

CONSERVATION GENETICS IN WILDLIFE MANAGEMENT

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INTRODUCTION

Prior to 1980, genetic techniques were not typically used in wildlife biology. With recent technological advances, straightforward and rather inexpensive genetic techniques have emerged, which can be directly applied to wildlife studies. In this chapter we discuss molecular genetic techniques and how they can be applied in wildlife biology. This material is intended for wildlife biologists and managers. Geneticists and those interested in detailed descriptions of each technique are referred to Avise (1994) and Hillis et al. (1996). Here we present a compilation of ideas, techniques, and applications of use to wildlife students and professionals seeking to use molecular genetic techniques.

Molecular Genetic Techniques

Nuclear vs. Mitochondrial Genomes

All genetic techniques and molecular markers described in this chapter examine portions of DNA at some scale. Two different genomes are used in genetic studies of animals. The nuclear genome is biparentally inherited and is found in the cell nucleus. It is large and not well mapped in most species. The mitochondrial genome is housed in the mitochondrion, an organelle involved in cellular metabolism. It is small compared to the nuclear genome and is a circular, maternally inherited molecule that has been well mapped in many species. Nuclear DNA on average evolves slowly although some portions (e.g.,

microsatellites) evolve quickly. Mitochondrial DNA (mtDNA) on average evolves more quickly than the nuclear genome and some areas (e.g. control region) evolve very rapidly. These features make mtDNA and some regions of nuclear DNA suitable targets for certain genetic studies (Avise 1994).

Investigating Genetic Variation

Some molecular techniques consider gene products (e.g., proteins), and some examine DNA variation at the nucleotide level (e.g., DNA sequencing, fragment analysis). In the past, analysis of certain proteins has been easy and economical; however, quantifying variation at the nucleotide level has become a more powerful molecular tool for population genetics and systematics. Some techniques look for differences in actual nucleotide sequence, while others infer relatedness based on analysis of fragments and restriction sites.

The advent of the Polymerase Chain Reaction (PCR) has revolutionized molecular biology. Essentially, PCR is a reaction in which a region of DNA is targeted and amplified exponentially (Avise 1994, Palumbi 1996). This reaction requires development of unique primers, which flank both sides of the targeted region of DNA. Once amplified to large quantities, the targeted region (usually between 100–2,000 base pairs) is available for study with a wide variety of molecular techniques. We briefly review several techniques that have been and are currently used in wildlife studies (Table 1). More detailed and excellent

Table 1. Applicability of common types of molecular markers for wildlife biologists. The number of X's indicates the relative applicability of each technique to a specific question (modified from Mace et al. 1996).

Type of marker	Taxonomic delineations	Regional/subspecific population structure	Genetic diversity and sub-population structure	Individual ID and paternity/maternity analysis
Allozymes	XXX	XXX	XXX	X
MtDNA sequences and RFLP	XXXX	XXXX	XX	X
Microsatellites	X	XX	XXXX	XXXX
Minisatellites	X	X	XX	XXXX
RAPD	X	XX	X	X
AFLP	X	X	XX	XXX

reviews of these and additional genetic markers available for studying genetic diversity in wildlife populations have been presented elsewhere (Avise 1994, Smith and Wayne 1996, Haig 1998).

Analysis of Gene Products

Protein electrophoresis is a technique that can be used to examine population subdivision or structure. Proteins are a series of amino acids joined by peptide bonds. Each amino acid has a distinctive side chain, some of which are either positively or negatively charged. Thus, when an electric current is present these proteins migrate differentially through a matrix based on their charge and on their size and shape. Proteins can then be visualized through histochemical staining or other methods (Murphy et al. 1996). Mutations cause changes in the DNA sequences of amino acids forming proteins, which in turn cause changes in the shape, net charge, and migration rates of proteins. Such changes can be revealed through electrophoresis and provide information showing variability between individuals, populations, or species. While inexpensive, this technique can examine only a small proportion of the variation present in the DNA that codes for the proteins; differences in proteins are not necessarily detected. The subset of proteins typically studied with this approach is called allozymes. These proteins, however, may be under selective pressure and may not represent the diversity and divergence present in other genes. Further, the tissue required for this type of analysis typically requires highly invasive/destructive sampling and is logistically difficult to manage in field situations.

