Origins and development of adaptation

Ciba Foundation symposium 102

1984

Pitman

London

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Chairman's introduction

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As natural scientists, it is our duty to listen to what Nature says but, unfortunately, Nature does not always speak clearly, so that we have to do experiments in order to improve her diction. When I was an undergraduate we were given, for the good of our souls, an essay on adaptation by Peter Medawar. The burden of this essay was that adaptation is a term full of pitfalls, describing sometimes a process, sometimes a general state, and sometimes a particular phenotypic condition. In its general connotation, adaptation merely means appropriateness, or propriety in the old sense of that word. Because improper organisms do not survive, the word is liable, if it is used loosely, to conceal a tautology. We have to be careful not to describe something as an adaptation merely because it exists and because organisms that show it survive. Yet there are degrees of propriety, and we know that in some particular environments some genotypes survive or reproduce better than others. The causes of these differentials are proper subjects for scientific study.

The common thread that I see in this symposium is the study of differentials brought about by the presence in the environment of lethal substances, produced either by humans or by other organisms. The effects of these substances have given us some of the strongest evidence in favour of natural selection.

It still seems to me extraordinary that bacteria, plants and animals can make so rapid an evolutionary response to the poisons produced by the human race. The answer must surely be that every organism has a long history of attempts upon its life, and comes equipped with a battery of defensive weapons that can be modified for use against new threats. But none of these threats is wholly new; the human race, in creating its poisons, has most often

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copied nature. Herbicides, insecticides and antibiotics are often modifications of the offensive and defensive weapons that organisms have used against each other. Thus, it is likely that coevolution—the alternation of offence and defence—will be a recurring theme at this symposium, and rightly so.

Evolutionary geneticists, in attempting to explain change or stability, have a natural tendency to measure what are called 'environmental factors' or 'environmental variables', and these are usually physical characteristics of the environment such as temperature, humidity or shade. Yet the most powerful evolutionary variables are likely to be the effects of other organisms. Such effects have often been ignored because they are so difficult to measure. There is, however, a great deal of evidence for their importance. The remarkable examples of cryptic coloration among prey species testify to the effectiveness of predators as agents of natural selection. These protective resemblances not only involve colour and pattern but also morphology and behaviour. The great array of complex arrangements that organisms have evolved as defences against parasites, from hairy leaves to the production of antibodies, testifies to the importance of parasitism in affecting the course of evolution. Similarly, the phenomena of character displacement and ecological replacement testify to the importance of competition. With respect to my own parasitism have hardly yet been touched by the experimenter (or, in the protein and DNA—I believe that parasitism will turn out to be the dominant evolutionary force. Mathematical models suggest that the coevolutionary chase between host and parasite can very efficiently generate genetic diversity under an unusually wide range of conditions. But the evolutionary genetics of parasitism has hardly yet been touched by the experimenter (or, in the current vernacular, the 'experimentalist').

There has lately been a depressing tendency to label scientific points of view as if they were political persuasions, and sometimes to behave as if they were. We are blighted by many '-isms'. Thus, if you believe in natural selection you are a 'selectionist'; if you believe in random genetic drift you are a 'neutralist'. There does not seem to be a label for those who believe in both.

This symposium is about adaptation, and it is no surprise that people who study adaptation are called 'adaptationists'. 'Adaptationists' have been accused by Stephen Gould and Richard Lewontin (1979) of many misdemeanours: 'We fault the adaptationist programme for its failure to distinguish current utility from reasons of origin; for its unwillingness to consider alternatives to adaptive stories; for its reliance upon plausibility alone as a criterion for accepting speculative tales; and for its failure to consider adequately such competing themes as random fixation of alleles, production of non-adaptive structures by developmental correlation with selected features, the separability of adaptation and natural selection, multiple adaptive peaks, and current utility as an epiphenomenon of non-adaptive structures'.

While students of adaptation may, from time to time, have committed all these sins, it seems to me that Gould and Lewontin have missed the very great strength of the Darwinian approach to evolution—that it makes us formulate testable hypotheses. If you believe that the phenotype is only some kind of epiphenomenon, or a neutral character, you have no motivation or incentive to do an experiment. Gould and Lewontin have neglected even to mention the experimental evidence for the evolution of adaptations by natural selection. Nevertheless, their list of vices seems a useful way to start this meeting. We must resolve to avoid the delights of easy 'plausibility' (without, I hope, cultivating implausibility); we must eschew just-so stories, and what they call the 'Panglossian paradigm', where everything is for the best in the best of all possible worlds; and we must assiduously seek alternative explanations of our phenomena. But, above all, we must do experiments, observe the results, and listen to what Nature tells us.

