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## METAL TOLERANCE IN PLANTS: PERFECTING AN EVOLUTIONARY PARADIGM

Janis Antonovics

*Botany Department*  
*Duke University*  
Durham, North Carolina 27706

### ABSTRACT

This paper reviews the studies that have been carried out in the past five years on the evolution of metal tolerance in plants. The number of species shown to evolve metal tolerance has increased. Evolution of metal tolerance has been shown to be a function of, among other things, time, metal concentration, and sensitivity of the species to the metal. Two species have been shown to colonize contaminated soils, apparently without evolution of tolerant races. Rigorous genetic studies have shown metal tolerance to be inherited in a polygenic matter with no maternal effects. The mechanisms of metal tolerance appear to be diverse, but frequently involve cellular sequestering of the toxic elements. The tolerance mechanism is metal specific. The use of screening techniques for estimating low frequency occurrence of tolerance has extended our knowledge of the origin of tolerance from non-tolerant populations. Considerably more studies are needed to clarify what limits selection for increased tolerance in already tolerant species or species with the potential to evolve tolerance. There have been relatively few studies on the evolution of isolating mechanisms and speciation on metal mines, even though such studies could be particularly rewarding—especially on naturally occurring metal outcrops that have been in existence for long periods of time.

### RÉSUMÉ

Cette communication passe en revue les études qui ont été réalisées au cours des cinq dernières années sur l'évolution de la tolérance aux métaux chez les plantes. Le nombre d'espèces qui ont acquis une tolérance aux métaux a augmenté. On a démontré que l'acquisition de la tolérance aux métaux dépend, entre autres, du temps, de la concentration métallique et de la sensibilité de l'espèce au métal. D'après les observations, on a constaté que deux espèces qui s'étaient implantées sur un sol contaminé n'ont pas produit de races tolérantes. Des études génétiques rigoureuses ont démontré que la tolérance aux métaux était transmise par polygénie sans effets par le parent femelle. Les mécanismes de tolérance aux métaux sont, semble-t-il, variés, mais comprennent fréquemment un emprisonnement par la cellule des éléments toxiques. Le mécanisme de tolérance est spécifique à un métal. Les techniques de dépistage utilisées

pour évaluer l'incidence peu fréquente de la tolérance ont augmenté considérablement nos connaissances en ce qui concerne l'origine de la tolérance chez les populations non-tolérantes. Il faudra effectuer beaucoup d'autres travaux pour expliquer ce qui limite la sélection pour une tolérance accrue chez des espèces déjà tolérantes ou des espèces pouvant acquérir une tolérance. Il y a eu relativement peu d'études sur l'évolution des mécanismes d'isolation et la spéciation due aux métaux, bien que de telles études pourraient être particulièrement fructueuses, spécialement sur des affleurements naturels de métaux qui existent depuis longtemps.

## INTRODUCTION

"The sequence and pattern of genetic change responsible for the colonization of metal contaminated areas is . . . one of the best documented cases of evolution in action"

Antonovics, Bradshaw and Turner (1971)

Current concerns with environmental issues stem in part from recent increases in the level of many elements or compounds on both a local and a global scale. The increased use of heavy metals in particular has led to a corresponding dissemination and increase of these elements in the environment. The best known of these are lead (National Academy of Sciences 1972), mainly from "anti-knock" compounds in car exhaust, and mercury (Swedish Royal Commission 1967), from its use as a fungicide in agriculture and industrial processing. However, increased quantities of a large number of other elements have recently been detected (Burkitt *et al.* 1972: Pb, Sn, Cd; Abdullah and Royle 1972: Mn, Fe, Cu, Pb, Cd, Ni, and Sn; Goodman and Roberts 1971: Zn, Pb, Cd, Cu, Ni; Ruhling and Tyler 1969: Pb, Cu, Sn, Ni, Cr; Cannon and Anderson 1971; Stanford *et al.* 1975). Metal pollution is no longer seen to be an isolated or anomalous phenomenon, but is an extremely pervasive syndrome in our environment. For example, Ernst *et al.* (1974) have shown that in one province, Westfalia in Germany, metal pollution is the result of metal mining, smelting, car exhausts, sewage sludge, and downwash from power lines.