Fragment Analysis

Fragment analysis comprises a group of different genetic techniques that explore nucleotide variation indirectly by comparing the size of DNA fragments electrophoretically. Although fragment analysis offers less resolution than direct DNA sequencing, it is cost effective when examining many individuals and many different loci. Among fragment analysis techniques, some cut (cleave) DNA in certain areas, (i.e., Restriction Fragment Length Polymorphisms [RFLPs] and minisatellite fingerprinting), while others amplify many different loci (Random

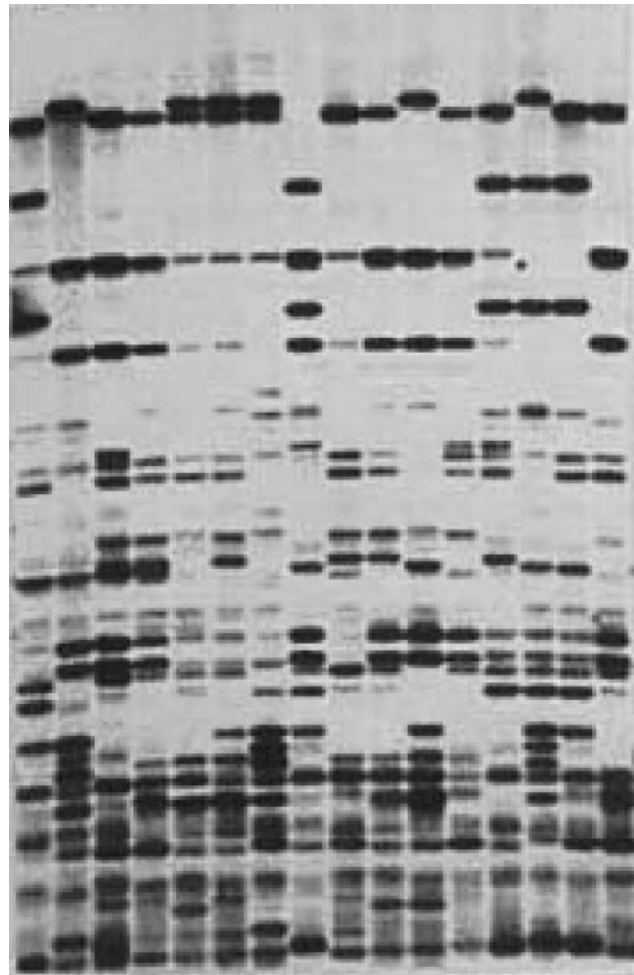


Fig. 1. Example of variation at multiple minisatellite loci. This illustration shows variation within and between families of pukeko (*Porphyrio porphyrio*) detected using markers pV47-2 and 3'HVR (from Lambert et al. 1994).

Amplified Polymorphic DNAs [RAPD], Amplified Fragment Length Polymorphisms [AFLP], and microsatellites). With the exception of microsatellites, these techniques produce multiple fragments (bands) per individual (Fig. 1). In these cases, individuals are compared by the extent of band sharing among individuals. These markers, with the exception of microsatellites, are considered dominant which refers to the fact that they document presence or absence of an allele. Codominant markers are those that reveal both alleles at a given locus (i.e., heterozygotes can be distinguished from homozygotes). Thus, they provide much more information and allow for the documentation of heterozygosity and tests of Hardy-Weinberg Equilibrium and Mendelian inheritance.

For RFLP analysis, the template DNA is typically a small portion of the nuclear or mitochondrial genome that has been amplified using PCR. RFLPs characterize genetic variation using restriction endonucleases, which are enzymes that cleave at specific locations within DNA sequences. Restriction enzymes cleave at a specific recognition sequence, usually 4–6 base pairs long. The enzyme EcoRI, for example, cuts between G and A when it comes across the sequence GAATTC. Thus, every string of GAATTC in the PCR product will be cut in the same loca-

tion and will produce many fragments of different sizes. Mutations that cause changes in the cleavage site (e.g., GATTC changed to GATAC) prevent the enzyme from cutting at that location thereby producing a different series of fragments (different numbers or sizes of fragments). The series of fragments is then compared to examine the similarity of individuals or populations.