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Adaptation of plants to soils containing toxic metals—a test for conceit

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Abstract. Darwin, and many biologists afterwards, have seen few, if any, limits to the processes of adaptation by evolutionary change. Perhaps we have been conceited. A study of heavy-metal tolerance, and other conditions to which evolutionary adaptation has occurred, should overwhelm us with evidence for limits to the evolutionary process and limits to the adaptation it achieves. These limits clearly arise from restrictions in the supply of genetic variability. Nearly all species are in a condition of *genostasis*, in which there is a lack of appropriate variability for further evolutionary change. It is the molecular biologist who, by understanding the architecture of genes, will ultimately be able to explain what failures and limitations in genetic architecture at the molecular level cause the limits to adaptation itself.

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At any stage in the development of an idea, conceit can take over—not moral but intellectual conceit, coming not so much from people but from ideas. The idea of adaptation as a result of the action of natural selection or random variation is, because of its powerful simplicity, perhaps one of the best examples. It is so simple an idea, with such powerful implications, that even at the beginning Charles Darwin wrote, in The Origin of Species (1859), 'I can see no limit to this power, in slowly and beautifully adapting each form to the most complex relations of life'.

A hundred years later we continue with this view, stressing, with greater realization, the extraordinary range of adaptations that the simple process has produced. It is therefore well worthwhile taking stock of our position, to see whether we are guilty of conceit. The process might not be quite what we thought it was. Unfortunately, because evolutionary adaptation is such an immense topic, we cannot look at the whole of it. I will therefore take one single environmental condition which has profound effects on plants, and in

which evolutionary adaptation clearly occurs, and will try to see what it can tell us about our understanding of the processes of adaptation.

Metal-contaminated environments

Environments have been contaminated by heavy metals such as lead, zinc and copper ever since the original magma of the earth solidified, but they have often been covered with innocuous superficial deposits. In recent years the occurrence of such environments has been enormously increased by mining, so that we now have areas all over the world, ranging from a few square metres to many hectares, where the soil is dominated by the presence of heavy metals. *Dominated* is a correct word, because heavy metals are extremely toxic to plants: 0.5 parts per million (p.p.m.) of copper, 12 p.p.m. of lead and 20 p.p.m. of zinc in solution will each completely stop plant root growth and cause plant death in a few weeks.

Heavy metals are immobile in soil, and the levels found in mining wastes are commonly of the order of 1% (i.e. 10 000 p.p.m.). Although only a small proportion of this is available to plants, these areas are extremely inhospitable and devoid of plants. If an ordinary plant species such as the common pasture grass, *Lolium perenne*, is established on these areas experimentally it may germinate and grow for a while, but it soon dies. We have, therefore, an environment which is not only extreme but also permanent. More importantly, because it is open and without plants, it is potentially available for colonization by plants. Since plants have effectively colonized every environment available to them, what happens?

Colonization as a result of evolution

It is an exaggeration to say that these metal-rich habitats are totally devoid of plants. In fact, a restricted number of species is to be found growing on them, species that are also found growing in normal environments uncontaminated by metal. It was Prat (1934) who first showed, for *Silene vulgaris* (red campion), that the populations growing on the metal-contaminated materials were different from populations of the same species growing on normal soils; they are tolerant to the metal toxicity, whereas normal populations are not (Fig. 1). We now know that this is true for all the species found growing on mine wastes which have been tested, such as *Agrostis tenuis* (bent grass), *Anthoxanthum odoratum* (sweet vernal grass), *Plantago lanceolata* (ribwort plantain) and *Rumex acetosella* (sheeps' sorrel). These species are only found in metal-contaminated sites because they have metal-tolerant populations;

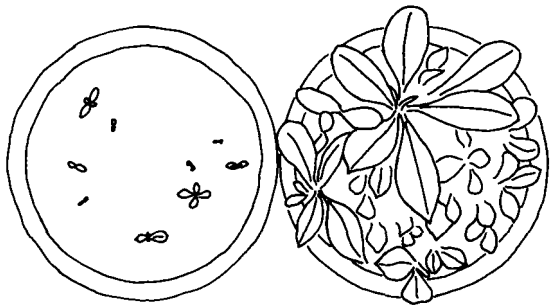

FIG. 1. The first evidence for the evolution of metal tolerance (or indeed for evolution in response to any pollutant): seedlings of normal (left) and copper-mine (right) populations of red campion (*Silene vulgaris*), growing on soil which has been treated with copper carbonate (drawn from Prat 1934).

their normal populations die rapidly when grown on metal-contaminated material. There is no clear evidence that any species is pre-adapted and thus has metal-tolerant normal populations.