The effects of such increases may be immediate and acute, or long term and chronic, depending on the level of the pollution: in both cases ecosystems respond at several levels. Individuals may show toxicity symptoms, and these are so frequently recorded in animals, plants, and humans that a review is beyond the scope of this introduction; indeed much of the present volume concerns itself with such effects on individuals. Above the individual level, populations may respond by undergoing evolutionary responses to metal pollution. These have been most extensively documented in the case of very

toxic levels of metal (Antonovics *et al.* 1971). Nevertheless, the role of evolutionary response in studies of metal contamination is often misunderstood or considered unimportant. For example, in the recent National Academy of Sciences (1972) Report, "Airborne Lead in Perspective", it is stated: "...there seems to be no reliable evidence that lead injures plants in nature. In fact extensive investigations summarized by Bradshaw *et al.* (1969) show that some species of plants have adapted to habitats near mining operations that contain lead, zinc, and copper in amounts that are toxic to non-adapted populations of the same species." There is clearly an internal contradiction in these statements, since it is precisely the injurious effects of lead that have resulted in natural selection for more tolerant genotypes; it is not further evidence of "beneficial" responses of plants to metal contamination analogous to trace element response.

The individual and population responses are translated at the species level into shifts in the community structure: different species have different direct reactions to increased metal level, and species also differ in their ability to evolve tolerance. The resultant changes in species composition have been studied most extensively in the communities growing on old mine-waste in Europe (Ernst 1965). The population and community effects of metal contamination interact in important ways. Colonization of contaminated mine waste has been generally contingent on the evolution of tolerant races or ecotypes. In over twenty species that had been studied (Antonovics *et al.* 1971), there was no known species with an "inherent" tolerance to high levels of metal (although several studies where this may be the case are discussed later). Evolutionary effects are also important for ecosystem studies, particularly as far as entry of metals into food chains is concerned, since metal-tolerant plants are likely to contribute more to metal uptake from the environment than non-tolerant members of the same species. Firstly, they are able to grow in areas containing larger concentrations of metal ions. As a consequence, metal tolerant plants may contain extremely high amounts of metal: for example, Prat and Komarek (1934) found individuals with 3.25% Cu in the ash, and Ernst (1965) records plants of *Thlaspi alpestre* containing more than 15,000 ppm Zn on a dry weight basis (for review see Antonovics *et al.* 1971). Secondly, there is evidence that metal tolerance may at times enhance uptake (Gregory 1965) since the mechanism of tolerance often involves complexing of the metals by cell constituents rather than their exclusion from the plant. Thirdly, there is evidence that the distribution of metal among plant parts may be different in the tolerant and non-tolerant genotypes (Peterson 1969).

In this paper I review explicitly the studies concerning evolutionary processes in contaminated areas that have been published since the review of

Antonovics *et al.* (1971). I will consider how these recent studies impinge on our ideas of metal tolerance as a phenomenon, and on our current ideas of evolutionary processes in natural populations.

#### EVIDENCE FOR RACES TOLERANT TO METALS

Both the number of species showing metal tolerant races, and the range of metals to which species can adapt, have been increased as a result of studies summarized in Table 1. Although relatively few species have been added to the list, several important new considerations to our view of intraspecific variation in metal tolerance have emerged.

