Genetic Susceptibility in Ecosystems: The Challenge for Ecotoxicology

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Environmental management is inevitably complicated by the large variation in susceptibility to chemical toxicity exhibited by the living components of ecosystems, a significant proportion of which is determined by genetic factors. This paper examines the concept of genetic susceptibility in ecosystems and suggests the existence of two distinct forms reflecting genetic changes at the level of the individual and at the level of population and community. The influence of genetic susceptibility on exposure-response curves is discussed and the consequent accuracy of data used for toxicity test-based risk assessments examined. The paper concludes by describing a possible biomarker-based approach to future studies of susceptibility in ecosystems, suggesting the use of modern molecular genetic methods. — Environ Health Perspect 105(Suppl 4):849–854 (1997)

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Introduction

Environmental management is undoubtedly a difficult occupation. The ability for researchers to accurately predict the consequences of exposures of natural ecosystems to particular levels of chemical contaminants is considered by many to be impossible. The traditional approach involves assessing the potential toxicity of chemicals in the laboratory under controlled conditions and then, with the judicious use of safety factors, ensuring that the concentrations of these chemicals in the environment do not exceed safe or acceptable levels.

A major criticism of this approach has been that it seldom considers the variability in susceptibility to chemical toxicity exhibited by the large number of animal and plant species found in the natural environment. Differences in susceptibility often can be attributed to abiotic factors that depend on the geography of the ecosystem in question, and the physicochemical form of the contaminating substance (1). However, a significant proportion of the variability in the response of living organisms, whether it be among individuals, populations or whole communities, can be attributed to their underlying genetic makeup.

Other contributors to SGOMSEC 12 have highlighted the biochemical and pathological effects of genetic susceptibility in human populations exposed to environmental hazards, and a range of both genotypic and phenotypic biomarkers for the assessment of this phenomenon has been proposed. Although several of these methods could be readily applied to similar studies of animal and plant populations, it is unlikely that such methods could form the sole basis of this type of research in the natural environment.

Research into both the nature and significance of genetic susceptibility in whole ecosystems is in its infancy and, unlike comparative studies in humans, mammals and other higher vertebrates, there are currently no tried and tested methods available for use with invertebrates and plants. However, ecotoxicologists are realizing that a better understanding of the genetic aspects of population, community and whole ecosystem responses to toxic chemical exposure is vital to future environmental management programs.

This paper addresses this important issue by investigating the nature of genetic susceptibility in ecosystems and its relevance to contemporary ecotoxicological research. We also discuss the use of appropriate biomarkers for studies of susceptibility in the natural environment.

Defining Genetic Susceptibility in Ecosystems

Genetic susceptibility in ecosystems is subtly different from that observed in human environmental and occupational medicine because of the huge species diversity and the large number of complex biological interactions involved. In our opinion a more appropriate approach would be to consider the study of susceptibility as a component part of contemporary ecotoxicology, where research is aimed at acquiring a better understanding of pollutant-induced biochemical and physiological events and where the relationships between these events and the consequences for individual phenotypes, populations, and communities are of paramount importance (2).

In ecotoxicological terms, genetic susceptibility in ecosystems may be considered to exist in one of two forms: the result of events contributing to a reduction the natural variation found in any gene pool or the result of the effects of genetic damage (gene mutation, alterations in gene expression, or selective effects of toxic chemicals on gene frequencies within exposed populations and communities) (3).

Both forms are relevant to ecotoxicology as they can greatly influence the Darwinian fitness of an affected individual exposed to toxic chemicals. It is these changes in fitness, whether expressed overtly as premature death or more insidiously as changes to reproductive capabilities, which can have far-reaching implications for populations, communities, or even whole ecosystems.

Differences in Susceptibility Due to Natural Genetic Variation

Genetic variation is vital to all living organisms—a large gene pool contributing to the overall survival of a population and ultimately the success of a species in a given ecological niche. Population geneticists believe that this variation can be so great that no two members of a population...
are exactly alike at all gene loci, with even clonal organisms differing as a result of somatic mutations occurring during development. This variation pattern is complicated further by events such as recombination, which can break up existing gene combinations, producing new genotypes in each succeeding generation. Thus, from a genetic point of view, each member of a population is probably unique, having a gene combination that will never be formed again during the species' history (4). This combination may involve hundreds, thousands, or even millions of gene sequences, each in turn coding for a similarly large number of polymorphic proteins. It is not surprising therefore to find that, within a population of animals or plants, these gene combinations can influence the susceptibility of the organism to toxic chemicals.