While RFLPs look for variation within a single targeted segment of DNA, other fragment-based methods examine variation throughout the genome. Minisatellites refer to portions of DNA that have variable numbers of tandem repeats (sometimes called VNTRs); the length of each repeat unit is approximately 20 base pairs long. Typically, genomic DNA is digested into many fragments with restriction enzymes. These fragments are then separated by size using electrophoresis. The number of fragments produced by this process precludes visualization of individual bands, so radioactive or fluorescent probes specific for the minisatellite repeat are used to visualize and compare these sequences (Jeffreys et al. 1988). Because such "repeats" are commonly repeated in the genome, it is not unusual for this technique to produce dozens of bands. Although DNA fingerprinting with minisatellites has typically involved analysis with restriction enzymes and labeled probes, PCR-based approaches are becoming more common.

Rapid amplified polymorphic DNAs (RAPD) provide an alternate approach to documenting variation among individuals by generating a set of PCR products (bands) using short, random PCR primers. Variation is presumably due to sequence changes in priming sites or length changes in the PCR products. Analysis involves examining the presence or absence of bands of a particular size. RAPD analysis has the advantage of being a multilocus technique, whereby hundreds of polymorphic loci can be scored with minimal *a priori* knowledge of the target genome. Two disadvantages with this technique limit its utility: it is a dominant marker system (heterozygotes cannot be distinguished from homozygotes), and its repeatability has been questioned (Ellsworth et al. 1993, Muralidharan and Wakeland 1993).

Amplified fragment length polymorphism (AFLP) analysis is another multilocus technique that involves randomly primed loci and requires no *a priori* knowledge of the target genome (Hill et al. 1996). Analysis of AFLPs involves cutting the genomic DNA with restriction enzymes, and ligating (attaching) short "adapters" of known sequence to the fragment ends. PCR is then used to selectively amplify subsets of these fragments. As with RAPDs, AFLPs produce a series of hundreds of bands on a gel. Scoring is based on the presence or absence of a particular PCR product. AFLP analysis is also a dominant marker system, but has the advantage of being highly repeatable and is being used with increasing frequency (Mueller and Wolfenbarger 1999).

Microsatellite analysis, another PCR-based technique, differs from most other fragment analyses because the attempt is to identify diploid (codominant) genotypes for specific loci. Like minisatellites, microsatellites are VNTRs; however, the repeated sequence is short (2–5 base pairs). Mutation rates of these regions are high and the number of alleles (versions of a particular sequence) per locus in a population is also typically high. Allelic varia-

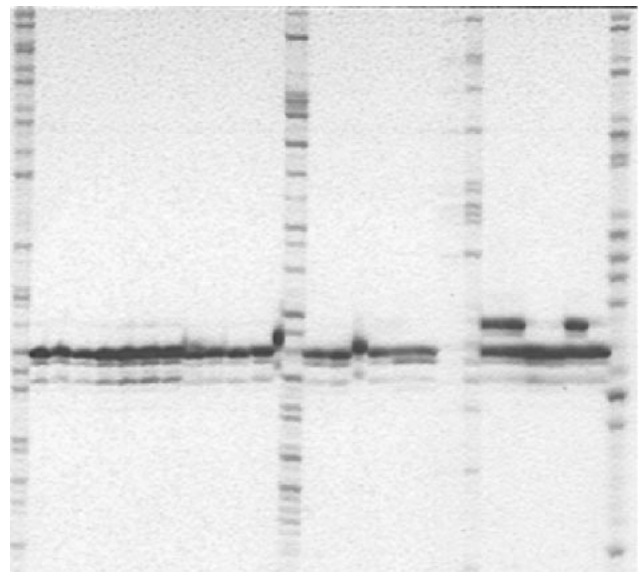


Fig. 2. Example of variation at single microsatellite loci. In this example, the microsatellite locus LST 1 is amplified in 24 individual Gunnison sage-grouse (*C. minimus*) from 3 different populations. Note that individuals in the first 2 populations are all monomorphic and homozygous for the same allele.

tion is usually in the form of length polymorphism, which can easily be detected on a high-resolution gel. Amplification results in either 1 (homozygote) or 2 (heterozygote) bands per individual (Fig. 2). Microsatellite primers are specific to a single locus and are usually specific to a particular species or group of closely related species. Because of this primer specificity, the development of primers for an individual species can be expensive. The advantages of microsatellite analysis include codominance and high levels of polymorphism. Typically, data from several microsatellite loci are used in a particular study.

