Adaptation to Metals in Widespread and Endemic Plants

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Bryophytes, including the mosses, liverworts, and hornworts, occur in a variety of habitats with high concentrations of metals and have other characteristics that are advantageous for studies of metal tolerance. Mosses may evolve genetically specialized, metal-tolerant races less frequently than flowering plants. Some species of mosses appear to have inherently high levels of metal tolerance even in individuals that have not been subjected to natural selection in contaminated environments. *Scopelophila cataractae*, one of the so-called copper mosses, not only tolerates extremely high concentrations of metals in its substrates, but requires these substrates for optimum growth. This species should be included in mechanistic studies of tolerance at the cellular and molecular levels. — Environ Health Perspect 102(Suppl 12):105–108 (1994)

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Bryophytes, broadly defined to include the mosses, liverworts, and hornworts, are unique among land plants in having a homosporous life cycle in which the haploid gametophyte phase is dominant. As a consequence of their unique life cycle, bryophytes potentially have a wider range of breeding systems than any other group of land plants. Approximately half of all mosses and two-thirds of all liverworts have gametophytes that produce gametangia of both sexes (1). Gametophytic self fertilization results in completely homozygous sporophytes in a single generation, a genetic process not possible in heterosporous plants. Spores produced by such sporophytes are genetically identical (barring mutation) and are therefore a form of asexual reproduction. This feature is attractive for research purposes because spores derived from intragametophytic selfing provide an abundant source of genetically identical propagules (2). I will describe, below, other features of bryophytes that make them attractive organisms for ecotoxicologic research.

The evolution of metal tolerance has become a model system for studying microevolutionary processes in plant populations (3–6). Research on a variety of taxa, mostly angiosperms, has shown that populations growing in contaminated environments generally represent genetically specialized tolerant races, tolerance can evolve in just a few generations, and high levels of tolerance tend to be metal-specific. Tolerance mechanisms are varied and the genetic basis for infraspecific differences in tolerance may also be varied. Major genes with modifiers seem to be involved in several species but additional genetic analyses are needed.

Tolerance in Widespread Mosses

Many species of bryophytes are known to tolerate habitats with high concentrations of environmental pollutants (7) and the effects of metals such as copper, zinc, and lead have been most thoroughly studied under experimental conditions. Genetically specialized ecotypes tolerant of copper have been described in the liverwort, *Solenostomum crenulatum* (8), and in the mosses *Funaria hygrometrica* (9) and *Ceratodon purpureus* (Jules and Shaw, unpublished data). Plants from an urban population of the liverwort, *Marchantia polymorpha*, were shown by Briggs (10) to be more tolerant of lead than plants from rural sites. These observations are in accord with those from numerous studies of flowering plants in which high levels of tolerance to metals are almost always restricted to plants growing in contaminated environments. The implication of this correlation is that increased tolerances are responses to natural selection at polluted sites.

Other studies on several species of mosses suggest that in addition to, or sometimes instead of, the evolution of genetically adapted ecotypes, inherently broad tolerances permit plants to colonize and thrive in metal-contaminated environments. Shaw (9) studied tolerance of cop-per, zinc, cadmium, and nickel in plants from eight populations of *Funaria hygrometrica*. There was strong evidence of ecotypic differentiation for copper tolerance; populations could be clearly segregated as either tolerant or relatively nontolerant. Tolerant plants were restricted to copper-enriched sites. These observations provide solid, if indirect, evidence of responses to natural selection for tolerance at contaminated sites.

The same populations were genetically variable with regard to cadmium and zinc tolerance, but the relationships between tolerance levels and concentrations of these metals in the natural habitats of populations were less clear than for copper. For cadmium, plants from one population that originated at a cadmium-enriched site were indeed relatively tolerant of this metal, but plants from several other populations growing at sites without cadmium enrichment were also tolerant. Plants that were tolerant of copper also tended to be tolerant of cadmium, even if cadmium was not present in high concentration in their substrates. This observation suggests cotolerances between metals, with obvious implications for mechanistic studies.

Plants also exhibited variation in tolerance of zinc, but there appeared to be no relationship between tolerance levels and zinc concentrations in natural substrates. Whatever the evolutionary basis for population differences, there was no evidence of a role for natural selection. Finally, the populations were indistinguishable in their responses to experimentally supplied nickel, despite the fact that one population originated at a site near Sudbury, Ontario, where nickel contamination of the soil was extreme. Sudbury plants did not appear to

possess genetically based physiologic adaptations to high nickel in their substrates.

