Population Genetic Structure and Ecotoxicology
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Electrophoretic analyses of population genetic structure, both in the laboratory and in the field, have documented significant shifts in allozyme genotype frequencies in a variety of aquatic taxa as a result of environmental impacts. Studies are documented which indicate that contaminants may select for individuals with tolerant allozyme genotypes, causing the potential loss of individuals with sensitive genotypes. This may diminish the genetic variability and fitness of affected populations and make them more susceptible to extinction following a subsequent stress. Future research involving population genetic structure and ecotoxicology should focus on determining the mechanism of sensitivity, documenting multigenerational effects of chronic laboratory exposure on population genetic composition, investigating whether previously stressed and genetically impacted populations are more susceptible to further natural and/or anthropogenic stressors, and establishing the utility of population genetic structure as a sensitive monitor of impacts in aquatic systems and their subsequent remediation. — Environ Health Perspect 102(Suppl 12):97–100 (1994)

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Research efforts in our laboratory have recently focused on examining, by gel electrophoresis (1–3), changes in the genetic structure of populations as an indicator of exposure to stress. This emphasis was prompted by our finding (4) extreme lack of genetic variation in yellow perch (Perca flavescentis) from the western and central basins of Lake Erie (one enzyme locus heterozygous of 54 loci studied in 161 individuals). The loci for phosphoglucomutase (PGM) and glucose-6-phosphate isomerase (GPI) are extremely variable in the Oneida Lake, New York and Lake Champlain, Vermont control populations; in invertebrates, allozymes at these loci were extremely sensitive to heavy metals (5–7). One mechanism suggested to explain the relationship between pollution tolerance and allozyme genotype was that heavy metals competitively inhibited magnesium-dependent allozymes differentially (8,9). Extensive contamination in Lake Erie by heavy metals including mercury, copper, and zinc may have selectively eliminated sensitive alleles resulting in the electrophoretically uniform perch population. The perch data also suggested that anthropogenic stress has the potential to alter significantly genetic structure of populations and that monitoring genetic structure may provide a sensitive indicator of the impact of stress on populations.

It was hypothesized that exposure to contaminants selects against sensitive genotypes and that elimination of genotypes narrows genetic diversity and significantly changes the genetic structure of the population. To test these hypotheses, a series of studies was initiated to demonstrate whether a relationship existed between changes in population genetic structure and exposure to contaminants in natural systems. In addition, we investigated whether a cause-and-effect relationship existed between contaminants and sensitivity to toxicity of individuals with different genotypes.

Some natural systems studied were a stream flowing through a uranium reprocessing facility in southwestern Ohio (10), a coal ash settling basin at the Department of Energy Savannah River site in South Carolina (11) and an acid-impacted river system in the Adirondack Mountains of New York (12). Selected examples of the patterns observed are presented.

Surface water runoff occurs from the production area of the uranium reprocessing facility towards a stream passing through the facility. Fish and selected benthic macroinvertebrates were sampled from sites above and below the production area. The stream had received a significant amount of radionuclide contamination from processing activities, and the levels of contamination has remained high, relative to upstream sites, in recent years. Patterns for PGM in stoneroller minnows (Campostoma anomalum) indicate a marked shift in allozyme frequency between upstream and downstream sites. The majority of the change occurred between sites 2 (immediately above the production area) and 3 (within the facility), over a distance of less than 500 meters. Examination of genotypic proportions indicates the same pattern with the PGM-BB genotype increasing significantly from upstream to downstream (Figure 1A).

If individuals vary in their sensitivity to the toxic effects of contaminants, then this "increased sensitivity" should be observable under laboratory conditions. Stonerollers were collected from upstream of the uranium reprocessing facility and also from a local stream; these fish were exposed in the laboratory to an acutely toxic concentration (0.3 mg/l) of copper sulfate. Dead fish were removed periodically, frozen, and subsequently analyzed electrophoretically. To determine if allozyme frequencies were associated with relative sensitivity to copper toxicity, stonerollers were grouped according to their time to death. The results of this toxicity test suggest that stonerollers with the PGM-AA and PGM-AB genotypes are more sensitive to copper toxicity than fishes with the PGM-BB genotype (Figure 1B). These findings parallel the field data. Thus, Campostoma with certain allozyme genotypes may be more sensitive to the toxic effects of specific contaminants and complex effluents than individuals with other genotypes.

The genetic structure of mosquitofish (Gambusia holbrooki) populations from two sites at the DOE Savannah River Site were compared: one site was contaminated...
with heavy metals and other elements from the 400D area power plant and the second was from a nearby reservoir (11). Sluiced coal ash from the large coal power plant is pumped to a settling basin, which then empties into a second basin with the effluent passing through a flume into a swampy area which flows down a narrow channel into nearby Beaver Dam Creek. Mosquitofish (Gambusia holbrooki) were collected from three sites receiving effluent originating from the power plant; all sites were characterized by high levels of As, Cd, Cr, Cu, and Zn in both the water column and sediments. Control samples were collected from Par Pond, a 1100 ha reservoir located approximately 20 km northeast of these sites. Electrophoretic patterns at three of the five loci examined and the percent heterozygous loci/individual document the highly significant reductions in genetic variability in mosquitofish exposed to effluent from the coal plant compared to the fish from Par Pond (Figure 2). All loci studied tend toward allelic fixation in the ash basin Gambusia sample. Reduced variation can result from founder effects; however, several studies of attempted bottlenecking (13), and genetic variability in mosquitofish from the Savannah River drainage (14,15), suggest that this is unlikely for the ash basin population. Laboratory data provide support for observations from field populations; the ash basin Gambusia genotypes that were fixed or approaching fixation for the GPI-2 and IDH-1 loci, respectively, are the same genotypes found to exhibit greatest tolerance to arsenic and mercury during laboratory exposures (16,17).