DNA Sequence Analysis

Direct DNA sequencing (nuclear or mitochondrial) is one of the most widely used techniques today because it is highly informative and, recently, has become much easier and less expensive to perform. It is also appealing because evolutionary processes can be modeled and integrated into analyses. Further, because the genome is so vast, the amount of information gleaned from sequencing may be quite large. DNA sequencing involves amplifying a target region and then creating a series of labeled (either radioactively or fluorescently) DNA fragments that correspond to each nucleotide in that region (Hillis et al. 1996). The DNA fragments are then separated using electrophoresis and visualized. Recent technological advances have automated the sequencing process using fluorescently labeled DNA fragments (reviewed by Hillis et al. 1996) that are read by a laser and interpreted by computer software.

Genetic Sampling

For genetic data to be used in a wildlife study, material must be collected from animals in the field. The type of material sampled, sample size, and sampling regime vary according to the questions being asked and the molecular markers being used (reviewed by Baverstock and Moritz 1996). DNA can be extracted from a variety of different

NOTE:
We never underline 1st letters of words that form acronyms; as you had: "variable numbers of tandem repeats"

tissue including muscle, heart, liver, blood, skin, hair, feathers, saliva, feces, urine, scales, bone, fins, eggshell membranes and, potentially, cervid antlers. DNA extraction techniques for most tissues are well established and involve the isolation of DNA from proteins and lipids using a digestion with the enzyme proteinase K followed by extraction with organic solvents (Sambrook et al. 1989). Modifications to traditional extraction methods, for example, are needed when using hair or feathers when the DNA is encased in the hardened tissue of the shaft and root (reviewed by Morin and Woodruff 1996). DNA has been successfully extracted and used from museum specimens (Mundy et al. 1997) although these techniques can be highly labor intensive and expensive. When considering what type of tissue to sample, several different factors must be addressed. It must first be decided what quantity and quality of DNA is needed to answer the question of interest. Second, the necessity, feasibility, and logistics of trapping and sampling animals must be examined. Finally, field preservation and sample storage issues should be addressed prior to the beginning of a study.

Some molecular techniques require a reasonable quantity of high quality DNA (e.g., sequencing large fragments of mtDNA, DNA-DNA hybridization, protein electrophoresis) while others (most PCR-based techniques) are much more forgiving. Samples of feathers, hair, feces, and urine may contain small amounts of DNA that may be of low quality (sheared into many fragments), whereas, blood, skin, and muscle tissue often yield DNA of high quantity and quality.

The logistics of trapping and sampling wildlife vary greatly depending upon the species of interest. Some species are relatively easy to trap and sample, while others are difficult and/or dangerous. Destructive sampling refers to instances where the organism is killed during the process of sampling such as for collection of muscle, heart, liver, or embryo. If an animal is killed (hunting) or found dead (road kill or disease) samples can easily be taken for genetic analysis. Nondestructive (or noninvasive) sampling occurs when a genetic sample can be obtained without sacrificing the animal. Feathers, blood, shell membranes from hatched eggs, skin, hair, feces, and urine can all be collected nondestructively and provide potential sources of DNA for genetic analysis. Genetic samples can also be gathered without having to handle the animal in question (e.g., feathers, hair, feces, and urine).

In most cases, genetic samples can be stored on ice, refrigerated, desiccated, collected into a preservative buffer, or frozen almost immediately after collection (Table 2). When fieldwork occurs in remote areas, sampling certain tissues (e.g., skin, feathers) may be more feasible than tissues such as blood. All samples should be kept cold or dry or placed in a preservation buffer. When working with blood, only a small amount is needed (5 drops) and should be mixed with a preservative such as EDTA or with a blood buffer storage solution such as Longmire Buffer. Muscle tissue should be either placed in a preservation buffer (e.g., buffer containing Tris/HCL, EDTA, NaCl, N Lauroyl sarcosine) or frozen immediately. Contour or wing feathers provide the best source of DNA but smaller downy feathers can suffice. Feathers from individual birds should be kept in separate bags. Eggshell membranes can also be a good source of DNA as long as

Table 2. Sources of DNA and how samples should be collected.

