Genotypic Toxicity: Implications for Individuals and Populations

Michael H. Depledge

Department of Biological Sciences, University of Plymouth, Plymouth, England

The goals of genetic ecotoxicology are discussed and redefined. New directions in which genotoxicity “effect” studies might be pursued are outlined. Recognition of the genotoxic disease syndrome in lower animals suggests that more attention should be given to exploring the relationships between DNA damage (adduct formation, gene mutations, etc.) and its manifestation at the level of individuals. Within a given population, not all individuals are equally susceptible to pollutant toxicity (including genotoxicity). It is proposed therefore, that more attention be paid to identifying the factors underlying interindividual variability in susceptibility. Examples are provided of specific cases in which differences in susceptibility to pollutants have been directly related to genotypic predisposition. This approach is also advocated for investigating the individual and population level consequences of genotoxic damage. The possibility of using phenotypic traits to recognise subsets of individuals within populations possessing similar genotypes is discussed. — Environ Health Perspect 102(Suppl 12):101–104 (1994)

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Introduction

Genetic toxicology is conventionally regarded as the study of the effects of chemicals and radiation on DNA and on mechanisms of inheritance in cells and whole organisms (1). The goal of studies in this area is to assess the risks to man posed by chemicals capable of inducing cancer, genetic diseases, and teratogenic abnormalities. However, in genetic ecotoxicology, threats to individual organisms are usually considered of less significance than threats to populations and communities. This shift of emphasis has great bearing on the types of investigations that must be performed to detect genotoxicity in natural populations. Genetic ecotoxicology is defined here as the study of pollutant-induced changes in genetic material in natural biota. Key objectives are to explore the extent to which ecosystems are contaminated with genotoxins and, more importantly, to identify adverse effects such as reduced fitness in individuals and alterations in gene frequencies and genetic diversity in populations and communities, associated with genotoxic exposure. So far, progress has been made principally in detection of exposure to genotoxins using a variety of biomarkers (2,3). Linking genotoxic exposure to adverse effects in individuals and populations has proven a more intractable problem. What follows is a brief discussion of some directions in which genotoxicity “effect” studies might usefully be pursued.

Genotoxicity versus Direct Chemical Toxicity

In higher vertebrates, binding of a pollutant chemical to DNA (DNA adduct formation) is thought to be the event that triggers the cascade of biochemical changes eventually leading to neoplasia and in some cases, malignancy (4). Some chemicals may also cause or contribute to the development of cancer by mechanisms other than DNA binding (5). Malignant tumors are uncommon, however, in lower vertebrates and especially invertebrates [which represent 95% of extant animal species (6)]. Instead, genetic damage is manifest as a suite of pathophysiological changes, the so-called genotoxic disease syndrome (7). This comprises impaired enzyme function, enhanced protein turnover, impaired general metabolism, impaired immune responses, production of initiators of cytotoxic injuries, inhibited growth, decreased scope of growth, decreased fecundity, and faster aging. Thus, there is an urgent need to identify the ways in which genotoxins modify genes and gene expression such that phenotypic characteristics are also altered, and to find ways of distinguishing between direct chemical toxicity and genotoxicity as components of damage.

Correlating Genotypes with Resistance or Susceptibility to Specific Pollutants

Futyuma (8) reviewed data from several fields of research which indicated that genetic mutations conferring resistance to pollutants occur independently of exposure and are therefore not induced by pollutants. This implies that susceptibility to pollutants arises by chance. However, the occurrence of the genotoxic disease syndrome (a direct consequence of genotoxic exposure) in which genotypic changes (mutations, modifications of gene expression, etc.) are detrimental to the fitness of the affected individual (7), indicates that, in this special case, genetic modifications do not occur at random nor independently of pollutant exposure.

The work of Baird et al. (9) is illustrative of the importance of genotypic differences in determining phenotypic resistance to pollutant exposure. The responses of different genotypes of the parthenogenetic cladoceran Daphnia magna to cadmium and 3,4-dichloroaniline (DCA) were compared. The results indicated the following:

• The sensitivity of different genotypes (clones) to cadmium varied by three orders of magnitude.
• The sensitivity of different genotypes to DCA varied by two orders of magnitude.
• Different genotypes were sensitive to cadmium and DCA.
• Individuals of some clones exhibited little variability in susceptibility to toxicity while others showed very marked differences, even though all animals were genetically identical and had been reared in homogeneous environments.

Thus, small genotypic differences (whether they occur naturally or as a result of modifications associated with genotoxins) appear to strongly influence susceptibility to...
stressors. Recently, studies by Møller et al. (10) have shown that environmental factors are capable of further modifying the susceptibility of a given clonal genotype to toxicants. Returning to the Daphnia example, a genotype that survives in cadmium-polluted conditions may be ill adapted to tolerate DCA exposure. Consequently, genetic diversity within a population may be eroded as exposure to mixtures of pollutants occurs. Such diversity reductions are associated with increased inbreeding, the consequences of which are massive declines in fertility, viability, and other fitness parameters as individuals within the affected population become more homozygous allowing expression of detrimental recessive genes (11,12).

