakespeare with the stature of Carnera. But he could not produce a race of angels. For the moral character or for the wings, he would have to await or produce suitable mutations.

Plant and animal breeders are well aware of Fisher's and Haldane's strictures, and well appreciate the consequences of genetic constraints. This paper has little new to offer them. They are dominated by the problem of finding new genetic variation.

But in studies of normal evolution there is little evidence that the problem is fully appreciated. Most work is directed to showing what evolution can do, and rarely what it cannot do. We are guilty of bias. We have to remember that what we see today is as much determined by evolutionary failure as by evolutionary success. We need to consider in more detail to what this failure can be attributed.

The failure requires our attention for practical reasons also. One is that the successful use of antibiotics and pesticides relies on lack of evolution of resistance by the target organism. We have been fortunate that such evolution has not been excessively common. But it is now becoming more and more prevalent. It is important that we know why this evolution either does or does not take place. A second is that in the future we have to expect global climatic change. In relation to this, although some evolution will occur in some species, as it has in relation to air and other forms of pollution, we must presume that, in general, evolutionary adaptation will be limited. Unless the genetic variability of the species affected can be increased, or migration enabled, substantial extinctions can be expected. The failure of evolution also requires our attention when we begin to release organisms whose genetic make-up has been radically and successfully altered by genetic engineering. The unlocking of these organisms from their previous evolutionary constraints could have serious repercussions.

For a century we have been mesmerized by the successes of evolution. It is time now that we paid equal attention to its failures. There are important reasons why we need to understand them.

I am most grateful to Mike Begon, Bryan Clarke, John Endler, Doug Futuyma, Tom McNeilly, John Maynard Smith, Geoff Parker and Brian Tomsett for their patient reading of early drafts of this paper and their invaluable comments, and to Arthur Cain for his stimulus and encouragement over many years.

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Figure 2. Photographs illustrating the evolutionary situation in the vicinity of the 90-year-old copper refinery at Prescot, Merseyside; (*b–e*) taken in the railway cutting that antedates the refinery and passes through the polluted refinery area. (*a*) A typical front garden; attempts to garden given up and only *Agrostis stolonifera* and *Festuca rubra* (both represented by tolerant populations) surviving; (*b*) typical polluted area; the cutting sides are occupied by only five species, here mainly the grass *Agrostis capillaris* (tolerant population); (*c*) typical unpolluted area further away; the cutting sides are occupied by a much greater variety of species; (*d*) close up of polluted area; pure stand of *Agrostis capillaris* with not more than 50% cover; seedlings from a garden tree of *Acer pseudoplatanus* (arrowed) attempting to invade but without success as they do not possess required variation in tolerance; (*e*) most heavily polluted area; cutting sides with large bare areas because pollution levels are too high for the level of tolerance that has been evolved; some new growth is now occurring because of reduced pollution from improved control measures.