It has become clearer that evolutionary response to metal pollution is a function of time and metal concentration (Wu and Bradshaw 1972). Plants growing on areas of lesser contamination will therefore be expected to have undergone variable evolutionary responses, depending on many factors such as time scale, concentration of metals in the environment, heritability of metal tolerance, sensitivity of the species to the metal, and breeding system and generation time of the parental species. For example, Wu and Antonovics (1975a) showed that whereas *Plantago lanceolata* taken from a lead contaminated roadside showed lead tolerance, *Cynodon dactylon* sampled from the same site showed no significantly increased tolerance when compared with control populations. Subsequent studies showed non-tolerant *P. lanceolata* to be more sensitive to lead (in terms of root growth inhibition) than *C. dactylon*. A contributory factor to the difference between the two species could also have been the fact that *C. dactylon* has profuse vegetative propagation and possibly therefore less genotype turnover during colonization. Similarly, McNaughton *et al.* (1974) found no evidence of significant differential growth response of populations of *Typha latifolia* from metal contaminated and uncontaminated soils, when grown reciprocally on the two soil types. Wiltshire (1974) examined a wide range of species from contaminated and uncontaminated soils with regard to their growth on control soils, and on soils high in Ni/Cr or Cu/Pb. In only two cases was there significantly better growth of the population from metal containing regions on metal containing soils (see Table 1). In all other comparisons the population from the metal containing soil normally grew better on the soils high in Ni/Cr or Cu/Pb, but the differences were not statistically significant (*Andropogon gayanus* var. *squamulatus*, Ni, Cu; *Andropogon schirensis*, Ni; *Aristida adscensionis*, Cu; *Heteropogon contortus*, Ni; *Bidens pilosa*, Ni; *Borreria senensis*, Ni; *Celosia trigyna*, Ni, Cu). Both studies suggest that colonization of contaminated soils may indeed be possible without evolution of tolerance, but

TABLE 1

Plant species showing races tolerant to heavy metals.

Species	Metal	Location	Author
<i>Andropogon scoparius</i>	As	Virginia, U.S.A.	Rocovich and West (1975)
<i>Plantago lanceolata</i> <sup>1</sup>	Pb	North Carolina, U.S.A.	Wu and Antonovics (1975)
<i>Eragrostis viscosa</i>	Ni/Cr	Rhodesia	Wiltshire (1974)
<i>Pogonarthria squarrosa</i>	Cu/Pb, Ni/Cr	Rhodesia	Wiltshire (1974)
<i>Agrostis stolonifera</i> <sup>1</sup>	Cu	Lancashire, U.K.	Wu and Bradshaw (1972)
<i>Marchantia polymorpha</i>	Pb	Scotland, U.K.	Briggs (1972)
<i>Lolium perenne</i> , <i>Arrhenatherum elatius</i> , <i>Dactylis glomerata</i> , <i>Poa trivialis</i> , <i>Cynosurus cristatus</i> .	Cu	Artificial selection	Gartside and McNeilly (1974c)
<i>Silene maritima</i>	Cu, Zn, Pb	U.K.	Baker (1974)

<sup>1</sup>Species previously shown to form tolerant races to other metals.

care must be taken in the interpretation of soil experiments, since not only may the effective toxicity of such soils be low, but the experimental conditions may also ameliorate the toxicity. For example, Wiltshire (1974) diluted the soils with sand and fertilized them with nutrient solutions.

In view of the wide ranging nature of metal contamination, it would be fascinating to document the extent and course of evolutionary response to metal pollution, even in areas of relatively low contamination. It may at first sight seem that this would be difficult to study since evolutionary response to relatively minor environmental changes may be slow and/or obscured by the "noise" of other local adaptations or individual genetic variation. This does not, however, imply that it is beyond resolution, particularly in a character such as metal tolerance which is clear cut and easily studied. Screening populations for frequency of incidence of tolerant genotypes is a very sensitive technique for detecting occurrence of such genotypes even in uncontaminated pastures. It has also been used for detecting extremely low level gene flow into pastures several kilometers distant from a large copper mine (Bradshaw *et al.* 1969; Khan 1969). It can therefore be used as a primary tool for detecting small-scale evolutionary shifts toward greater metal tolerance.