It follows from the above that each organism has a particular predisposition to pollutant toxicity (including genotoxicity). In man, genetic variability among individuals is immense. The DNA present in the haploid human genome corresponds to ca. 3 million average-size gene sequences which code for ca. 20,000 polymorphic proteins (13). Some of these proteins will inevitably influence individual susceptibility to toxicant exposure. Leaving aside responses to direct chemical toxicity, genotoxicity varies markedly among individuals. For example, Rudiger (14) demonstrated that the individual risk of developing cancer following exposure to genotoxic agents is determined not only by exposure, but by the ability to cope with the genotoxic burden. Among a range of cellular defense mechanisms, DNA repair is particularly important. This was demonstrated by the increased cancer incidence in several genetic disorders that are characterized by a specific DNA repair defect. Similarly, even small differences in the efficiency of DNA repair mechanisms among normal individuals can account for differences in susceptibility to genotoxicity. Hemminki (15) showed that for a given level of genotoxin exposure, interindividual variation in DNA adduct binding may vary up to 10-fold.

The particular genotypes at risk in a population exposed to pollutants will depend on their phenotypic attributes and the pollutant chemicals in question. Luoma (15) pointed out that for the induction of genetically based resistance in animal populations, a pollutant must be present in biologically available quantities sufficient to limit the reproductive success of a proportion of the individuals in the population. Previously, such considerations have lain outside the field of genetic ecotoxicology. It is argued here that there are compelling reasons to begin to address this issue.

**Establishing Mechanistic Links between Genetic Composition and Phenotypic Attributes**

Evidence presented above demonstrates that genotoxicity should not be regarded as a special kind of biological damage, but as an integral component of the summated adverse effects of pollutants that give rise to changes in genotype and gene frequencies in exposed populations. Establishing correlations between specific genotypic attributes (whether naturally occurring or resulting from genotoxic modification) and susceptibility to particular toxicants is a potentially valuable approach for detecting ecologically significant, pollutant-induced genetic change.

At first sight, it appears to be a daunting task to identify genes, gene complexes and modulators of gene expression which actually determine the characteristics of enzymes, metabolic processes, detoxification mechanisms, and excretory systems associated with tolerant or susceptible phenotypes. There are, however, grounds for optimism. Physiologic traits reflect the functioning of underlying metabolic pathways, which in turn arise from structured interactions among the enzyme products of genes (16). Consequently, all physiologic characteristics of organisms are ultimately genetically determined, even though they exhibit a high degree of variability in response to environmental fluctuations. Fortunately, metabolic structure is so highly conserved that, despite this variability, specific physiologic differences among individuals can be attributed to polymorphisms at a single gene, or in a gene complex, or to the presence of multiple copies of a single gene (17). For example, differences in resistance among Drosophila exposed to ethanol in the environment are related to specific genetic variants coding for aldehyde dehydrogenase (18). The occurrence of metal-tolerant populations of the plant Silene vulgaris is governed by a single gene, although the level of resistance can be modulated by external factors (19). Some populations of invertebrates are metal tolerant by virtue of the amplification of metallothionein genes (20). Experiments involving mammalian cells have also revealed metallothionein gene amplification following metal exposure (21). Other studies have demonstrated the role of specific genetic changes in the development of resistance to pesticides among insects (22). By way of contrast, an unfavorable change in genetic composition with adverse phenotypic consequences is the appearance of mutated c-K-ras oncogenes in fish exposed to polyaromatic hydrocarbons and polychlorinated hydrocarbons. Mutation of this gene is correlated with pathophysiologic changes in liver structure and function (23).

In summary, there is already clear evidence that it is possible to relate specific genetic changes to particular physiologic consequences. Great efforts should now be made to further apply this approach to the problem of identifying the phenotypic consequences of genotoxic damage.