Tissue type	Amount	Quantity of DNA	Quality of DNA	Preservation method
Blood	5–10 drops	High	Good	EDTA coated tubes, Lysis Buffer (Longmire)
Muscle	Square 2 cm on a side	High	Good	Buffer
Feather	At least 1	Low	Good	Dry
Egg shell membranes	As much as is possible	Depends	Good	Dry
Hair	At least 1	Low	Good	Dry
Scat	Variable	Low	Poor	Ethanol or Dry
Teeth	Variable	Low	Depends	Dry
Bone	Variable	Low	Depends	Dry

there is vascularization of the membrane. Each membrane should be stored dry in separate bags. Hair, bone, and teeth can be used as a DNA source if they are stored dry. For hair, only the follicle is needed. Scat can also be used but the quantity and quality of DNA are often low. Scat should be preserved in either liquid ethanol or with silica beads. Detailed protocols for sample collections and descriptions of buffer are available at <http://www.absc.usgs.gov/research/genetics/sampling.doc>.

TAXONOMY

Species/Subspecies Identification

Taxonomists have been categorizing organisms into hierarchical groups ranging from kingdom and phylum levels to genus and species for hundreds of years. Classifications have been defined using morphological and behavioral characteristics. Taxonomic delineations derived only from morphological characteristics can be erroneous (Avice 1989) as they can either fail to recognize distant forms (Avice and Nelson 1989) or they can recognize forms that exhibit little evolutionary differentiation (Laerm et al. 1982). While classifications based on morphology and behavior have been acceptable, use of genetics can often resolve discrepancies and refine taxonomic definitions. However, using genetic data alone for taxonomic classifications is not advised.

While most taxonomic definitions are somewhat arbitrary (subspecies, genera, order), classification at the species level is perceived to be based on real, evolutionary units (Dobzhansky 1970). Because the species definition is integral to the Endangered Species Act (ESA) and the protection and management of many species, we briefly mention species definitions. Many definitions of species exist (reviewed by Avice 1994); the 2 most commonly used are the biological species concept (Dobzhansky 1937) and the phylogenetic species concept (Cracraft 1983). The major difference between these 2 species concepts is that the biological species concept emphasizes reproductive isolation and the resultant limitation and/or preclusion of gene flow, while the phylogenetic species concept defines species solely using genetic data.

Genetic data can be used to address the species question regardless of which definition is used. Documenting an absence of gene flow among sympatric populations is one piece of evidence that can be used along with morphological and behavioral data to suggest delineation of a species. Constructing phylogenetic relationships among individuals to examine whether a monophyletic group exists can also be achieved by comparing DNA sequences.

Until recently, genetic information was difficult and expensive to acquire and, at times, could only be used to resolve differences between distantly related species. Protein electrophoresis (allozymes) became a useful genetic tool to distinguish differences between some species but is less useful when delineating the taxonomic relationship among closely related organisms (whether they are species, subspecies, etc.). The advent of PCR and automated sequencing has made it relatively straightforward to collect data at a high resolution in a cost-effective manner from a large number and variety of organisms. Further, sequence data from genes evolving at widely different rates can be gathered, which allows for taxonomic comparisons at immensely different levels (from kingdom/phylum/class to genus/species/subspecies). This allows for re-evaluation of taxonomic status using genetic information or for the addition of supplementary data to unresolved taxonomic questions.

There are several molecular techniques with which to assess taxonomic relationships (e.g., DNA-DNA hybridization, protein electrophoresis). Perhaps the most widely used and most applicable to questions in wildlife biology is analysis of the mtDNA sequence. The mitochondrial genome is small (15,000–20,000 base pairs) and contains approximately 37 genes although the order of these genes is not constant (Avice 1994). It is maternally inherited and does not recombine, as does nuclear DNA. While comparisons of the gene order of mtDNA have been used in investigations of taxa, direct comparison of sequences has proved to be an effective technique in finer level taxonomic questions (among more closely related species) (Avice 1994) which are much more common wildlife management concerns. Mitochondrial DNA is well mapped in many animals (Bibb et al. 1981, Anderson et al. 1982, Roe et al. 1985) and evolves 5–10 times faster than single copy nuclear genes (Brown et al. 1979, 1982). It also contains a noncoding control region in which some areas are even more variable (4–5 times more variable than mtDNA as a whole) that can be used to delineate closely related species and populations (Greenberg et al. 1983). Each mtDNA gene evolves at a different rate allowing for different level comparisons using genes with different mutation rates.