In the past, much tolerance testing has relied on a limited knowledge of the relevant toxic elements in mine soil. Usually there has only been soil analysis for the one or two elements most likely to be in high toxic levels, as inferred from mineralogical and geological observations. This has led to anomalies such as that reported by Barker (1967, personal communication). In a study of the tolerance of a lead-tolerant strain of *Agrostis tenuis*, he discovered an appreciable co-tolerance to copper. This had never before been detected. However, subsequent soil analysis revealed copper as an associated element in the waste tailings from which this particular strain originated. Multiple elemental analysis of soil in which the plants are growing, or of the plants themselves (Walter *et al.* 1974), could enable simultaneous testing for tolerance to a number of elements in the proportion in which they occur in the soil. There is evidence that mixtures of elements are considerably more toxic to non-tolerant plants than are the elements singly, and this could prove to be a particularly sensitive technique for detecting evolutionary responses to lower concentrations of metals.

There have been several studies on metal tolerance of micro-organisms. Stokes (1975) in the present volume, reports enhanced metal tolerance in strains of *Scenedesmus* and *Chlorella* from lakes contaminated with nickel and copper from smelter emissions. Small (1971, 1972) showed that fungi (for example, *Sclerotinia homoeocarpa*, dollar spot) controlled by mercurial application on golf-course greens had evolved resistance to mercury and

cadmium. Phytotoxic effects of the fungicide applications were also noted, and it is possible that the grasses of golf-course greens are developing tolerance to these metals, particularly since the species commonly used on golf courses (namely red fescue, *Festuca rubra*; creeping bent grass, *Agrostis stolonifera*; and velvet bent grass, *Agrostis tenuis*) are well known to evolve tolerance to a number of different metals. Mercury tolerance has also been reported in bacteria (Vaituzis *et al.* 1975). Nyberg (1975) has reported the presence of copper-resistant strains of *Paramecium aurelia* among strains isolated from a wide range of natural habitats and localities. He suggests that this enhanced copper tolerance may be adaptive to conditions from which the strains come.

### GENETICS OF METAL TOLERANCE

Several studies have considerably strengthened our understanding of the genetics of metal tolerance in natural populations of higher plants. Gartside and McNeilly (1974a) performed diallel crosses among plants of *Anthoxanthum odoratum* and *Agrostis tenuis* sampled from a zinc mine and an adjacent uncontaminated pasture. In both species, the results showed zinc tolerance to be clearly inherited with partial dominance of the tolerance; there were no significant maternal effects. It was suggested from the continuous nature of the F<sub>2</sub> segregations that several genes were involved in the tolerance. Gartside and McNeilly (1974c) tested the inheritance of copper tolerance in artificially selected material, using single parent/progeny regressions from polycross material. In all species studied (*Lolium perenne*, *Agrostis tenuis*, *Arrhenatherum elatius*, and *Dactylis glomerata*), copper tolerance was highly heritable (0.66-0.88).

The apparent facility with which species have evolved tolerance to heavy metals has led people to question, at least informally, whether we are seeing here some kind of Lamarckian or conditioning effect rather than the process of natural selection (cf. Reilly and Stone 1971; Rocovich and West 1975). Previous evidence (that tolerance is transmitted to seed progeny, remains constant through several cycles of cloning on normal soil, and cannot be induced in non-tolerant material by conditioning) argued strongly against Lamarckian-type processes. The rigorous studies of Gartside and McNeilly (1974a, b) on the genetics of tolerance confirm that there are no maternal effects and that tolerance shows an inheritance pattern typical of a normal quantitative trait. It is interesting in this regard to note that tolerance was preserved through several "cell generations" of tissue culture of copper and zinc tolerant *Agrostis stolonifera*, and was identical in plants regenerated from

shoot-callus or root-callus tissue from the same individual (Wu and Antonovics 1976b).

In spite of these advances in our understanding of the genetics of tolerance, important questions remain. It is puzzling that tolerance should be highly metal specific (Wu and Antonovics 1975a), yet be under polygenic control. There have been no studies of crosses between plants of a single species that are tolerant to different metals. Such studies might throw considerable light on the nature of the metal tolerance specificity. Metal tolerance has undoubtedly been evolved independently in different regions of the world. For example, *Plantago lanceolata* has zinc tolerant races in the U.S. and Europe (Wu and Antonovics 1975a). It would be interesting to see if the same or similar genes determine tolerance to a particular metal in different regions, or whether different genes (and possibly tolerance mechanisms) can produce tolerance in one species. Similarly, in view of the possibility of producing hybrids among some tolerant species (e.g. *Agrostis tenuis*, *A. stolonifera*, and *A. canina*), it would be interesting to know whether essentially homologous genes are involved in the tolerance mechanism of related species. This is undoubtedly a more difficult, but nonetheless intriguing question to answer.