The tolerance is largely specific to individual metals, and is almost certainly due to more than one mechanism, e.g. binding in the cell wall or isolation in the vacuole (Brookes et al 1981). It is generally determined by either a few or several nuclear genes, and has a high heritability (Bradshaw & McNeilly 1981).

The evolutionary process—rapid and local

Existence of tolerant populations is not by itself proof either of evolution or of a Darwinian process, although with our existing knowledge it is difficult to envisage any other origin. Although detailed testing has not been done, there is no evidence of a Lamarckian origin by induction—an individual plant cannot be trained to tolerate metals—and tolerance is not lost in culture in non-toxic conditions.

There is, instead, very good evidence that the character of tolerance can be rapidly developed in populations as a result of selection acting on heritable variation that occurs within populations which are not tolerant. If normal populations of a species such as *A. tenuis* are sown on a copper-contaminated soil, although nearly all the seedlings die, a few (about three in 1000) survive and grow successfully (Fig. 2). These can be shown to be copper-tolerant and to give rise to tolerant offspring when intercrossed (Walley et al 1974); this has now been demonstrated for at least six species.

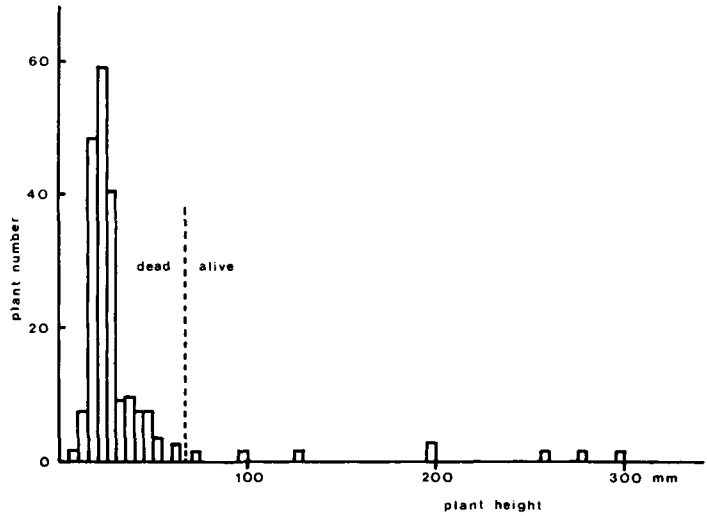

FIG. 2. The frequency distribution of growth of seedlings of a normal population of bent grass (*Agrostis tenuis*) on copper-contaminated soil, after 6 months, showing the survival of only a very few individuals. These seedlings are copper-tolerant and give rise to tolerant offspring, demonstrating that substantial copper tolerance can be selected in a single generation (from Walley et al 1974).

It is clear that exactly this process occurs in natural conditions. When a natural population is first subjected to metal contamination, enormous numbers of individuals die, but a few survive. There is selection for copper-tolerant individuals and a tolerant population is built up. This has been demonstrated in populations of *Agrostis stolonifera* adjacent to a copper refinery (Wu et al 1975a). The process can occur with great rapidity, in one or two generations, as in artificial selection experiments (Fig. 3). The rate-limiting factor appears to be the speed with which the tolerant survivors can develop vegetatively and sexually to give rise to a complete population (Bradshaw 1975). In less than 10 years, 50% of full tolerance can be achieved in *A. stolonifera*, despite its long generation time; this is quite sufficient for it to survive in levels of copper contamination that are lethal to normal populations of the grass.