Variation in susceptibility has not gone unnoticed in ecotoxicological research (5). In 1986, Futyma (6) reviewed data from several fields of research that indicated the importance of naturally occurring mutations in determining resistance (and conversely susceptibility) to pollutants, and numerous other studies have investigated the important influence of genotype on the variations in susceptibility to chemicals found in a range of microorganisms, animals and plants (6–9).

One of the more interesting studies was carried out by Baird et al. (10). It illustrates the importance of genotypic differences in determining the susceptibility of the cladoceran Daphnia magna to cadmium and 3,4-dichloroaniline (DCA). These authors found that sensitivity among clones to both cadmium and DCA could vary by as much as three orders of magnitude and that different clones were sensitive to different toxicants. An equally significant finding of this research was that individuals of some clones exhibited little variability in susceptibility to toxicity, while others showed very marked differences, indicating that even very small genetic differences can strongly influence susceptibility to stressors.

**Susceptibility Arising from Contaminant-induced Genetic Damage**

The considerable differences in the responses of living organisms to toxic insults, brought about by natural genetic variation, obviously affects our present ability to accurately predict the reaction of natural populations and communities to pollutant chemicals. More disturbingly, a second type of genetic susceptibility is also apparent in the natural environment, the direct result of genetic events driven by previous exposures to toxic chemicals. Discussion of these genetic changes can be seen as being particularly timely considering the mounting concerns about chemical alterations to the nature and integrity of the genetic material of natural biota, and the consequent changes in both inter- and intraspecies biodiversity that appear to be taking place worldwide (11).

A consequence of the large amount of interindividual genetic variation in natural populations and the subsequent variations in susceptibility is the possibility that a chemical, or mixture of chemicals, may exert a selective pressure on individuals which then may be reflected in the genotypic makeup of impacted populations and, in turn, changes in population densities and biodiversity within communities and ecosystems. A number of such changes, which are manifest in the evolution of genetically resistant populations at chronically polluted sites, have been recorded.

Pollutant-induced selection of resistant genotypes has been demonstrated for fish (12), polychaetes (13–16), insects (7,17), and a range of plants (8,18). Although the long-term ecological impact of such selection is not known with certainty, there are a number of possible implications of ecotoxicological significance.

For example, the selective pressure of toxic chemical exposure may ultimately result in major reductions in genetic diversity at pollution-impacted sites. These reductions in turn could lead to known genetic phenomena such as in-breeding depression and may also result in the loss of evolutionary adaptability to novel environmental stresses due to the reduced genetic variation in a small population (4).

The process involved is straightforward. If a previously large population is reduced to a small size through toxic effects, individuals may be forced to mate with close relatives and often experience reduced fecundity and viability of offspring. This phenomenon is known as in-breeding depression (4) and has been found in a number of animal populations. The mechanisms involved are unknown for most species, but probably reflect homozygous deleterious recessive mutations at a number of gene loci. Notable studies of in-breeding depression in natural animal populations have been carried out on the African cichlid (Acinonyx jubatus), Florida panther (Felicus concolor coryi), and various species of whale (19).

The loss of genetic variation observed with decreasing population size is due to increases of genetic drift. In the absence of forces maintaining genetic variation, such as mutation, migration, and selection favoring heterozygotes, there can be a significant drop in the level of heterozygosity (37% of its original value in 2n generations, where n = effective population size) (4). A population may lose its heterozygosity at a much faster rate if mating occurs nonrandomly among close relatives.

In an ecotoxicological context, if the genetic variation of a population is measured by the level of heterozygosity, and if in turn, the heterozygosity of a population is associated with improved survivorship when faced with environmental stress, then any reductions in heterozygosity in populations exposed to toxic chemicals or other anthropogenic stressors may herald ecosystem disturbance.

A number of studies have tested this hypothesis. Hawkins et al. (20) claimed that a positive relationship exists between the growth rates of the blue mussel (Mytilus edulis) and heterozygosity measured at polymorphic enzyme loci, whereas work by Nevo et al. (21) indicated that, in the case of marine gastropods, broad-niche, genetically rich (highly heterozygous) species display significantly higher survivorship after exposure to multiple inorganic and organic pollutants than their narrow-niche, genetically poor, congeneric counterparts. In contrast, field studies in which the gene frequencies of mosquitofish (Gambusia holbrooki) populations at clean and heavy metal polluted sites were examined revealed that heterozygosity (determined on the basis of three allozyme loci) was markedly reduced in fish inhabiting the polluted areas (12).