#### MECHANISM OF METAL TOLERANCE

Recent studies on metal uptake have generally confirmed that metal-tolerant plants take up large quantities of metal (Reilly and Reilly 1972; Reilly 1969; Schiller 1974; Wiltshire 1972; Baker 1974) yet species may well differ in their tolerance mechanisms. For example, Reilly (1967) reports that in the Zambian flora one can distinguish those species which accumulate relatively large amounts of copper (e.g. *Becium homblei*: 324 ppm in shoots on soil containing 2,300 ppm Cu) from those which exclude copper very effectively (e.g. *Trachypogon spicatus*: 15 ppm in leaves on soil containing 7,250 ppm Cu). In a later study it was suggested that even in the former species (*Becium homblei*) the tolerance mechanism may be in part "external" (Reilly and Stone 1971). *Becium homblei* accumulates considerably more copper in its leaves than in the roots. At the end of the growing season, the leaves wither and with the stems are burnt away during regular bush fires, thus removing much of the copper from the plant. If the stems escape burning, then newly produced leaves are chlorotic because the stems still contain copper accumulated in the previous season.

It has also been confirmed that the mechanisms of zinc and copper tolerance are independent of each other. Wu and Antonovics (1975b) showed



that in a zinc and copper tolerant clone of *Agrostis stolonifera*, uptake of the two metal ions was independent: the amount of copper taken up was the same regardless of zinc concentration in the solution, and *vice versa*. This was in spite of the fact that there was a strong interaction between the two metals with regard to toxicity as measured by effect on root elongation.

The nature of the internal tolerance mechanisms have been investigated in *Agrostis stolonifera*, *Silene cucubalis*, and *Becium homblei*, as well as in the previously studied species, *Agrostis tenuis*. The results have confirmed that cellular sequestering of the metal ions is involved, and that there seems to be no increased resistance to metals by enzymes involved in normal metabolism. Mathys (1975) showed that there was no difference between tolerant and non-tolerant plants of *Silene cucubalis* (*in vitro*) in the effect of zinc or copper on the activity of nitrate reductase, malate dehydrogenase, isocitrate dehydrogenase, or on glucose-6-phosphate dehydrogenase. However when plants were grown *in vivo* in nutrient solutions containing different levels of zinc, there were large differences between the clones in their enzyme activities. In the zinc tolerant clone, zinc addition to the nutrient medium greatly increased nitrate reductase activity; whereas in the non-tolerant clones, the nitrate reductase activity was reduced. Activity of the other enzymes was influenced to a lesser extent. Malate dehydrogenase activity seemed unchanged in all populations; isocitrate dehydrogenase activity was increased in the tolerant population, but decreased in the others; and glucose-6-phosphate dehydrogenase was decreased in the tolerant population, but increased in the others.

Similar results were obtained by Wu *et al.* (1975b) in a study of copper tolerant *Agrostis stolonifera*. There were no differences between a copper tolerant and non-tolerant clone with regard to the effect *in vitro* of copper on overall respiratory rate and on malate dehydrogenase activity. However, *in vivo* pretreatment with copper resulted in an inhibition of the respiratory rate and malate dehydrogenase activity of the non-tolerant clone; but this was not so of the tolerant clone. When malate dehydrogenase activity was expressed as a fraction of total protein content there were no differences between tolerant and non-tolerant plants: this suggests an overall difference in the protein contents of the two clones.

