

Somatic and Heritable Effects of Environmental Genotoxins and the Emergence of Evolutionary Toxicology

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The genetic effects of environmental pollutants include mutations in somatic cells or germinal cells that are the direct result of exposure to toxicants. Biomarkers that detect such mutagenic effects have been developed and tested in field studies on wildlife populations. However, another class of genetic effects resulting from pollution exposure exists. Specifically, changes in allele frequencies of populations will occur as a result of population bottlenecks, inbreeding, or selection at loci critical for survival in polluted environments. We describe how such genetic alterations can be studied at the population level using the techniques of molecular genetics, and we predict the development of a new field, evolutionary toxicology, that will address such issues. — *Environ Health Perspect* 102(Suppl 12):25–28 (1994)

Key words: somatic effects, heritable effects, genetic toxicology, ecotoxicology, evolutionary toxicology

Introduction

Contamination of the environment by anthropogenic chemicals has become a world-wide problem. Numerous environmental insults including depletion of ozone resulting from the presence of atmospheric pollutants, contamination of vast areas by radioactivity released from the Chernobyl accident, contamination of remote Arctic whale populations by airborne pollutants, and pollution of the oceans by dumping and industrial run-off are all indicative of problems of a global scale which will require unprecedented cooperation among nations to alleviate or control. Innovative and costly control of industrial, military, and consumer activities will be required to solve these problems. However, equally daunting challenges face the scientist who must provide enlightened, and effective,

information in support of environmental decision makers. In particular, a clear understanding of all the dangers posed by environmental pollutants to both human health and ecologic systems are needed.

Traditional approaches used in environmental toxicology are fourfold. These include: ranking of relative toxicities of chemicals, detection and quantification of toxic chemicals in the environment, study of mechanisms of chemical toxicity, and determination of no-observable-effect levels in the laboratory. While these approaches are still useful, they are unfortunately too narrow in scope, and their validity is being seriously questioned (1). Traditional studies in wildlife toxicology, which primarily focus on the deleterious ecologic effects of pollution on natural populations (2), are also useful but still do not provide a complete picture of the issues. The field of biomarker research has developed in recent years to add yet another piece to our maturing concept of the effects of pollutants on organisms and the environment (3–7).

In this article, we briefly summarize previous and ongoing studies in our laboratory of one aspect of biomarker research, genotoxic effects on somatic tissues in wildlife populations (8–10). We will define somatic effects and heritable effects as two subclasses of mutagenic effects and describe how each can be detected in natural populations. We also show how the field of molecular genetics will contribute to an understanding of the effects of pollutants on population genetics and ultimately lead to the recognition of the field of evolutionary toxicology. This new field will deal

largely with the emergent effects of environmental toxins. That is, evolutionary effects are changes at the DNA sequence level that are not necessarily the direct result of a mutation induced by the pollutant. Rather, they are the result of organisms adapting to a polluted environment and thus are fundamentally different in nature, and emergent from, lower-level processes such as ecologic effects and toxic effects.

Detection of Somatic Mutations in Wildlife Populations

Somatic effects are here defined as DNA alterations resulting from exposure to environmental mutagens that are expressed in any tissue or organ but not in the germinal tissues and not passed on to the next generation. Somatic mutations can result in reduced viability, which could lead to reduced survivorship and lower reproductive output. Ultimately these responses may result in ecologic effects such as reduced population density, population extirpation, or other higher order effects. Techniques to effectively detect the presence of somatic mutations have been developed and tested in laboratory and field studies. Responses evaluated in our laboratory include chromosome analysis, micronucleus analysis, and flow-cytometric analysis. A series of studies was conducted to investigate the use of cytologic and cytometric techniques to detect genotoxic damage in wildlife populations (Table 1). These studies have included species of mammals, birds, and reptiles occurring in

This article was presented at the Napa Conference on Genetic and Molecular Ecotoxicology held 12–15 October 1993 in Yountville, California.