In summary, changes in genetic susceptibility in natural populations may have important ecosystem consequences, but a great deal of research on the genetic alterations that affect individual and population responses to toxic insult still remains to be done. It is hoped that through a better understanding of the mechanisms underlying these events, ecotoxicologists may ultimately acquire more pertinent information for use in both risk assessment and environmental monitoring protocols.

**The Significance of Genetic Susceptibility in Ecotoxicology**

The variations in susceptibility described above and their consequences for individuals, populations, and communities can...
have a marked effect on the outcome of ecotoxicological studies. The use of data gathered from both laboratory-based assay and field-based monitoring experiments may therefore be significantly affected by our present lack of knowledge of this area of environmental research.

An example of how variations in organism susceptibility can influence important ecotoxicological protocols was recently provided by Depledge (22). He highlighted the affects of susceptibility on a central paradigm of ecotoxicology, namely, the dose–response paradigm. Applied research stemming from the paradigm enables lethal and sublethal concentrations of chemical toxicants to be determined for selected organisms in controlled conditions in the laboratory, and subsequently influences predictions of chemical affects on organisms in the natural environment.

The core component of the dose–response paradigm is the dose–response curve (Figure 1). The graph shows that, for a population of cells or individuals, a low dose of a chemical compound initiates a response in only the most susceptible individuals within the population. As the dose increases, many more individuals respond, but at very high doses, only a few resistant individuals are left that are capable of responding.

This relationship, extensively used in medical toxicology, has been applied, with some minor modifications, to ecotoxicological studies, and is currently used to obtain both absolute and relative estimates of chemical toxicity. The most obvious modification is replacement of dose with exposure concentration, since only the latter is known in ecotoxicological studies.

From each exposure–response experiment, a value is usually derived from the curve that reflects the exposure concentration that produces mortality in 50% of the test population—the so-called LC50 (value a on graph). Values for LC50 are then used to compare the relative toxicities of different chemicals and form the basis (with appropriate safety factors) for setting safe limits for discharge and environmental levels of chemical compounds.

Variations in susceptibility, resulting from abiotic or biotic factors, are one of the major drawbacks to the use of the dose–response paradigm, as the levels of phenotypic heterogeneity in natural populations are often absent from populations of organisms used in the laboratory (23). Although the removal of such variability is helpful in increasing the repeatability of these procedures and therefore the ease of ranking chemical toxicity, it is difficult to envision what the results of such tests tell us about the likely consequences for genotypically and phenotypically diverse natural populations when exposed to the test chemical in the environment. The dangers of constructing a dose–response curve using a population of organisms that does not reflect the variability of the susceptibility found in natural populations are quite considerable; LC50 values determined using populations exhibiting phenotypic homogeneity may overestimate or (more seriously) underestimate the susceptibility of the organisms exposed to the particular compound under study. This is also illustrated in Figure 1.

It can be seen that if the susceptibility of a natural population is represented by a curve constructed using the data points 1 to 3, then the resultant LC50 value is lower than that predicted using the laboratory test population. Conversely, if the level of susceptibility of the natural population is represented by a curve constructed using the data points 4 to 6, the resultant LC50 value is lower than that predicted by the laboratory test organisms.

It is also a matter of some concern that toxicity test data, collected using dose–response experiments on individuals, is used to predict consequences at the level of populations and communities (22). It could be argued that the discrepancies between the dose–response curve obtained in laboratory tests and the actual effects of chemical toxicants on natural populations can be compensated for by the application of suitably large safety factors, and also that the questions posed above are too subtle for inclusion in environmental management strategies. From a scientific point of view, these arguments are flawed in that a safety factor that is essentially an educated guess should not be used to compensate for a lack of knowledge about vulnerable/susceptible organisms within a population.