Once an appropriate gene is chosen for the taxonomic issue in question, DNA sequence from that region is obtained and the relationship among individuals is inferred by comparing the DNA sequences. Metrics, such as the percent sequence divergence, provide some measure of how similar or different the DNA sequences may be. Genetic distances or phylogenetic relationships (trees) are then estimated using either algorithms (e.g., unweighted pair group method) or optimality criterion (e.g., parsimony, maximum likelihood). These methods are well established and reviewed extensively by Miyamoto and Cracraft (1991) and Swofford et al. (1996). Nucleotide substitution

patterns in the mitochondrial control region are quite elaborate and models that estimate the rate of nucleotide substitutions have been developed (Tamura and Nei 1993, Tamura 1994). Modeling substitution rates circumvents violations of assumptions used by parsimony methods.

Using genetic data to address taxonomic questions becomes important for wildlife management primarily at the species and subspecies level. Wildlife managers are often charged with managing species and subspecies while these definitions are yet unresolved. Further, some subspecies (and even species) are difficult to distinguish in the field without extensive morphological measurements and comparisons with museum type specimens (e.g., Prebles meadow jumping mouse, *Zapus hudsonius preblei*) or detailed analyses of behavior or song (Southwestern willow flycatcher, *Empidonax traillii extimus*).

The ESA and other national and international environmental programs charge managers with protection of species, subspecies, and distinct population segments that are deemed threatened or endangered. At times, little is known about the taxonomic status of species or subspecies that are petitioned to be listed as threatened or endangered. This classification is also important for recovery of the species/subspecies because funding priorities generally are based on taxonomic status (O'Brien and Mayr 1991). Taxonomic delineations are often based only on morphological characteristics and could be refined by adding behavioral and genetic characteristics.

The taxonomic status of many different species has recently been re-evaluated using genetic data. For example, the status of sage grouse (*Centrocercus* spp.) has recently been examined using behavioral, morphological, and genetic data resulting in the recognition of a new species (Box 1). Other examples include the Kemp's Ridley sea turtle (*Lepidochelys kempii*), which has been recognized as a separate species qualifying for protection under ESA because of data from a recent mtDNA study (Bowen et al. 1991). The taxonomic status of right whales (*Eubalaena* spp.), which has historically been based on a single morphological character in the orbital region of the skull, has been redefined as the result of analysis of mtDNA data (Rosenbaum et al. 2000a).

Hybridization

Defining "hybrid" is as perplexing as is definition of the term species. Classically, "hybridization" and "introgression" are used to describe interbreeding between 2 distinct species. However, because a definitive definition of a species is still nonexistent, "hybridization" is sometimes relaxed to include interbreeding between 2 groups that are genetically different while introgression refers to the movement of genes between 2 genetically differentiated groups (Avice 1994). Hybridization can be positive or negative (Haig 1998). In a positive sense, hybridization events can increase the overall genetic diversity of a taxonomic group; it can produce increased fitness (hybrid vigor) in some cases, and can produce progeny that are more adaptable than either parent. However, in some instances, hybrids can have reduced viability and fertility. Further, the effects of outbreeding depression (decrease in fitness due to a loss of alleles which are locally adaptive) on a species due to a hybridization event can be quite negative. Because true hybrids are generally not protected by the

Box 1. Using genetics to help define taxonomic definitions for sage-grouse.