At the same time the evolution can be extremely localized. Because strong selection for tolerance can counteract any immigration of genes from non-tolerant populations, contaminated areas as little as 20 m across can maintain tolerant populations. The transition from a tolerant to a non-tolerant population can occur over 5 m or less when there are sharp

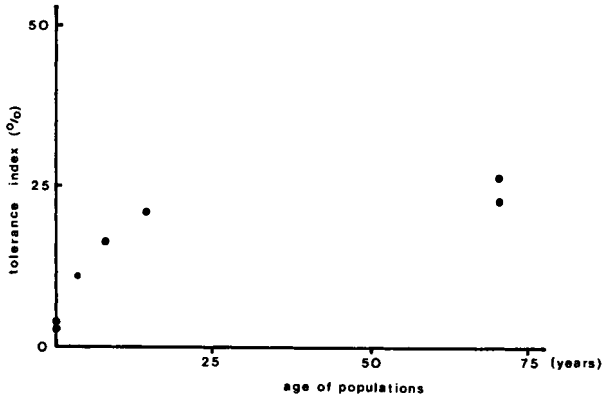

FIG. 3. The mean copper tolerance of populations of creeping bent grass (*Agrostis stolonifera*) exposed to copper contamination for different lengths of time in the neighbourhood of a copper refinery at Prescot, Merseyside. Copper tolerance can thus evolve in natural situations in a few years (from Wu et al 1975a).

boundaries between contaminated and normal soils (Antonovics & Bradshaw 1970). The adaptation can follow the pattern of the environment very closely.

The cost of tolerance

Tolerance does appear to have a cost in terms of fitness when plants are growing in the absence of heavy-metal contamination. In some cases, for instance zinc tolerance in *A. odoratum*, tolerant plants are distinctly slower growing than non-tolerant plants. Even in species where this does not happen, tolerant plants, when put into competition with non-tolerants on ordinary soils, perform significantly less well than non-tolerants (Cook et al 1972, Morishima & Oka 1977).

However, most of these differences come from observations on plants that derive from different populations. Their differences in growth rate or competitive ability may not be the direct effect of tolerance, but may be due to the evolution of differences in response to other characteristics of the environment; for example, slow growth is an adaptation to the low nutrient supply which is common in metal-contaminated habitats. For this reason it is interesting to discover that in populations on normal soils adjacent to, but outside, metal-contaminated areas, frequencies of metal-tolerant individuals are low (although they may be somewhat elevated from levels in normal populations). This implies a selection against tolerance and therefore against

its accumulation due to gene flow. There is elegant evidence of such selection in *A. tenuis* outside a small copper mine in North Wales (McNeilly 1968) (Fig. 4). This fits in with the fact that tolerance has never been found in species on uncontaminated soils. There is clearly a cost to the tolerance that is at present available in plant species. For copper-tolerant individuals the explanation appears to be an enhanced requirement for copper under normal conditions, presumably brought about by the copper-tolerance mechanism.

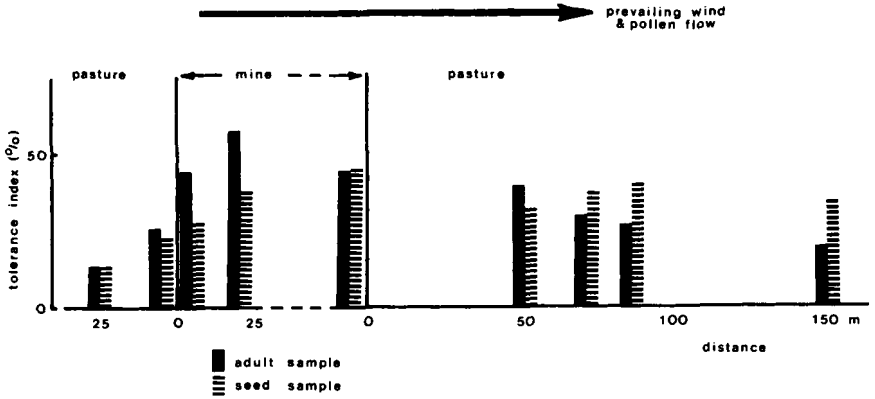

FIG. 4. Copper tolerance in adult and naturally produced seed populations of bent grass (*Agrostis tenuis*) at Drws-y-Coed copper mine: copper tolerance is more or less limited to the very small mine site, although the seed populations show that some genes for tolerance escape from the mine area and are selected against (from McNeilly 1968).