Despite evidence of copper tolerant ecotypes in *F. hygrometrica*, data on tolerances of other metals suggest that adaptation is less metal-specific than is the case for most flowering plants that have been studied. Whether this is a general feature of mosses is yet to be determined; but studies on another moss, *Bryum argenteum*, suggest that broadly tolerant "general purpose genotypes" may be the rule. Plants from seven populations of *B. argenteum* showed indistinguishable growth responses to media supplemented with copper, zinc, lead, and nickel, despite the fact that the experimental plants originated at diverse sites including both highly polluted and clean environments (*11,12*). The degree of tolerance of *B. argenteum* to copper and zinc was intermediate between the levels of tolerance exhibited by tolerant and nontolerant races of Funaria. It may be that this inherent, relatively high level of tolerance exhibited by all *B. argenteum* individuals makes the evolution of specialized races unnecessary. A comparison of tolerance mechanisms in *Bryum* and *Funaria,* at both the molecular and cellular levels, would be insightful.

It has been observed on several occasions that sexual reproduction (as evidenced by formation of sporophytes) appears to be inhibited in mosses growing in polluted environments (*13,14*). Much of the evidence is anecdotal, however, and additional studies are required to determine even the proximate causes for this phenomenon. A failure to produce sporophytes could result from several factors, including inhibition of gametangial formation, abnormal production of gametes, and poor development of sporophytes subsequent to fertilization. In *Ceratodon purpureus*, plants grown on contaminated soil produced fewer gametangia than those grown on normal soils (*14*).

Virtually all moss gametophytes have a high potential for asexual regeneration because most or all cells are totipotent. In fact, one of the ways we routinely regenerate mosses in the laboratory for experimental purposes is to dry the gametophytes, grind them to a powder, and sprinkle the powder onto a suitable substrate. Gametophytic plants of the rare "copper moss," *Scopelophila cataractae,* have been regenerated after 8 years of dry storage as herbarium specimens (*15*). Many mosses also form specialized structures such as gemmae for asexual reproduction. One such species, *Bryum bicolor,* exhibits genetically based

variation among populations in gemma formation, as well as highly plastic responses to soil type (*16*). Plants grown on metal-contaminated mine soil formed three to five or more times the number of gemmae, on average, than plants grown on noncontaminated soils. The degree of substrate-induced plasticity was so great that genetically based differences between populations would be obscured in field investigations. This might be an interesting system for studies on the effects of substrate metals on gene expression.

Copper Mosses

The so-called "copper mosses" comprise a group of about ten unrelated species of mosses and liverworts that show an ecological association with substrate copper or other metals (*17–19*). These species share a number of interesting features: very broad but highly disjunctive geographic ranges spanning several continents, a general rarity throughout their ranges, and a rarity or complete absence of sexual reproduction in many geographic areas. The two best known copper mosses are *Mielichhoferia elongata* and *Scopelophila cataractae. M. elongata* was described as a new species from an abandoned Austrian copper mine in 1817; and it has subsequently been reported from naturally occurring copper-enriched substrates elsewhere in the Alps (*20*), Scandinavia (*21*), Great Britain (*22*), and arctic North America (*23*). Herbarium collections of *M. elongata* from Scandinavia have been used to identify sites with previously unknown copper deposits (*17*). Many North American sites where the species has been found, however, lack unusually high concentrations of copper (AJ Shaw, unpublished data).

Plants from five American populations, grown under experimental conditions, do not exhibit high levels of tolerance to copper (AJ Shaw, unpublished data). Like *Bryum argenteum*, described earlier, *M. elongata* exhibits a level of copper tolerance intermediate between genetically specialized tolerant and nontolerant races of *F. hygrometrica.* There was no evidence from experiments that *M. elongata* requires unusually high concentrations of copper for optimum growth.

S. cataractae, on the other hand, is almost completely restricted to sites with extremely high concentrations of substrate metals (*19*). The species occurs on abandoned mine tailings and around sites of extreme industrial pollution in North America, Europe, central Asia, and Japan. In Japan, *S. cataractae* is most common in

the city of Tokyo where it occurs on soils around Buddhist temples where copper leaches from metal roofs. Sexual reproduction does not occur over large areas of its intercontinental range. In the United States, for example, where *S. cataractae* occurs disjunctively in Pennsylvania, North Carolina, Tennessee, Texas, Arizona, and California, the sporophyte generation has never been observed.

Experimental studies have shown that, in contrast to *M. elongata, S. cataractae* does not grow well in the absence of substrate metal contamination (*15,24*). Plants from five North American populations were equally tolerant of contaminated mine soil despite that fact that one of those included in the experiment originated at a rare site for the species where the soil was not enriched with metals. *S. cataractae* apparently has an inherently high tolerance of, and even a requirement for, high metal concentrations in its substrate. Plants not only produced the greatest cover area and biomass on mine soil, but were also morphologically most robust on that soil treatment. The pattern of morphologic plasticity, with largest dimensions formed on mine soil, was opposite the pattern shown even by metal-tolerant races of *F. hygrometrica,* which were stunted by the same mine soil (*25*).