The tolerance mechanism of *Becium homblei* appears also to be internal. About 17% of the copper is found tightly bound to the cell wall fraction, about 63% is tightly bound to water soluble peptide-copper complexes, while about 20% is in loosely bound water soluble form (Reilly *et al.* 1970; Reilly 1972).

The studies of Mathys (1973) on German populations of *Agrostis tenuis* were interesting because of the difference in his results from those of Turner

and Gregory (1967) on Welsh populations of the same species. Plants from the German zinc tolerant population took up less zinc than the controls, and there was relatively more uptake into the shoots than there was in the British populations. He confirmed that the cell wall was, at least in part, involved in the tolerance mechanism of this species to zinc: as the level of zinc in culture solution increased, so the amount of zinc bound to the cell wall fraction of the root increased in the tolerant strain, but decreased in the two controls. At the higher zinc concentration, just over 50% of the zinc was bound to the cell wall fraction of the root. In the shoot, the amount of zinc bound to be cell wall fraction did not increase even though the total zinc content of the shoot increased; this was true of both the tolerant and non-tolerant strain, suggesting that the mechanism of shoot tolerance was different from that of the roots. Some interesting interactions with uptake of other metals were noted. Increasing zinc concentration increased the uptake of Fe, Ca, Mg, K, and Na; but Mn uptake decreased. Cation exchange capacity remained unchanged throughout, and it was suggested that Zn and Mn may be competing for some common carrier.

## EVOLUTION OF METAL TOLERANCE

### (a) The Evolution of Metal Tolerance *de novo*

By screening large amounts of seed for growth and survival on mixtures of mine and normal soil, it has been possible to show that non-tolerant populations of many mine species contain an appreciable frequency of tolerant individuals (see Antonovics *et al.* 1971; Bradshaw 1970; Walley *et al.* 1974). This has raised important questions about the origin of metal tolerance *de novo*.

1 Do different species differ in their potential to evolve metal tolerance?

2 Do some species have the potential to evolve metal tolerance but fail to do so under natural conditions? If so, why?

3 What are the relative roles of mutation and recombination in generating tolerant individuals in non-tolerant populations?

4 Do tolerant genotypes have an adaptive role in "normal" populations, or are they for some other reason preadapted to colonize contaminated soils?

Recent studies have gone a long way towards answering some of these questions. Gartside and McNeilly (1974c) showed that different species differed greatly in their potential to evolve copper tolerance. *Agrostis tenuis* readily evolved tolerance, as expected; but several species not normally found

on metal mines also produced either highly tolerant survivors (*Dactylis glomerata*) or survivors with an enhanced tolerance (*Poa trivialis*, *Lolium perenne*, *Cynosurus cristatus*, *Arrhenatherum elatius*). Other species (*Anthoxanthum odoratum*, *Plantago lanceolata*, *Trifolium repens*) produced no survivors at all, even though the two former species are known to evolve tolerance to zinc and lead. It is interesting to ask why some species with the potential to evolve tolerance do not in nature colonize mine soils. The species studied above are all common pasture species in Britain growing around mining areas: proximity to mine sites is therefore not a major determinant. It seems more likely, as suggested by Gartside and McNeilly (1974c), that these species are unable to tolerate other attributes of the mine environment. Selection for characters other than metal tolerance on mine sites has been frequently recorded (Antonovics *et al.* 1971; Lefèbvre 1971a,b).

This is confirmed by the frequent distributional anomalies of mine species. For example, Craig (1972) pointed out that *Agrostis tenuis* does not colonize lead mines in the Tyndrum region of Scotland even though it is common in the pastures. The mines instead are exclusively colonized by *A. canina*. *Anthoxanthum odoratum* (which has been well studied because of its abundance on an interesting lead-zinc mine at Trelogan, North Wales) is rarely found on lead mines in general; only one isolated individual was found on one mine out of nearly fifty that were visited in central Wales (Antonovics, unpublished).