Limits to adaptation

The occurrence of tolerant plants in metal-contaminated environments might seem to imply that tolerance is all-powerful, but this is not so; it has distinct limits. This is manifest in the field by the fact that there are, commonly, areas of metal contamination that are not colonized, even by metal-tolerant plants. Sometimes this must be due to some other factor, such as extreme deficiency of a nutrient. But it can obviously be caused also by limits in the evolution of tolerance despite powerful selection pressures. Although comparisons of copper-tolerant and normal plants show that the tolerance mechanism has a remarkable ability to complex copper and to prevent its being translocated, there comes a point at which the tolerance mechanism is overcome; this can easily be demonstrated in solution culture (Wu et al 1975b). 'Super-tolerant' populations on areas of very high metal contamination have never been found.

The limits of variability

So far I have treated the adaptation of species for survival in metal-contaminated environments rather as if all species can evolve tolerance. But this is patently not true. Only certain species colonize these habitats (Antonovics et al 1971), and where a pre-existing flora has become subject to metal contamination, only a few species persist and evolve tolerance; although the rest have had the opportunity to do so, they appear to have failed to achieve it (Bradshaw 1975).

This failure to develop tolerance raises an important point. These latter species did not lack for selection, and so the only alternative possibility that will account for the lack of evolution is that they lacked for appropriate variability. This must be true for innumerable species and contaminated habitats. Material of a wide range of species will constantly be arriving in contaminated habitats by migration and will therefore be subject to selection; yet these species do not establish tolerant populations.

As we have seen, when normal populations of *A. tenuis*, a species that does evolve tolerance, are screened for tolerance, a very low frequency of at least partially tolerant individuals is found, on which natural selection can act. If a range of species is examined, it is found that those that occur in metal-contaminated environments and evolve tolerant populations also possess variability for tolerance in their normal populations (Gartside & McNeilly 1974). We have now looked at this in more detail, using a more sensitive screening technique (Table 1) involving the ability of individual seedlings to root in copper solution, in water culture, when supported on a raft of polyethylene beads. For the 15 species tested there is no case of a species that evolves tolerance but which does not possess variability for tolerance in its normal populations—although, in some species that possess variability for tolerance, tolerant populations have not yet been found. Evolution cannot proceed if the appropriate variability is not present, and for tolerance this variability is apparently not found in all species.

Relevance to other adaptive situations

Because metal contamination provides a rather extreme habitat, it is tempting to dismiss what we can learn from the evolution of tolerance as irrelevant to the processes of adaptation to other, more normal, environments. But it must be remembered that most habitats are extreme in one characteristic or another; there is severe competition in fertile habitats, severe nutrient deficiency in calcareous habitats, lack of light in woodlands, and high salinity in salt marshes. Indeed, the fact that only a limited number of characteristic

TABLE 1 The percentage of copper-tolerant individuals found in normal populations of various grass species, in relation to the presence of the species on copper-polluted waste and whether the plants collected were tolerant of copper

Species	*% occurrence of tolerant individuals*	*Presence of species on mines:*		*Tolerance of collected adult plants*
		On waste	*At margins*	
Holcus lanatus	0.16	+	+	+
Agrostis tenuis	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 spp.	0.0	−	+	−
Arrhenatherum elatius	0.0	−	+	−

+, presence; −, absence. Only those species which possess copper-tolerant individuals in their normal populations evolve tolerant populations which colonize mine waste. (Data of C. Ingram.)

species occurs in every habitat is proof that the habitats are severe for other species, which would otherwise be present.

All the characteristic features of the evolutionary process that allows plants to grow in metal-contaminated environments can be matched by examples from other environments. Table 2 gives some examples, although it cannot do full justice to unpublished information that is within people's experience, particularly plant breeders. Indeed, the whole experience of plant breeding is that once the genes for the development of a new character, or the enhancement of an old one, are available, rapid progress is possible. But normally the great difficulty lies in finding the desirable genes.

Discussion—the limits to evolution

If we put all this information and the deductions from it together, there are certain unavoidable conclusions which bear on the nature of adaptation due to evolutionary processes.

We must realize that all habitats, without exception, have the potential to generate selection pressures. This is obvious when we consider all the species

TABLE 2 Some examples of adaptation to environments other than those contaminated by metals, which illustrate the same principles of adaptation

Colonization as a result of evolution	
High altitudes	*Achillea borealis*
Low-fertility habitats	*Trifolium repens*
Serpentine soils	*Gilia capitata*
Rapid and local evolution	
Park-grass-fertilizer experiment	*Anthoxanthum odoratum*
Vernal pools	*Veronica peregrina*
Grazing	*Dactylis glomerata*, *Trifolium repens*
Evolutionary cost	
Life-cycles	*Poa annua*
Soil calcium	*Festuca ovina*
Latitude	*Oxyria digyna*
Limits to adaptation	
Yield	chickens
Disease resistance	*Triticum, Solanum, Hordeum*
Alpine climate	*Melandrium rubrum, Ranunculus acris*
Limits to variability	
Herbicide resistance	*Stellaria media, Bellis perennis*
Disease resistance	*Triticum, Solanum, Oryza*
Flower colour	*Delphinium*

that do not occur in a given habitat; those which continue to immigrate but fail to establish demonstrate the existence of coefficients of selection against them equal to 1.0.

