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# Genetic Diversity as an Indicator of Ecosystem Condition and Sustainability

## Utility for Regional Assessments of Stream Condition in the Eastern United States

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## Preface

In 1995, the U.S. Environmental Protection Agency's National Exposure Research Laboratory (NERL) enhanced its ecological research and development efforts to support the delivery of the "next generation" of biological indicators. These new ecological condition and stressor diagnostic indicators would follow the risk paradigm organization of the U.S. EPA's Office of Research and Development (ORD). ORD's Ecological Research Strategy (US EPA, 1998) called for the "development of a set of indicators for estuarine, stream, and lake systems that can be interpreted relative to status and changes in fundamental ecological and hydrologic processes that influence and constrain the integrity and sustainability of these systems." Genetic diversity indicator research was initiated in NERL to address this objective.

In 1998, ORD's National Center for Environmental Research (NCER) requested proposals from the academic research community for Science to Achieve Results (STAR) grants focused on "Ecological Indicators". Research sponsored by these grants emphasized genetic diversity and landscape ecology, both of which can be interpreted at a number of geographic scales, a requirement for the next generation of ecological indicators. In May 2000, NCER sponsored a review of genetic diversity science including both ORD scientists and STAR grant recipients during which a roundtable discussion delineated contributions as unique to either academic or federal laboratories. Large, regional-scale evaluations of genetic diversity within species, deemed beyond the scope of academic laboratories, were seen to be appropriate for federal laboratories and easily incorporated into existing environmental monitoring studies such as the Environmental Monitoring and Assessment Program (EMAP). These views were mirrored by recommendations in the July 1999 NSF Task Force on the Environment document "Environmental Science and Engineering for the 21st Century" which called for genetic diversity research within federal laboratories. Current advances in molecular biological science and technology have converged with classical genetic research, large-scale field biological monitoring, and remote sensing capabilities to provide unprecedented opportunities for multifaceted studies of species population structure and dynamics. These types of studies are critical to understanding the integrity and sustainability of ecosystems.

This report chronicles significant strides made in the development of an ecological indicator based on genetic diversity that is suitable for environmental monitoring studies at a range of geographic scales. Implementation of regional studies of genetic diversity required development of protocols for inclusion of genetic sampling in field studies and large-scale laboratory throughput of these samples. Robust statistical methods facilitated the meaningful interpretation of genetic diversity DNA fingerprinting data in the context of other environmental data collected concurrently. Documentation of these procedures for measuring genetic diversity is presented herein, along with the background and rationale for employing genetic diversity as an ecological indicator. Case studies are presented which demonstrate the application of genetic diversity in two field-monitoring efforts. Finally, recommendations are given for genetic diversity study design and technology transfer based on field and laboratory experience with large-scale studies.



## Summary

Genetic diversity is a fundamental component of biodiversity and is as critical to sustainability of our natural resources as are diversity of species and ecosystems. It encompasses all of the genetically determined differences that occur between individuals of a species. Virtually all species are composed of populations that exist somewhat independently of each other, and thus genetic diversity exists both within and among populations. Levels of genetic diversity in any one population are determined primarily by four forces: (1) mutation, the ultimate source of all genetic diversity; (2) migration, the exchange of individuals between populations; (3) natural selection, the removal of "unfit" individuals from the population; and (4) genetic drift, random changes in gene frequency each generation due to limited numbers of breeding adults. The natural history of a species and the structure and dynamics of populations provide the arena in which these forces interact to drive evolutionary adaptation of populations to their environments. Thus, natural and anthropogenic environmental changes lead to changes in genetic diversity, both within and among populations, and genetic diversity measurement can provide insights into the consequences of environmental changes.

Genetic diversity can be measured by examining common morphological or morphometric traits. Such observable characteristics often result from the interaction of many genes, the expression of which is influenced by environmental factors. Assessments of molecular markers based directly on DNA have simple inheritance patterns and are not influenced by environmental factors. This simplification of the genetic system allows precise estimates of genetic diversity for any one marker and, by assessing many markers, can provide more precise estimates of overall levels of genetic diversity within and among populations of a species. Mathematical tools have been developed that allow diagnosis of the relative strengths of the four genetic forces and, indirectly, properties of populations, such as population size, breeding structure, and dispersal abilities.

Measurement of genetic diversity with molecular markers can add value to assessments of ecological condition derived from other ecological indicators, such as landscape and species assemblage indicators. Population parameters can be effectively estimated with molecular markers and used to characterize the geographic structure and connectivity of populations critical to interpreting data for ecological assessments. Genetic diversity also serves as an independent indicator of environmental condition. Environmental stressors typically reduce genetic diversity, primarily through the forces of selection and genetic drift, so that a recent reduction in genetic diversity is indicative of deteriorating environmental condition. As an indicator of ecological condition, genetic diversity integrates the genetic effects of multiple sources and is cumulative over time. In addition, it is a naturally 'scalable' indicator, as the geographic structuring of genetic diversity at the population, watershed, and regional levels is easily inferred.