Our genetic toxicology studies have been accomplished using equipment in the Center for Biosystematics and Biodiversity, Department of Wildlife and Fisheries Sciences, Texas A&M University. The Center was established by a grant from the National Science Foundation. The population genetics study of Steller sea lions was conducted at LGL Ecological Genetics, Inc., in collaboration with Dr. Thomas Loughlin, and funded by the National Marine Fisheries Service. This article is contribution no. 26 of the Center for Biosystematics and Biodiversity.

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Table 1. Somatic mutations in wildlife populations caused by exposure to environmental mutagens: selected studies.

Species	Site	Type of pollutant	Assay	Reference
Cotton rat	Fireman's Training School	Mixture ^a	Flow cytometry (FCM) and chromosomes ^b	McBee et al. (22)
White-footed mouse	Fireman's Training School	Mixture ^a	Chromosomes and FCM	McBee and Bickham (23)
Merriam's kangaroo rat	Nevada Test Site	Radiation	Micronucleus ^c	
Black-crowned night heron	Sabin National Wildlife Refuge, LA, Houston Ship Channel	Mixture ^d	FCM ^e	
Pond slider (turtle)	Savannah River Site	Radiation	FCM	Bickham et al. (24), Lamb et al. (25,26)

^aPetrochemicals, heavy metals. ^bTB Lyne (unpublished). ^cT Lamb (unpublished). ^dPetroleum contaminants. ^eT Custer (unpublished).

terrestrial and aquatic habitats in environments containing a variety of genotoxic agents including radioactivity, heavy metals, petrochemical wastes, etc. (Table 1). These studies demonstrate that the mutagenic effects of environmental genotoxins are detectable in indigenous wildlife populations, using various cytogenetic and cytometric techniques, and that the effects can be replicated over time (8,9). Moreover, the effects of environmental mutagens closely parallel observed effects in laboratory dosage studies (11,12) despite inherent difficulties associated with field exposures. These included the facts that controls are much less precise in field studies, the nature of exposure is radically different (chronic exposure in field studies, acute exposure in laboratory studies), and in the field organisms are often exposed to complex mixtures whereas pure chemicals are used in the laboratory studies.

Detection of Heritable Effects in Natural Populations

Heritable effects fall into two discrete categories: the first is mutations induced in germ cells as a result of exposure to a mutagen; the second is selection or other population-level processes that arise from the stress caused by exposure to a polluted environment. Usually, heritable effects are considered to be mutations that occur in gametes, as a result of exposure to environmental mutagens, that are passed to the offspring of an exposed individual. Such heritable effects are of particular concern because they could continue to be expressed in populations long after removal of the causative contaminants. However, the genetic makeup of populations also can be altered indirectly by toxic exposure as a result of somatic damage that leads to ecologic and population genetic effects. Therefore, even nonmutagenic toxicants can have genetic effects if they result in alteration of allele frequencies of populations by this process.

Recent advances in molecular biology have greatly increased our ability to study the genetics of natural populations and hence to detect the changes in allele frequencies that could result from toxic expo-

sure. In particular, we will discuss PCR-based assays using automated DNA sequence analyses on Steller sea lions as an example of the use of a neutral genetic marker, mitochondrial DNA (mtDNA), to document levels of genetic variability within and among populations. Subsequently, we describe how these same techniques could be applied in studies of genes that are functionally relevant to toxic exposure.

Steller sea lions were once an abundant marine mammal found throughout the north Pacific (13). Unfortunately, their census numbers have declined precipitously since the early 1960s—an approximately 39 to 48% decline for the species (14). Some regions, such as southeastern Alaska, have maintained or slightly increased their population numbers, whereas other regions, such as the Aleutian Islands, have declined by as much as 80% (15,16). The reasons for this decline are not well understood, but various human activities have been suggested, including commercial fishing, pollution, and hunting. Bickham et al. (17) recently conducted a study on the population genetics of this species to determine the levels of genetic variability within and among populations. The goal of this study was to determine if genetically differentiated stocks of sea lions exist as well as to determine if genetic markers could be discovered that would allow the identification of the genetic stocks for individuals taken away from their rookeries.