The high frequency of pre-adapted tolerant types occurring in non-tolerant populations suggests that mutation *per se* is not responsible. Estimation of mutation rates in species that are poorly understood genetically is fraught with difficulties; however, with increasing development of techniques of plant tissue culture, it may be possible, using selective techniques, to estimate mutation rates in cell suspensions or callus tissue. Wu and Antonovics (1976) developed techniques for growing callus tissue from tolerant and non-tolerant *Agrostis stolonifera*. Adult plants could easily be regenerated, and they retained the degree of tolerance of the individuals from which they were derived. Metal tolerant genotypes in normal populations are likely to be the result of chance amplification (through segregation and recombination) of some existing variation. Many individual plants in natural populations produce sufficient seed to give an adequate sample size for screening of tolerant individuals within families. Identification and subsequent characterization of individuals with a high propensity to produce tolerant seed may point to ecological, morphological, or physiological attributes of the preadapted state.

(b) The Process of Selection During Colonization of the Mine Habitat.

Once colonization of a contaminated area has occurred and a tolerant population is established, the process of selection will continue but be more complex than a simple selective screening of more tolerant genotypes. Contribution of a particular individual to future generations will depend on its survivorship, reproductive output, and quality of the seeds (level of inbreeding, seed size) produced. The process of colonization of contaminated areas was described explicitly by Wu and Bradshaw (1972) and Wu *et al.* (1975a). Populations of increasing age show increasing plant cover and increasing tolerance of the component genotypes. In the more toxic sites, only *Agrostis tenuis*, a species capable of evolving a high degree of tolerance, has survived. A considerable time period is needed for the production and spread of fully tolerant genotypes. This may be the result of a limited variability and limited heritability of metal tolerance in the original population. Gartside and McNeilly (1974) have shown that it is possible to predict how many generations would be needed to evolve full tolerance in a number of different species. In addition to this, however, is the problem that metal mine populations have to be adapted, not to just metal toxicity, but to a wide range of other characters also. Simultaneous selection is difficult for many such traits and can seriously retard the process of range extension into a harsh habitat such as a mine site (Antonovics 1976). Given that we can estimate selection coefficients acting on particular characters (e.g. by parent-offspring comparisons), it is pertinent to ask how selection acts on a complex trait made up of several such characters. This will be determined by the genetic and phenotypic correlations among the characters, and by the nature of the contributions of the characters to overall fitness (e.g. whether it is multiplicative or additive). At present there is very little information on how selection acts on such complex traits in nature: the extreme nature of many mine sites, and the clear adaptive significance of many of the characters to metal tolerant plants, suggests that these plants may prove valuable in analyzing the complexities of natural selection.

We know that in many species there seems to be a limit to selection for tolerance in natural populations: studies of metal tolerant communities consistently distinguish groups of species that can colonize different levels of toxicity (Antonovics *et al.* 1971). It would be particularly interesting therefore to study the effects of selection for increased tolerance in already tolerant plants. This may bring into clearer perspective the nature of correlated responses to selection for tolerance, particularly if there are correlated responses which are the direct effects of the tolerance genes themselves. Direct effects of such genes may be greater metal binding and

hence greater trace element requirements on normal soil; or they may have a general "vigor" effect, which results from resources or normal functions being diverted to production of metal binding complexes. Several recent studies have confirmed that metal tolerant races are competitively inferior to non-tolerant races on normal soil (Cook *et al.* 1972; Hickey and McNeilly 1975). However, it is not clear whether the lowered competitive ability is due to the direct effects of tolerance or to the presence of other characters (e.g. more prostrate growth habit) selected as a result of other features of the mine environment.