Such species cannot have failed to adapt, and therefore to survive, because of a lack of selection. Their failure must be due to a lack of appropriate variability, that is, of such variability as would allow them to survive in that environment. The species that do occur in a particular environment are those that possess the appropriate variability. This variability is thus not universal to all species; only certain species possess it.

If the appropriate variability is present, then it is selected rapidly. Because of high coefficients of selection, evolutionary adaptation will occur within a few generations. If it does not occur rapidly, because the variability allows only very small improvements in fitness or because there is some cost due to pleiotropy or linkage, for instance, then it is, again, because the *appropriate* variability is not present. Inappropriate variability includes genes with weak effects, genes with disadvantageous pleiotropic effects, or genes linked too closely to other disadvantageous genes.

It follows that, for every character, any population has effectively reached an evolutionary plateau, determined not by selection but by lack of appropri-

ate variation—a condition of *genostasis* (Bradshaw 1984). This genostasis must be almost universal, and the incidence and degree of adaptation, whether found on old mine workings or generally in nature, must be the outcome of what genetic variability is available. The genostatic condition applies, of course, only to the particular character on which selection has been acting: there can be plenty of variability in other parts of the genotype.

Lack of progress in achieving a particular goal in an artificial selection programme, or lack of further improvements in fitness characters in natural populations, often appears to be due to overdominance or to other forms of non-additive gene action. Fitness characters often show striking genetic variability but low heritability (Falconer 1981). While this may be the immediate cause of lack of adaptation, it is not the ultimate cause, which is lack of appropriate, in this case additive, variation. Similarly, where stabilizing selection appears to be the cause of a lack of change in a character, it is perfectly possible that there is really a lack of variability that would allow directional selection to have effects. A better turtle, after all, would surely be possible!

Of course it must follow that where a population or species has not already been subject to particular selection pressures it is likely to possess potential variability for selection—that is, for the characters in question. It is perhaps significant that most of the best examples of evolution in action are where new selection pressures are operating: for instance, in industrial melanism in moths, in resistance to pesticides in insects and in heavy-metal tolerance (Bishop & Cook 1981).

Conclusion

I would conclude that we should not allow the original Darwinian concept of evolution to lead us to conceit. Evolution may be able to achieve remarkable results, but what should bother us is how much it does *not* achieve, despite all the pressures of natural selection.

This suggests that to understand adaptation in the future our attention should turn even more from what is outside to what is inside organisms. The key to understanding evolution lies in understanding the architecture of characters or, more particularly, the construction of characters. It is the molecular biologists who, by understanding the ways in which genes that produce new functions (and therefore new adaptations) are built up by random events within a pre-existing structure, may be able to explain how some things are possible and others are not.

It is unlikely that we shall ever be able to predict future evolution because of the stochastic elements of the mutation process. But surely the key to

understanding how adaptations do, and do not, occur must lie within the architecture of genes.

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DISCUSSION

Clarke: A.J. Cain (1964), in his essay on 'The perfection of animals', said that evolution could more or less do what was necessary, when required. Perhaps you would disagree about this. Among the grasses, is there any ecological or taxonomic sense in your list (Table 1) of species that have or do not have available variation in tolerance?

Bradshaw: We have been unable to make any sense of the occurrence of variability. When we reviewed what was known about tolerance to different metals in all species (including all the species found on metal-contaminated sites whether or not they were known to be tolerant) previous ideas that we had had about links with particular families of flowering plants evaporated. There are no legumes that are metal-tolerant in Europe, but in other parts of the world they can grow at very high metal levels, e.g. in Zimbabwe, where legumes are some of the most common species on metal-contaminated sites. Yet certain families contain no examples of metal-tolerant individuals. The numbers involved are, however, rather small so the results could just be a matter of chance.

Hartl: If genes for heavy-metal tolerance are detrimental in uncontaminated soil, then why do certain species maintain the genetic variation?