Automated nucleotide sequence analysis was used to study a 256 bp segment of the control region of the mtDNA from 225 sea lions representing six geographic regions, including Russia, the Aleutian Islands, eastern and western Gulf of Alaska, southeastern Alaska, and Oregon. Mitochondrial DNA was chosen for study because it is haploid and clonally inherited through maternal lineages (18). A portion of the control region was selected for analysis because of its extremely rapid rate of evolution and because it clearly represents a neutral marker with no known function (19,20).

The sea lion populations were highly variable with a total of 52 haplotypes being

observed among the samples. Strong regional differentiation was apparent in haplotype distribution, which suggested the evolutionary divergence of a western form, including Russian, Aleutian, and Gulf of Alaskan populations, from an eastern form including southeastern Alaskan and Oregon populations. The sea lion study demonstrates that fine-scale population genetics studies can be accomplished using high-resolution techniques on a scale not possible even a few years ago. As a result, changes in the genetic makeup of sea lion populations that could result from inbreeding or genetic drift caused by the observed population decline can be detected. Because the genetic marker we used was ostensibly neutral and not likely to be open to natural selection, stochastic processes such as these should result in the observed reduction of variability in mtDNA. Potentially this loss could be paralleled in genetic loci that are important to the maintenance of the fitness of the individuals within these populations. We anticipate conducting similar studies in the future on vertebrate populations from which we have documented somatic effects resulting from toxic exposure (Table 1).

Whereas neutral loci such as the mtDNA control region would be sensitive primarily to historic factors such as population bottlenecks, some loci could reasonably be expected to be open to selection due to their performing a function critical to survival in a polluted environment. Such loci, which code for functional biomarkers according to the terminology of Depledge (1), could include DNA repair enzymes which are responsible for the repair of damage caused by environmental mutagens. If allelic variation occurs in such repair enzymes or their control elements, selection could favor particular alleles at polluted sites if the favored alleles allowed for increased survivorship which resulted in higher reproductive success. It is important to remember that biomarker studies conducted on natural populations in chronically polluted environments (Table 1) are dealing only with the survivors—the segment of the original population inhabiting the area that

has survived and successfully reproduced. It is possible that such populations are genetically distinct from progenitor populations and from populations at pristine reference sites. To investigate this process of adaptation to polluted environments, the development of PCR primers for genes such as various DNA repair enzymes, as well as other loci involved in chemical detoxification, such as metallothionein, should be developed. Subsequently, population genetics studies such as that described above for Steller sea lions can be conducted using these functional loci as biomarkers to detect whether or not particular alleles are favored in polluted environments.

Conclusions and Future Prospects

Figure 1 presents our understanding of the stages that a population of organisms experiences starting with initial exposure to chronic pollution and leading to adaptation of the population resulting from selection at functionally relevant loci. The field of environmental genotoxicology includes the study of genetic alterations that are both somatic and heritable. Whereas somatic alterations are due to the direct interactions of a mutagen with the DNA of an organism, heritable effects either can be due to direct interaction or else result from selection or stochastic processes that result from toxic stress not necessarily of a genotoxic nature.

A clear understanding of the entire process whereby environmental pollution affects animal populations requires investigations ranging from the molecular level through the population level. Indeed, population ecologic effects have long been recognized to be indicators of environmental pollution. However, population genetics or evolutionary effects are emergent properties of processes at lower levels of biologic organization (Figure 1). In particular, ecologic phenomena such as population bottlenecks, the disruption of social structure, and other behavioral effects, can lead to population genetic effects such as the reduction of genetic variability by inbreeding and genetic drift. Likewise, evolutionary effects, such as selection at functionally relevant loci, are emergent phenomena that result from ecologic processes such as decline in reproductive rates and survivorship.