**Can Physiologic Traits Be Used to Identify Genotypic Characteristics?**

Since specific genes or gene complexes are related to particular phenotypic characteristics, it is interesting to speculate whether the reverse pathway might be followed, namely, identifying subsets of organisms with similar phenotypic attributes that can then be shown to have similar (or the same) genetic characteristics. This concept is embodied in the so called "physiotype approach" described elsewhere (24). Among the physiotype traits that organisms possess, biochemical/physiologic traits are of most relevance to survival in polluted conditions [hence the term "physiotypes" (24)]. For a given genotype, a range of phenotypic outcomes are possible depending on environmental history and prevailing conditions. This range was referred to as the "norm of reaction" by Dobzhansky (25). In a given population, the "norms of reaction" of all genotypes overlap (26), but if a selection pressure is applied, then only subsets of genotypes that confer suitable phenotypic characteristics will persist. This concept has been explored using a variety of biochemical/physiologic characteristics to recognize subsets of individuals (physiotypes) within populations that were then exposed to chemical toxicity. An example is shown in Figure 1. Using the shore crab, Carcinus maenas, physiotypic groupings were assigned on the basis of hemolymph protein concentrations measured on 200 µl hemolymph samples taken nondestructively. Physiotype 1 comprised individuals with hemolymph protein concentrations lying from −3 to −1 standard deviations below the mean for the population. Physiotype 2 comprised animals with hemolymph protein concentrations between −1 standard deviation and the mean. Physiotypes 3 and 4 were assigned on the same basis using hemolymph protein concentrations from the mean to +1 standard deviation and from +1 to +3 respec-
tively. All crabs were then exposed to 0.75 mg/l copper (as copper chloride) for 20 days. Hemolymph protein concentrations and survivorship were determined on days 0, 6, 13, and 20 of exposure. A full report of these experiments will be presented elsewhere (Gyorkos and Depledge, unpublished data), but the findings relevant here are that the number of individuals belonging to physiotype 1 increased as the number belonging to physiotype 4 decreased over the exposure period. Furthermore, 58 of the original 88 animals died during copper exposure, with most of the mortality occurring among individuals belonging to the original (day 0) physiotypic groupings 1 and 2. Thus, individuals with similar physiologically attributes in clean conditions do appear to exhibit similarities in their responses to pollutant exposure. Efforts are now underway to examine the genetic basis of these similarities and to determine why, in this case, hemolymph protein concentration is correlated with survivorship.

**Homozgyosity and Heterozygosity in Relation to Genotoxicity**

Establishing mechanistic links between specific genotoxic damage and phenotypic consequences will undoubtedly take many years of painstaking research. There may be other less specific tools, however, that might prove useful. As was alluded to above, investigation of the genetic basis of biochemical/physiologic phenotypic variability has usually been ignored or regarded as irrelevant in the past. Only now has an understanding begun to emerge of how physiological attributes conferred by the genotypic influence fitness.

Over the last 10 years considerable progress has been made in this area with the identification of a positive relationship between individual growth and heterozygosity measured at polymorphic enzyme loci (27). For example, Nevo et al. (28) examined the relationship between genetic diversity and the extent of resistance to pollutants. Correlation of genetic diversity and pollution was tested by comparing the effects of pollution on populations having low and high degrees of heterozygosity. It was concluded that for marine gastropods, broad-niche, genetically rich, species display significantly higher survivorship than narrow-niche, genetically poor, congenetic species, after exposure to multiple inorganic and organic pollutants (28). This finding has interesting implications for the determination of an optimum strategy for evaluating species best suited as genetic monitors of pollution. In highly heterozygous species, changes in isoenzyme expression might prove useful in assessing pollutants to which organisms are exposed, as well as providing insight into expected changes in phenotypic attributes. This approach may be especially valuable in studies of genotoxicity, since it allows questions to be posed such as, “does genotoxicity result in altered isoenzyme expression?” and “do genotoxins interfere with the expression of heterozygosity and homozygosity to different extents with significant phenotypic consequences?” Answers to these questions are not yet available, but the pioneering work of Hawkins et al. (29) indicated that in the blue mussel, *Mytilus edulis*, exposed to copper in the laboratory, genotype-dependent mortality occurred. Those individuals expressing a higher degree of heterozygosity survived longest. Survivorship was also associated with low protein turnover times. Kurelec (7) refers to increased rates of protein turnover as one of the key features of the genotoxic disease syndrome. Small changes in protein turnover rate have been shown to have great significance for energy metabolism and fitness (27). The most heterozygous individuals have the lowest protein turnover times and routine metabolic maintenance costs, and hence the greatest fitness. It is tempting to conclude, therefore, that if genotoxicity influences gene expression such that protein turnover increases, then survivorship in stressful conditions would decline. Of course, such a conclusion is tentative, but this line of research might prove valuable in ascertaining whether genotoxins can indeed influence the degree of heterozygosity expressed, and consequently, the phenotypic attributes that are manifest.

**Summary and Conclusions**

From the foregoing account, it is clear that there are fundamental differences in the objectives and concerns of genetic toxicology and genetic ecotoxicology. This has arisen because in the latter field, genetic damage is manifest primarily as alterations in phenotypic attributes and Darwinian fitness parameters rather than as neoplasia, teratogenic effects, and genetic diseases. It has been demonstrated that different genotypes (whether arising naturally or as a result of modification by genotoxins) show differences in susceptibility to natural and anthropogenic stressors. In some cases, the mechanistic basis by which changes in gene expression confer susceptibility or resistance have been established. However, it may also be possible to use biochemical/physiologic phenotypic characteristics to identify underlying similarities among genotypes. This approach is currently being explored.

In the future, firmer links must be established between genotoxic exposure (as indicated by a variety of sophisticated bio-marker techniques) and the phenotypic consequences of exposure in individuals and populations.
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