In the North Branch of the Moose River, a part of the Black River Drainage located in the Adirondack Park, New York, genetic variability and genotypic frequencies were compared (12) among populations of the central mudmimnow (Umbra limi) collected from sites varying in exposure to acid deposition. Populations were compared between reference and acid-stressed (low pH/high Al) sites to determine if persistence in stressed habitats was accompanied by detectable and consistent genetic shifts. Central mudmimnow populations from stressed sites were characterized by significantly lower heterozygosity levels than reference sites (12). In addition, at stressed sites two genetic loci exhibited consistent, and three loci showed moderately consistent, genotypic shifts relative to patterns at control sites; populations at acid-stressed sites were characterized by higher frequencies of one particular allozyme suggesting the effects of selection (12).

In many states, biological sampling has been implemented to assess water quality. Studies were recently conducted (18,19) to determine whether genetic structure of bluntnose (Pimephales notatus) and stoneroller minnow (Campostoma anom-
alum) populations could serve as an effective biomarker of exposure to anthropogenic stressors by comparing genetic measures with other biological indicators of water quality. Fish were collected from sites (15 for bluntnose, 14 for stoneroller) on different streams (8 for bluntnose, 7 for stoneroller) by the Ohio Environmental Protection Agency as part of their stream water quality evaluation program. Values for Index of Biotic Integrity (IBI) and Invertebrate Community Index (ICI) were determined for these sites. Starch gel electrophoresis was used to collect genetic data for variable enzyme loci. For both species, genetic diversity measures were not associated with site IBI or ICI values. However, the range of site IBI and ICI values was limited; future studies must ensure an extensive range of site IBI and ICI values. For Pimephales notatus, data suggested that the proportion of individuals not expressing EST-3 could be used to predict IBI; the proportion of nonexpression increased with decreasing IBI. Also, allele frequency differences were observed between sites on the Little Scio River, the one stream with a large difference in IBI and ICI values between sites. Data suggested that water quality, as measured by IBI and ICI, may predict genetic differentiation among sites on a stream. For Campostoma anomalum, allele and genotype frequencies were significantly different at the point source compared to sites up- and downstream. These data and genetic distance data for ston rollers suggest that point-source impacts altered genetic structure. These preliminary studies reinforce our contention that allele and genotype frequencies may be an indicator of water quality that is sensitive enough to detect change prior to species loss.

It is essential to extend studies of population genetic structure and genotoxicity to empirically demonstrate the effects of single-pulsed acute, and low-level chronic, exposures on subsequent survival of populations. Experiments in progress in our laboratories involve both acute and chronic exposures of fathead minnows (Pimephales promelas) to a PAH (fluoranthene) (Diamond et al., unpublished) and acute exposures to a metal (copper) (Schleuter et al., unpublished). Failure time analysis was conducted during the fluoranthene acute exposure, and GPI-1 genotype was significantly related to time to death; survivors were placed in clean water, and raised to sexual maturity, and to date two generations have been reared from the survivors. Both generations of offspring have had a high frequency of opercular deformities (30–70%) and less resistance to subsequent acute fluoranthene exposures than naive minnows. In the copper exposures, genotypes at five loci were related to time to death. Offspring of fluoranthene-exposed minnows exhibited greater resistance to copper than naive minnows. Pimephales in the chronic exposure to fluoranthene have just produced their first broods; we anticipate following at least four generations under chronic exposure. Data to date indicate reduced fecundity and survivorship; electrophoretic analyses have not yet been conducted. Offspring from chronic exposures will be subjected to failure time analysis through both acute exposures to fluoranthene in the laboratory and in situ exposures in a PAH-contaminated stream to assay for development of resistance.

Future research on the relationship of population genetic structure to ecotoxicology must focus in a number of areas (11, 20). First, it is imperative that additional investigations identify "sensitive" genotypes from field sites and document that these genotypes are more sensitive than others to specific contaminants in controlled laboratory studies. Relatively few such experiments have been conducted, and the generality of the correlation must be tested by field-laboratory assessments for several types of contaminants and a diversity of organisms. Data from these studies will also provide the evidence needed to use analyses of population genetic structure as a monitor of remediation; as remediation progresses genotypic proportions should return to levels found in unimpacted sites. Second, we have determined that there are links between toxicity and allozyme genotype; much research is needed to determine the biochemical mechanism of sensitivity which may lead to a better comprehension of the genetic basis and inheritance of tolerance to toxicity. Third, additional studies like the P. promelas example discussed above, are required that link particular resistant allozyme genotypes with individual and population fitness. Fourth, our investigation of Gambusia in the ash basin indicates that we must be concerned about the loss of genetic diversity within populations and species due to anthropogenic impacts. This loss may make the taxa more susceptible to future natural and/or anthropogenic stress and lead to extinction. Extinction is depauperate populations should be challenged with a variety of stressors in controlled laboratory experiments to determine whether they exhibit increased susceptibility to extinction. Finally, it must be determined whether to include analyses of population genetic structure as part of long-term environmental monitoring programs. If this is to be accomplished, baseline genetic data for natural populations must first be accumulated by surveying taxa for polymorphic isozymes and establishing normal local, regional and temporal fluctuations.

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