Bradshaw: This is an annoyingly difficult problem to answer; it is a contradiction. We don't know enough about the processes that produce variability. There are two possibilities. There is some evidence (Urquhart 1971) that metal tolerance is variable in its dominance and that initially it may be recessive but may vary in different populations (there is evidence, almost, of an evolution of dominance). So in this case the variability would be 'sheltered' in those populations where the genes are recessive. The other possibility is that mutation rate is important: mutation would 'feed in' genes against selection, the effects of which would be quite low when the genes are rare. The difficulty in this case is to measure the mutation rate in outbreeding species, which we have been working with until now. We would like to study this further by looking at mutation in tissue culture, where the tolerance is also expressed.

Gressel: Some genera, such as *Lolium* (the ryegrasses) seem to have picked up resistance (or tolerance) to many different things, and may therefore possess more adaptivity than the 'average' species. *L. perenne* has adapted to the herbicides paraquat and dalapon (Faulkner 1982) and to sulphur dioxide (Horsman et al 1978). *Lolium rigidum* has adapted to diclofop methyl (S. Knight 1983, personal communication). *Poa annua* has also evolved tolerance to a few herbicides. Is there something special about these?

Bradshaw: Because *L. perenne* is an important cultivated crop plant, people have simply studied it more. Any species is able to evolve many different things (which, in a sense, argues against what I said in my paper) but, on the other hand, not all species can do everything—of that there is clear evidence.

Davies: In the experiments where you found that one group gave rise to tolerant derivatives and another group did not (Table 1), were you testing all species on a soil with a constant concentration of the copper ion?

Bradshaw: Yes. The concentration of copper was $0.2\,\mu g\,cm^{-3}$.

Davies: Did the group that do not give rise to tolerant derivatives have a

much lower basal level of resistance (in terms of parts per million) to the copper ion?

Bradshaw: Gartside & McNeilly (1974) found that this was true to a certain extent, but the results did not go far enough to be conclusive. They tested *L. perenne* for copper tolerance and found, at what appeared to be low copper levels, certain signs of tolerant individuals. But, interestingly, that variability turned out not to be heritable.

Davies: It's really a question of the level of metal susceptibility of the plant. One cannot expect to find a species becoming tolerant to a concentration that is simply much too high for it.

Bradshaw: I agree. But in our work we found that if we started screening at lower levels of contamination, the individuals that we picked out as being tolerant possessed a variability that was not heritable. It is only at the higher levels of contamination that one sees variation that is distinctly heritable. We have never found, as I mentioned, any species that clearly evolve tolerance in nature and which do not have variability that is well expressed within their normal populations.

Davies: What is the difference in susceptibility to copper, lead or zinc for those two groups of plants?

Bradshaw: In their normal populations, the two groups have roughly similar susceptibilities, but one group of species has individuals in their population that are tolerant and the other group of species does not.

Davies: But that is only true at the concentration of copper that you tested; tolerance depends on concentration.

Graham-Bryce: You emphasized that the process of colonization is rapid, but it is important to establish the nature of the process to assess the significance of this rapidity. In your illustration (Fig. 1) of red campion (*Silene vulgaris*) growth you ask whether a local tolerant population evolved or whether it was there in the first place. Is this not merely a semantic point, because that work presumably demonstrates the selection of highly tolerant individuals from a heterogeneous population as a result of an extreme selection pressure? One would surely expect such a process to happen as soon as the selection pressure is applied. What seems more interesting is that examination of subsequent performance under heavy-metal stress indicated what might be termed a 'drift in susceptibility'—for example, the increased root growth in the population from a mine site, compared with that in the initially selected individuals. Is that a result of some subsequent adaptation?

Bradshaw: As far as our work can show (and we have never studied many cycles of selection), in one cycle of selection one can build up a population that has 50–70% of the tolerance of a mine population, but it depends on the species. This perhaps relates to the genetic basis of the variability that is available in the population. Fig. 3 (p 8) shows that, after the first cycle of

selection, further selection gives rise to a predominantly tolerant population.

Georgopoulos: You were speaking about flowering plants, but fungi that are pathogenic to plants have been controlled with copper for about the last hundred years. This has never caused any problems. Do you believe that the appropriate variability is not present in the fungi?