**Biologic Markers of Genetic Susceptibility in Ecosystems**

Ecotoxicologists have realized for some time that biologic markers, or as they are more commonly known, biomarkers, are powerful tools for the investigating contaminant exposure and effects on living organisms (24). Using a range of suitably characterized biological measures or indicators of toxic exposure, it is possible to study a great number of the different consequences of environmental contamination (25–27). The theoretical basis for using biomarkers in ecotoxicological research is demonstrated in Table 1, an amalgamation of the work of

| Level of organization | Type of effect | Type of marker | Time scale |
|-----------------------|---------------|----------------|------------|
| Biological molecules  | Molecular effects | Markers of exposure | Seconds |
| Organelles            | Tissue and cellular effects | Markers of effect | Minutes |
| Cells                 | Physiological and health and disease effects | Markers of ecological change | Hours |
| Tissues               | Impaired reproductive capability | | Months |
| Organs                | Decline in reproduction | | |
| Individuals           | Reduced census numbers | | |
| Populations           | Intbreeding | | |
| Communities           | Population decline | | |
| Ecosystems            | Reduction of genetic variation | | |
|                       | Selection at loci conferring resistance | | |
|                       | Possible extinction | Markers of evolutionary change | Years |
|                       | Reductions in biodiversity | | |
Depledge (22) and Bickham and Smolen (28) and Figure 2 (29), which serves to illustrate the key features of this approach.

Table 1 shows the hierarchy of pollutant effects in relation to specific levels of biological organization, and indicates the type of biomarker that may be employed to study such effects. If a collection of biomarkers of each type are used together, and these markers have been suitably characterized and found to reflect toxic exposure at their specific levels of biological organization, they may be then be used in experiments to determine the response of an individual or population to a specific toxicant.

Figure 2 indicates that biomarkers may also provide clues to the causal link between the presence of a contaminant or group of contaminants and an ecological response at the community or whole ecosystem level. This figure suggests a way that biomarkers may be employed in the assessment of ecosystem integrity and draws attention to the analogy between the sequence of responses of individual organisms and the responses of populations and communities to increasing pollutant loads. Although it must be said that it is not known whether a linear model such as that shown in Figure 2 is suitable for describing ecosystem effects of chemical contaminants or to what extent the structural and functional instability of ecosystems interferes with this model, to date, other better models have not been proposed (29).

To be of greatest value in determining the implications for ecosystems of exposure to pollution, biologic markers should be chosen so that they reflect changes in the fitness of an organism or, in more simple terms, changes that affect its overall reproductive capability (premature death, ability to mate, fecundity, viability of offspring, etc.), as these can have the greatest influence on effects at the higher levels of biological organization.

Fitness of a living organism after toxic chemical exposure can be influenced quite significantly by the types of genetic susceptibility present in ecosystems; it is therefore logical to consider the study of these phenomena as an important ecotoxicological endeavor.

Specific biomarker methodologies used in ecotoxicological studies are shown in Table 2. This diagram replaces the hierarchy of possible pollutant effects shown in Table 1, with a similar hierarchy of biologic markers that can be used for the assessment and monitoring of toxic effects on living organisms. Markers at levels below that of individual (molecules, organelles, cells, and tissue) are generally the most costly and more specific molecular biological and biochemical assays; the nonspecific markers are the physiological and behavioral analyses, and ecological methods. Effects of pollutants on natural environments are usually detected initially...
by the lower cost markers high in the hierarchy. The lower hierarchy, more specific methods, are employed to provide information on the specific mechanisms involved and possibly to reveal the nature of the contaminating compound (24).

A similar strategy would be employed to study the effects of genetic susceptibility and could obviously include the majority of markers shown in Table 2. Studies should be comparative and highlight the differences in responses found in individuals, populations and communities. However, in conjunction with these studies, attempts should be made to confirm and characterize the genetic basis for this variation in response. For this purpose we propose the use of biomarkers reflecting changes at the level of DNA.

We feel that two types of genotypic markers will offer the best prospect for progress in this area of ecotoxicology, both reflecting changes in the genetic makeup of the living components of ecosystems: markers indicating alterations at the level of gene sequences crucial to the response of organisms to toxic insult (Table 2, group A) and those which reflect potentially deleterious changes in population genetics (Table 2, group B).

Alterations to Genes Crucial in the Response to Toxic Insult

Modern molecular biological studies reveal a number of proteins that play a vital role in the cellular response to physical and chemical insults. These proteins include the family of heat-shock proteins (HSP) (30), the cell regulatory proteins such as p53 (31), and oncoproteins (32), as well as a number of important enzymes involved in DNA repair. Also included under this heading are the various detoxification enzymes such as the mixed-function oxidases (MFO) and the cytochrome P450 family (27).

