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Perspective on conservation genetics

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Overview

Conservation genetics is an applied science. Much as electrical engineering is the application of principles of physics to building rockets, satellites and television stations, conservation genetics takes the methods and theories of molecular biology, genetics and evolution, and interprets the natural history of a threatened population, the hope being to provide useful clues about a population's genetic structure that can be valuable in developing an effective management strategy. When combined with demographic, ecological, behavioral, and physiological characteristics of endangered species, genetic data has emerged as a unifying component for interpreting past history, present status, and future prospects for threatened populations facing extinction. A notable change has occurred in the last decade in developing conservation plans. Before 1980, endangered species protection emphasized ecological and demographic considerations. Today, genetics, reproductive physiology, clinical medicine, and infectious disease are agenda items on nearly all conservation management plans.

Molecular descriptions of the quality and quantity of genetic diversity in populations really began when Lewontin and Hubby (1966) estimated the average genomic heterozygosity of populations of *Drosophila pseudoobscura* using 25 protein and allelic isozyme (allozyme) loci. Their study stimulated similar estimates in hundreds of species, each looking at up to 50 allozyme loci for genetically controlled variation (Nevo, 1984). Most species displayed 15–50% of their loci as polymorphic and the average heterozygosity was between 2 and 15%. Much of the early discussion of the data dealt with how much random mutational variation a population could tolerate (Muller's genetic load concept), and later on whether the patterns of variation supported an adaptive or a selectively neutral explanation. The conservation community took notice of such studies when Bonnell and Selander (1974) discovered that the endangered Northern elephant seal displayed no variation in a survey of 24 allozyme loci. These authors interpreted their

results as a consequence of an 18th century population bottleneck caused by hunting pressure on the species. Their conclusions have been confirmed more recently with additional allozyme loci plus DNA sequence analysis of mitochondrial (mt) DNA in a study that revealed that Northern elephant seals had less than 5% of the genomic variation that occurs in the Southern elephant seals (Hoelzel et al., 1993). The relevance of this finding to the future potential of elephant seals, however, was not obvious because the species has recovered to some 120 000 seals since it was afforded protection in 1922 by the governments of the U.S.A. and Mexico. It was Ralls et al. (1979) who demonstrated the severe cost of close inbreeding to wildlife biologists when they screened the breeding records of 24 captive wildlife species and showed that in every case but one, infant mortality was greater when the parents were related.

My own group became involved in conservation issues in the early 1980s when I entered a collaboration with Mitch Bush, clinical veterinarian at the National Zoo, and David Wildt, a reproductive physiologist then at NIH, to study the African cheetah (*Acinonyx jubatus*), a species that was rather reluctant to breed in captivity. We discovered that the cheetah also had remarkably low allozyme variation (90–95% less than other cat species), and extended our observations to track variation with two-dimensional gel electrophoresis of fibroblast proteins, diversity at the major histocompatibility complex (MHC) using both surgical skin grafts (14 out of 14 were accepted) and DNA variation, and morphological asymmetry of cranial specimens (O'Brien et al., 1985; Yuhki and O'Brien, 1990; Wayne et al., 1986). Cheetahs were also found to have a low sperm count and an elevated level of developmental abnormalities in their sperm (*circa* 70% compared to about 30% in African lions or domestic cats). This offered a physiological explanation for the breeding difficulties which were documented from studbook records of some 800 cheetahs in captivity (Marker and O'Brien, 1989). We hypothesized that the cheetah's ancestors probably passed through one or more population bottlenecks followed by inbreeding that dramatically reduced the species' allotment of diversity. By back-calculation based on the amount of variation at two rapidly evolving genomic families (mitochondrial DNA and multi-locus DNA fingerprinting) and the estimated per locus mutation rate, we estimated the time of the proposed bottleneck as toward the end of the Pleistocene (about 10 000 years ago) when there occurred an extensive extinction of large vertebrates in North America, Europe, Asia, and Australia (Menotti-Raymond and O'Brien, 1993). The procedure simply presumes that the bottleneck which reduced allozyme and MHC diversity also lowered mtDNA and DNA fingerprint variation to zero (as it has in Asian lions, Channel Island foxes, and Florida panthers in recent demographic collapses); then, by measuring the amount of accumulated

diversity on mtDNA and minisatellite loci, we can estimate the time required to repopulate diversity to today's levels.

The cheetah's difficulties did not stop there. One of the lessons that had been learned from inbred mice and livestock is that inbreeding sometimes causes an increased sensitivity of strains to infectious pathogens. The reason for this has to do with the evolution of the immune system. Several of the loci that mediate immune defenses seem to depend on extensive allelic variation within outbred populations as sort of a "moving target" for rapidly evolving pathogens; the idea is that when a virus genetically changes to overcome the defenses of a single individual, it will not be as effective in another genetically different individual. This explanation seems to be the driving force for enormous genetic diversity at the major histocompatibility complex, whose role it is to recognize and serve up foreign virus peptides to T-lymphocytes as a prelude to cell-mediated immune destruction of infected cells. The cheetah provided a vivid natural example of this scenario when we encountered a devastating epizootic of feline infectious peritonitis at an Oregon cheetah breeding facility in the mid 1980s. An outbreak of this virus in cheetahs resulted in 100% morbidity (symptoms) and 60% mortality over a 3-year period (Heeney et al., 1990). This represented the worst outbreak of this incurable disease in any feline species; in domestic cats the incidence is seldom greater than 5% morbidity. The conclusion that the cheetah's nearly homogeneous response to lethal peritonitis virus was related to its genetic homogeneity, particularly at the MHC, was inescapable.