The importance of genetic diversity to long-term sustainability is widely accepted, although the efficacy of molecular measures of genetic diversity for diagnosing extinction risk remains unclear and needs further investigation. Standing levels of genetic diversity in populations contribute to long-term sustainability in several ways. First, the ability of populations to adapt to changing environments is directly dependent on the amount of genetic diversity they possess. Second, small populations that lose genetic diversity may experience fitness reductions and increased extinction risk. Finally, populations that are adapted to

local conditions may become less fit if individuals from other areas that are adapted to different conditions are allowed to interbreed with them; the ensuing reduction in genetic diversity between populations influences long-term sustainability of the species.

This report documents research undertaken to determine if the theoretical promise of genetic diversity as an ecological indicator is realized in real-world applications. Results of two case studies confirm that genetic diversity is a useful indicator of environmental condition. The first case study incorporated the genetic diversity indicator in a larger Regional Environmental Monitoring and Assessment Program study of the Eastern Cornbelt Plains Ecoregion, done in collaboration with US EPA Region 5 and Ohio EPA. Genetic diversity of a small cyprinid minnow, the central stoneroller (*Campostoma anomalum*), was measured at 91 sites in nine watersheds using the RAPD (random amplified polymorphic DNA) fingerprinting technique. Although the RAPD technique was chosen primarily for ease of technology transfer, experiences with the technique suggested that it might not be robust to the normal variations in equipment and technical skills that exist among different laboratories. Nonetheless, the genetic diversity data obtained proved highly informative. Although sample sizes varied and were sometimes small (3-10 individuals per site), large differences in genetic diversity within sites and among sites were detected. Significant differences in the average levels of genetic diversity within populations were observed among major river drainage basins, leading to the conclusion that populations of stonerollers are highly differentiated within the Eastern Cornbelt Plains Ecoregion and there is geographical structuring of these populations within and among watersheds. Genetic diversity is related to environmental condition, particularly impacts from urbanization, channelization, and impaired riparian zones. Expected relationships between genetic diversity and existing ecological indicators such as the IBI and QHEI were seen, although the small degree of correlation suggests that the genetic diversity indicator provides supporting and not highly redundant information for environmental condition assessments.

The second case study examined the genetic diversity indicator applied to populations of the creek chub (*Semotilus atromaculatus*) in a small region of western Pennsylvania and West Virginia underlain by coal-bearing geology and for which the history of coal mining operations is known. Samples of between 9 and 28 creek chubs were collected from 10 sites within 4 watersheds. Two molecular methods were used: the amplified fragment length polymorphism (AFLP) fingerprinting technique was used to assess diversity in the nuclear genome, while a portion of the mitochondrial genome was assessed using DNA sequencing. Mitochondrial DNA differences showed a strong spatial component. The nuclear DNA also differentiated the populations although the genetic structure was not as strong as that seen in the mitochondrial DNA. Environmental factors (derived from principal components analysis of 25 key environmental measurements) accounted for about half of the differences in mitochondrial DNA diversity and virtually all of the differences in nuclear DNA (AFLP) diversity.

These two case studies clearly demonstrate that genetic diversity can serve as an indicator of environmental condition. They also provided the practical experience upon which recommendations for future implementation are based.

At present, genetic diversity indicators will be used most effectively if they are incorporated into multi-indicator assessments at large, regional scales. Typically, reduced genetic diversity in particular popula-

tions is inferred from assessments of spatially separated populations, although it can be detected more easily through temporal monitoring. Thus, incorporation of genetic diversity indicators into monitoring programs at intensively studied index sites will be useful. Regional genetic diversity data can then serve as baseline data for future monitoring of temporal patterns in genetic diversity at different spatial scales.

A number of different molecular technologies can be used for genetic diversity analysis, including allozyme, DNA fingerprint, microsatellite DNA, and mitochondrial DNA fragment or sequence analysis. At present, it appears that the most cost-effective strategy is to incorporate microsatellite markers into existing or planned ecological assessments. It may be beneficial to supplement microsatellite analysis with mitochondrial DNA analysis since mitochondrial DNA can yield complementary information. This approach is the most technologically challenging of all the genetic diversity assessment options. Thus, the recommendation is that a "three-laboratory approach" be used to obtain and interpret genetic diversity data. The lead lab would be the regional field lab, which would be responsible for design of the assessment, field collections, and preparation of DNA samples. A marker development laboratory would design molecular markers specific for the target species identified by the regional lab. A genetic analysis laboratory would use the molecular markers and DNA samples obtained from the other labs to perform the genetic diversity assessment and, together with the regional laboratory, derive the ecological assessment.