Metal-tolerant plants and toxic sites have been valuable for evolutionary studies by virtue of the simplified, often historically well documented, ecological situation they represent. The habitat is often clearly defined, species diversity and interactions are reduced, and the predominant selective agent, plus response (tolerance to metal toxicity), is easily recognized and quantified. Recent developments in population genetics have been stimulated by the development of electrophoresis techniques for analyzing genetic variability at the enzyme level; but a balanced understanding of the relationship between such variation and natural selection remains elusive. Metal tolerance studies provide a unique opportunity to bring classical concepts of natural selection into relationship with modern developments in population genetics. To interpret a clearly adaptive, quantitative character in terms of single gene frequencies would be a major breakthrough in population genetics. A necessary prelude to such studies may be the localization and characterization of the cellular processes and enzymes responsible for tolerance (see next section). One of the problems with identifying enzyme systems responsible for metal tolerance is that tolerant and non-tolerant plants differ also in a large number of morphological and physiological attributes (Antonovics *et al.* 1971). The isozyme studies that have been carried out on tolerant and non-tolerant populations have indeed revealed differences between tolerant and non-tolerant populations (Wu 1973); but these seem to be only indirectly related to the tolerance mechanisms themselves. The analysis of metal tolerance electrophoretically may therefore not be a simple task; but it is an approach that could help to bridge two very important dimensions in our concept of adaptation.

### (c) The Role of Isolating Mechanisms and Speciation

The role of isolating mechanisms and the evolution of tolerant races has been examined further by Craig (1972) and Lefèbvre (1973). Craig (1972) studied a very small spoil heap, about 10 m in diameter, that carried a lead tolerant population of *Agrostis canina*. Contrary to expectation, this population showed no evidence of having evolved isolating mechanisms (flowering

time, self-fertility), although a phenotypically-induced difference in flowering time between mine and surrounding pasture populations was present. A computer simulation of gene flow into this mine (Craig and Antonovics 1976) suggested that the greater density (nearly thirty-fold) of plants on the mine was sufficient to isolate it effectively from the surrounding pasture; pressures for the evolution of isolating mechanisms were, therefore, very weak or inoperative.

The work of Lefèbvre (1973) on *Armeria maritima* questioned the role of the previously observed self-fertility, either as an isolating mechanism or as an adaptation to colonization. Self-fertility evident in artificial self-fertilization experiments using bagging techniques, could not be detected in natural populations using marker genes for estimation. There was no clear explanation for this difference between experimental and natural populations.

In view of such contradictions, there is clearly a need for further study and documentation of the occurrence of isolating mechanisms between tolerant and non-tolerant populations at mine boundaries: this raises the very important question of whether we are in fact seeing here the beginnings of species formation. It further raises the question of what role does gene flow play in this process: what is the role of founder effects and "genetic revolution" in producing new breeding systems and isolated types: or what is the role of direct adaptation to a unique ecological circumstance in producing new species.

These questions become even more intriguing when one considers the large number of endemic species, or species frequently confined to contaminated soils that are not the product of recent mining but occur naturally and had been in existence over geological time. Such areas occur for example in Katanga (Duvigneaud and Denaeyer-de Smet 1963), Rhodesia (Wild 1968, 1970, 1974a, b, c, d), and Zambia (Reilly 1967; Reilly and Reilly 1972). The study of species on well established metal contaminated soils should prove very rewarding; but, as with many studies of evolutionary situations, information on past events may be lost, but it is crucial for interpretation of present day patterns. Given that we can recognize different "kinds" of populations (particularly 'putative' species) on contaminated soils, it is pertinent to establish the following relationships (see also Wild 1971, for discussion).

**A. Taxonomic affinities:** In Europe, unique specific epithets have been applied to metal tolerant races, as judged by both interbreeding and resemblance when grown under standard conditions (Antonovics *et al.* 1971; Lefèbvre 1971). Conversely, populations that by classical taxonomic criteria are still one species, may show isolating mechanisms and at least partial cross-incompatibility (McNeilly and Antonovics 1968).

**B. Ecological status of related taxa:** It is important to know both the potential and actual amount of gene flow between taxa (degree of sympatry), whether intermediate forms exist in intermediate habitats, and to what extent differences between taxa are direct adaptations to the local habitats.

**C. Time scale involved:** Given that two taxa are related, and one occurs on the contaminated area while another occurs on normal soil, it is pertinent to ask what time scale has been involved in generating the evolutionary difference between the taxa. Evidence from biogeography would be crucial here. Other methods (estimation of genetic distance using isozyme data) may also shed light on the historical aspects of the evolutionary process.

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