The lessons from the studies of cheetah genetics were clear. First, there were certainly undiscovered perils that can threaten populations that were not so apparent as ecological parameters. Second, when populations drop to very low numbers, as most endangered species do, if they do not go extinct they still could suffer genetic depletion when inbreeding is close and persistent. Third, although every population bottleneck is different, they all carry the risk of inbreeding depression, the expression of congenital abnormalities resulting from homozygosity of deleterious genes. Fourth, in addition to these heritable defects, inbreeding homogenizes variation at abundantly polymorphic genes that mediate immune response, increasing the population's risk of extinction from any pathogen that can overcome the immune defences of one individual.

Unfortunately, there are now several examples of endangered species and subspecies that have experienced population crashes followed by inbreeding and subsequent genetic depletion (O'Brien and Evermann, 1988). The most dramatic case we have observed is that of the Florida panther, a small relict population of about 30 pumas that survive today in the Big Cypress-Everglades ecosystem in South Florida (Roelke et al., 1993). This tiny population has reduced genomic variation, tail

kinks, worse sperm than cheetahs, over 80% cryptorchidism, fatal congenital heart defects, and a huge parasite-pathogen load. A recent management workshop on Florida panther recovery recommended the introduction of a formerly adjacent puma subspecies from Texas as an immediate one-generation attempt to overcome the deadly consequences of inbreeding in this population.

I tend to view the history of conservation genetics as consisting of phases driven largely by available technology. The first might have been the period of allozyme studies in which population geneticists told us how to interpret these data in terms of overall genomic variation. The second phase involved the application of DNA variation using restriction enzymes, notable in mitochondrial DNA and in nuclear DNA fingerprints. The mtDNA analysis was ideal for detecting population differentiation below the species level, because of its asexual transmission mode (through matriline) and its rapid accumulation of mutational differences relative to nuclear coding genes (Avise et al., 1987). DNA fingerprints were particularly useful in assessing parentage and kinship in free-ranging populations (Gilbert et al., 1991). In addition, overall DNA fingerprint variation of different populations is very sensitive to recent (within a few thousand years) bottlenecks and inbreeding events. The third technological advance involves population analysis of small quantities of DNA by direct amplification using the polymerase chain reaction. This method plus advancing technology has made direct DNA sequencing of nuclear and mitochondrial DNA feasible with population samples. In addition, the discovery of satellite families of nearly 100 000 hypervariable di-, tri- and tetra-nucleotide repeats dispersed in the mammalian genome offers enormous power for tracking individual and population differentiation.

The next phase is in the future. Today's methods largely track genomic variation that is of little adaptive consequence, but is useful as an index of overall variation. The selective pressures that affect population variation or that may influence survival are difficult to identify precisely because there are simply too many possibilities over 3×10^9 base pairs of the mammalian genome. The explosion in human gene mapping, driven by the human genome project will soon change that. As the 50 000–100 000 coding genes of man become identified and genetically mapped, the candidate genes for adaptation will be suggested. Further, the comparative gene mapping efforts in mammal species not only would contribute to an understanding of the patterns and forces of genome evolution, but also expand the interpretive power of gene linkages in all species by linkage homology to well defined genome segments of mouse and man. This leads to the exciting prospect of testing hypotheses about genetic adaptation during evolution and even discovering the exact gene defects that threaten small inbred populations. Over the past 30 years genetics research has progressed

from an emphasis on deductive reasoning of inheritance patterns to watching gene action directly. In the future, we may even be considering the reversal of historic accidents by well-reasoned genetic engineering.

As I write these words en route to a giant panda management plan workshop in Cheng Du, China, I am reminded that genetics is only one of several considerations in a goal of species conservation. Just obtaining reagents for charismatic endangered species can be difficult due to cultural differences and innate human sensitivities. Conservation workers have known for decades that only by anticipating a "win-win" situation where apparently conflicting interests can be accommodated can valuable data be collected, interpreted, and implemented in a world dominated by *Homo sapiens*.

The giant panda situation is painfully familiar: low numbers, human development, habitat depredation. The demographic data are clear, but the genetic structure is not. How much diversity remains in the species compared to other ursid species? Does maternal behavior influence overall diversity or, conversely, the number of lethal equivalents per genome? Are any of the 25 isolated populations descendants of an inbreeding event? How extreme is the depletion? Is it enough to consider opening migration corridors? What about disease outbreaks? Would we risk disease spread to abrogate genetic depletion? Is there genetic differentiation sufficient to merit subspecies designation and thereby separate population management? If a population shows genetic depletion, what are the fitness consequences: bad, like in the Florida panther, or modest, like in elephant seals? Every case is different, but I believe we have finally come to recognize the scope of conservation's challenge. It may be a twist of irony that the same advancing technology that led to the threat to many species may have a role in designing protection plans that may reverse the extinction process.

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