Although five populations of *S. cataractae* exhibited indistinguishable responses to mine soil, the populations were highly variable in their "tolerance" of soils lacking contamination (Figure 1). This is precisely opposite the response typical of most plants, where individuals are relatively similar in growth on normal soil, but are variable in tolerance of metal-enriched substrates. *S. cataractae* is an ecologically unique plant that should be investigated further. The species is easy to grow and would be suitable for mechanistic studies of metal tolerance. Phytochelatins, widely known from other metal-tolerant plants (*26*), were not detected in *S. cataractae* during preliminary studies (Steffens and Shaw, unpublished data).

Relevance to Ecological Toxicology

My work on the evolution of metal tolerance in mosses has not been conducted in the context of ecotoxicologic research; but the results have relevance, if indirect, to the field. Toxicologic research spans all levels of biological organization. Health risks to individuals, both human and other animals, are best assessed at the levels of molecules and cells. Mechanistic studies that

CONTROL SOIL   MIX SOIL   MINE SOIL
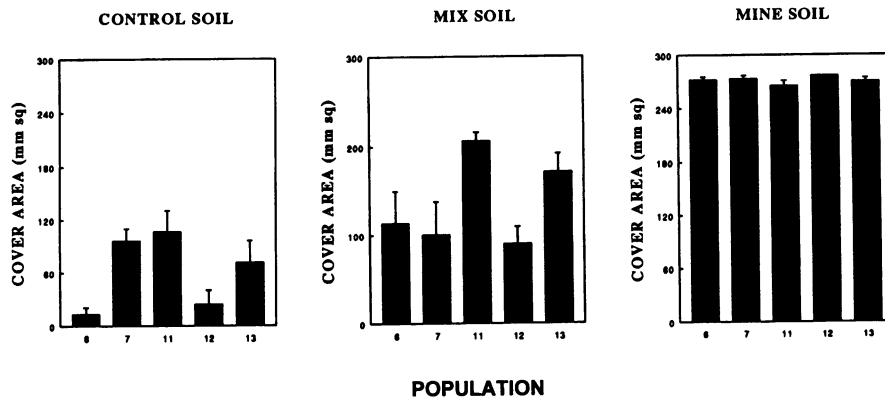
POPULATION

Figure 1. Mean cover area (+ SE) for plants from five populations of *Scopelophila cataractae* grown on three soils differing in metal concentrations. Population numbers (x-axis) correspond to numbers reported elsewhere (15).

directly address interactions between pollutants and DNA, or pollutants and primary metabolites, are essential. Risks to populations and ecosystems, however, occur when impacts at the individual level result in tangible effects at these higher levels of organization. If the ultimate issue in studies of medical toxicology is the survival of the individual, then the comparable issue in ecologic toxicology is the survival of species in natural ecosystems.

The two levels of toxicologic research (individual and ecosystem) are obviously related since the death of a species (extinction) is by definition the death of all individuals comprising that species. Nevertheless, in practice it is not so simple to relate the effects of toxins on the health of individuals to the health and survival of a whole species. Evolutionary biologists, and especially conservation biologists, have grappled for years with the question of what factors are most crucial to the long-term survival of species. This question has

been addressed by paleontologists (27), population geneticists (28), and ecologists (29). The problem, of course, is that there are many reasons a species may go extinct. Stated most simply, populations decline when rates of mortality exceed birth rates for extended periods. Toxicologic studies must address both processes. The consequences of increased mortality, or decreased fecundity, are most severe in small populations. Sublethal pollutants can cause the "first strike" (27) to an abundant species, after which a variety of factors, including stochastic events, can lead to extinction. Also, species with restricted geographic ranges are more likely to be permanently impacted by environmental changes than are widespread taxa.

Less obvious, but equally insidious, effects of pollutants are changes in the genetic structure of species. It is clear from studies of both plants and animals that natural selection for increased tolerances of pollutants in contaminated habitats leads

to profound changes in gene pools (30). Increased tolerances constitute only one aspect of such changes. Physical linkage between genes, as well as pleiotropic effects, inevitably lead to correlated changes in other traits not under direct selection. In the case of metal tolerances in plants, decreased competitive abilities have been demonstrated in tolerant races (31). Other correlated responses, including increased susceptibility to diseases, or, in the case of animals, behavioral changes, are likely, though poorly understood. Ecologic toxicology has to account for infraspecific genetic changes for a complete understanding of the long term effects of pollutants.

One approach to the elucidation of both direct and indirect consequences of natural selection for increased tolerances of pollutants is to establish artificial selection experiments. Successful establishment of lines exhibiting differences in tolerance levels confirms the potential of a species for adaptation to increased pollutant concentrations. Furthermore, observed responses permit estimation of "realized heritabilities" (32), from which quantitative predictions about rates of phenotypic change in nature can be made. Selected and unselected lines can be compared at all levels of biologic organization ranging from molecular modifications through cellular detoxification mechanisms to whole-organism fecundity, longevity, and mortality. Differences between lines in behavior, competitive abilities, and disease resistances, among other traits, permit ecologic predictions about changes in community and ecosystem dynamics. Although such experiments comprise just one approach to multidisciplinary studies of ecotoxicology, I consider this to be a fruitful avenue for

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