Bradshaw: I would like to know that myself! It provides a very good example of what I'm arguing for. Thankfully, these important pathogens do not seem able to evolve copper tolerance. *Scytalidium*, on the other hand, is well known as a species that *can* evolve copper tolerance, although in the somewhat different environment of liquid culture (Starkey 1973). Yeasts can also do this.

Elliott: What is known about the mechanism of the tolerance? Are natural chelating agents present to dispose of the metal ions, rendering them inactive?

Bradshaw: There are two lines of evidence, as I mentioned. One is that tolerance is connected with substances such as proteins in the cell wall, and that the metal is complexed by them in the cell wall (Turner & Marshall 1972). Another line of evidence suggests that the metal enters the cell and is isolated in the vacuole. Other, general tolerance mechanisms have been suggested to depend on malate (Ernst 1975). All the suggestions made about mechanisms have, naturally but unfortunately, been suggested for specific cases. The final answer is not clear. But whatever happens, metal accumulation in the plant is restricted to the root. In tolerant plants there is normally more metal in the root than in non-tolerant plants.

Hutchinson: Not only does metal accumulate in the root but the actual physiological basis of tolerance resides there, in several investigated cases. Some interesting reciprocal grafting experiments have been done by J.C. Brown in the USA. Sensitive and tolerant clones to iron-deficiency were grafted. Irrespective of which graft was made, the tolerance was really determined by the root stock, i.e. sensitive root stocks gave sensitive tops even if the top was from a resistant plant.

What we have been discussing reminds me of the development, in rats, of resistance to warfarin, in that it has originated independently in several separate geographic locations. The same plant species, in both North America and Europe, have been shown to develop metal-tolerant populations. There is clearly very widespread genetic potential in some of these genera, such as the grass *Agrostis*, which has produced resistance to copper, lead and zinc in the USA, Canada, the UK and Germany quite independently.

I have another point about tolerance specificity, with which Professor Bradshaw may not agree. Quite regularly we are finding, i.e. with two grass and two unicellular algal species, that the specificity of metal tolerance breaks down considerably. Co-tolerances seem to develop; that is, one can select for tolerance to one particular metal, e.g. zinc, and coincidentally one will also find

tolerances to lead and cadmium associated. Co-tolerances occur for which the metals were not elevated in the environment and could not, therefore, have been selected for in the usual way (see Hutchinson, this volume). So, rather than having to have an energy-expensive physiological mechanism with absolute specificity for each different metal, the plant can have some general biochemical mechanisms that can handle groups of chemically similar elements. One such example is when copper confers an enhanced tolerance to silver (Cox & Hutchinson 1980, Stokes et al 1973).

Bradshaw: I don't disagree with you. We have been looking at populations by crude analysis and have found that tolerance to individual heavy metals appears to be specific. But more refined techniques reveal that a copper-tolerant population that has never had any experience of lead or zinc has a low, but significant, level of tolerance to lead and zinc also. It seems that there is perhaps both a generalized tolerance operating at a low level of contamination and specific tolerance able to operate at higher levels. This rather suggests that there may be two different mechanisms operating.

Kuć: I find it difficult to come to grips with the terms tolerant, intolerant, and lack of tolerance. This is because many of these metal ions are actually *nutrients*, in trace quantities, and are absolutely essential to the metabolism of the plant. At one extreme one could say that all plants have not only a tolerance but a need for the ions. In defining tolerance one should indicate the amount of the contaminant concerned. Thus, plants can be considered tolerant if they grow and develop normally (whatever 'normally' means) at a certain level of the contaminant; if they do not, then they can be considered intolerant. The difficulty is in deciding what this level should be. Furthermore, tolerance to a given contaminant will also depend on environmental conditions other than the quantity of the contaminating substance in the environment of the plant.

Bradshaw: Obviously one is guilty of simplification when one starts to talk about a *tolerant* individual. That same individual could be found *not* to be tolerant by dying when exposed to a little more copper. But nevertheless, relative to normal plants, the individual can be considered to have a level of tolerance that is distinguishable. This problem also applies to any consideration of tolerance to antibiotics or herbicides. It also goes back to my consideration of the limits of tolerance. In areas such as the middle of the copper mine at Parys Mountain, copper-tolerant *Agrostis* fails to grow in the most toxic areas. One might have expected to find plants with super-tolerance to copper, but they do not occur. Plants from that habitat don't seem to be any better than plants that come from less extreme habitats. There seems to be one level of tolerance that the mechanism can achieve, and no more, implying a limit to the adaptation that can be achieved.

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