In conclusion, within the field of environmental toxicology assays have been developed (such as the cytologic and cytometric assays we have used) that are adequate to detect the somatic effects of environmental genotoxins, as well as a conceptual approach (the field of biomarker research) in which to apply these assays in

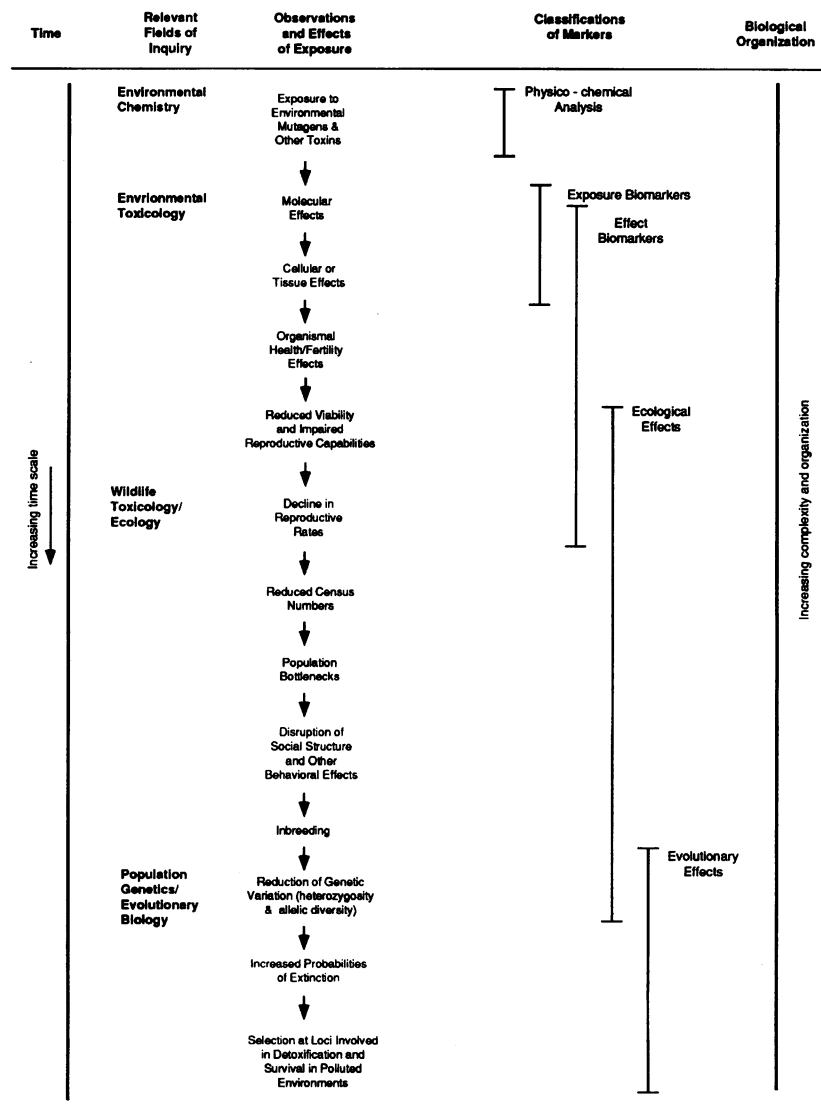


Figure 1. Schematic illustration of a possible sequence of steps leading from the exposure of individuals to environmental mutagens to the expression of evolutionary effects.

field studies. Appropriate assays to detect the ecologic effects of environmental contaminants are also available. Indeed, the fields of environmental toxicology and wildlife toxicology intergrade and are not necessarily easily distinguished.

It is apparent that the fields of population genetics and evolutionary biology have the potential to extend our understanding of the effects of environmental pollutants on the biota. In a recent editorial, Dieter (21) posed the question, "Are there specific pollutants or categories of pollutants that influence evolutionary processes, and, if so, to what extent can these effects be quantified?" Likewise, Depledge (1) has emphasized the need to understand and to measure the effects of environmental pollutants on the

Darwinian fitness of organisms. We conclude that the answer to Dieter's question is yes and that it is likely that most toxic chemicals in the environment will affect evolutionary processes. Moreover, modern procedures of molecular genetics, as applied to natural populations, offer the hope that such effects can be readily detected. We predict that a new field, evolutionary toxicology, will emerge to address these issues.

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