Research carried out mainly in populations of humans and other vertebrates has revealed that mutations in the genes coding for these proteins, whether they cause protein polymorphisms or a lack of functional protein, can have major consequences in those individuals exposed to a whole range of toxic compounds. In the case of mutations in the genes coding for p53 and different detoxification proteins, a possible consequence is the development of life-threatening pathophysiological conditions (31).

Alterations in genes coding for cellular defense proteins are therefore important in ecotoxicology due to the serious effects they could have on the fitness of pollutant-exposed organisms. They must also be seen as a major determinant of genetic susceptibility. Research into the molecular determinants of cellular defense in animals and plants, especially those selected as keystone species for ecological risk assessment, is therefore a priority.

Biomarkers Reflecting Changes in Genetic Variation

The second class of biologic markers important for the assessment of genetic susceptibility in ecosystems includes those which are capable of indicating changes in genetic variation in populations and communities. Such changes can be detected in the laboratory by using one or more of the large range of genetic profiling techniques which are now available. Profiling techniques include more traditional methods of population genetics such as electrophoretic analysis of allelic enzymes (allozymes) (12), and the more contemporary molecular biological methods. Genetic fingerprinting methods such as analyses involving locus-specific DNA or RNA probes have also found favor with environmental biologists (33); other genomic profiling methods such as restriction fragment length polymorphism (RFLP) and, more recently, randomly amplified polymorphism detection (RAPD) analyses (34) may also be employed.

Mitochondrial DNA is particularly useful in population genetics studies as it is haploid and clonally inherited through maternal lineages (35). The analysis of the mitochondrial DNA sequences of individuals from natural populations is a powerful method for studying genotypic distribution in ecosystems. A good example is provided by the work of Bicham and Smolen (28). These authors sequenced a 256 base-pair segment of the control region of mitochondrial DNA from 225 sea lions captured at various locations around the Pacific rim of North America. The study revealed differences in haplotype distribution among populations of sea lions, and the authors concluded that because the genetic marker used was ostensibly neutral, mitochondrial DNA analyses could be developed into a method for detecting anthropogenic population decline and the consequent effects on genotypes brought about by events such as inbreeding.

Of more importance to ecotoxicological research, this work serves to demonstrate that fine-scale population genetics studies can be accomplished using high resolution molecular techniques on a large scale. Other methods include profiling areas on genomes known as minisatellites which contain variable numbers of repeat nucleotide sequences (36).

Microsatellite analysis has already been employed in many studies of conservation genetics and shows considerable potential for measuring levels of genetic variation within populations Triggs et al. (37), describe the DNA fingerprinting analysis of the genetic similarity between populations of the blue duck (Hymenolaimus malacorhynchos) using two minisatellite probes. High levels of genetic similarity found within duck populations contrasted the decrease in similarity found as geographic separation between samples increased, indicating limited dispersal and inbreeding within populations.

Modern molecular genetic methodologies have much to offer to ecotoxicologists. They can be used with most organisms with little prior knowledge of the species' biology, and when coupled to specific nucleic acid amplification protocols, can be undertaken using small amounts of tissue. This means that previously neglected organisms, especially the huge number of invertebrate and plant species, could now be included in ecotoxicological studies.

Future Prospects for Research into Genetic Susceptibility

The revolution occurring in molecular biological methodologies offers much to future ecotoxicological studies in general and to studies of genetic susceptibility in particular. Contemporary methods now used to study altered gene expression are of particular interest, as these methods may offer a novel route to the isolation and characterization of new biomarker molecules and may further be modified as methods for genotypic analysis.

This is particularly interesting to us in our laboratory in Plymouth, where we are presently investigating the use of the recently developed method of mRNA differential display (38) in studies of pollutant-induced molecular change in invertebrate populations. The differential display method produces a snapshot of the total gene expression that can be used to compare molecular events occurring in cells or tissues from organisms exposed to toxic chemicals with those of the same species from an uncontaminated environment.
The real power in this method however, lies in the subsequent ability to recover differentially displayed DNA fragments and characterize them using DNA sequencing and hybridization experiments.

The benefits of using this technology are 2-fold; this method could be used both to search for novel biomarker molecules, and as the method operates at the level of gene expression, to fulfill a role as a combined phenotype–genotype assay. Used in conjunction with other indicators of organismal fitness, this type of assay may prove useful in studies of the genetic basis of susceptibility in a wide range of plant and animal taxa.

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