Large-scale habitat loss and degradation have resulted in the decline of sage-grouse populations throughout their range (Braun 1998) and have caused an increased concern over their status. Historically, sage-grouse were classified into 2 subspecies: eastern (*Centrocercus urophasianus urophasianus*) and western (*C. u. phaios*) sage-grouse based on plumage and coloration differences in 8 individuals collected from Washington, Oregon, and California (Aldrich 1946). The western sage-grouse presumably occurred in southern British Columbia, central Washington, east-central Oregon, and northeastern California (Aldrich 1946). Populations in other areas of the range were considered to be eastern sage-grouse. The validity of this taxonomic distinction has been questioned (Johnsgard 1983). Recently, sage-grouse from southwestern Colorado and southeastern Utah were found to be morphologically (Hupp and Braun 1991), behaviorally (Young et al. 1994), and genetically (Kahn et al. 1999, Oyler-McCance et al. 1999) different from sage-grouse throughout the rest of the range. This led to description of a new species, the Gunnison sage grouse (*C. minimus*) (Young et al. 2000).

With the validity of the 2 present subspecies in question, Benedict et al. (2003) sequenced a rapidly evolving portion of the control region of the mtDNA for 16 populations of sage-grouse on both sides of the subspecific boundary. The sequencing results provide no genetic support for the subspecies distinction. The authors suggest that further morphological and behavioral comparisons need to be conducted before overturning the subspecific classifications. This study did, however, identify a population of sage-grouse in the Lyon, Nevada and Mono, California area that was genetically unique from all other sage-grouse populations sampled throughout the species' range (Benedict et al. 2003). This group of sage-grouse is currently being studied morphologically and behaviorally.



Comparison of greater sage-grouse (left) and Gunnison sage-grouse (right).

ESA, hybridization provides interesting challenges for those charged with management and protection of species (O'Brien and Mayr 1991).

Molecular techniques provide an increasingly more accurate estimation of taxonomic relationships and history of gene flow (Haig 1998). These techniques are being used to address questions of hybridization, introgression, and taxonomic status. For example, large canids occupying the southeastern United States have long been classified as the red wolf (*Canis rufus*). Extinction of red wolves in the wild has led to serious conservation efforts to pre-

serve and restore them into the wild. However, mtDNA data and microsatellite data both strongly suggest the red wolf is a hybrid between the gray wolf (*C. lupus*) and coyote (*C. latrans*) (Wayne and Jenks 1991, Roy et al. 1994). The hybrid origin of the red wolf has led to debate over its eligibility for protection under the ESA.

Molecular techniques can also be used to identify the maternity and paternity of hybrids. Aldridge et al. (2001) described 2 sage grouse \times sharp-tailed grouse (*Centrocercus urophasianus* \times *Tympanuchus phasianellus*) hybrids in Alberta. Using analysis of mtDNA control

region sequence, they demonstrated the mother of each hybrid was a sage grouse rather than a sharp-tailed grouse. Similarly, hybrids resulting from crosses in both directions of blue (*Balaenoptera musculus*) and fin whales (*B. physalus*) have been documented using both nuclear and mtDNA (Arnason et al. 1991, Spilliaert et al. 1991).

Evolutionary Significant Units and Management Units

Given that genetic analysis can help refine taxonomic relationships, how else can genetic data be used to address management issues? Recently, there has been debate about how to objectively prioritize conservation or management value below the species level. This discussion began with Ryder (1986:9) who defined the term Evolutionary Significant Unit (ESU) as “a subset of the inclusive entity species, which possess genetic attributes significant for the present and future generations of the species in question.” In an attempt to develop an operational definition more useful to managers, Waples (1991) defined ESUs using 2 criteria. A population or groups of populations had to demonstrate substantial reproductive isolation from other populations of the same species and, at nuclear loci, it had to show significant divergence of allele frequencies. Moritz (1994b:373) further defined ESUs as “a population (or set of populations) that is reciprocally monophyletic for mtDNA alleles” and “shows significant divergence of allele frequencies at nuclear loci.” Moritz (1994a) defined a second unit called a Management Unit as a group with less separation than an ESU but deserving of specific management attention. This unit was defined to have significant divergence of nuclear or mtDNA allele frequencies regardless of the phylogenetic differentiation of alleles. While Moritz’s ESUs protect distinct units allowing for preservation of their long-term genetic variability, his Management Unit concept allows for shorter-term conservation goals. Several other scientists have put forth alternate ideas on the concept of ESUs (Dizon et al. 1992, Avise 1994, Vogler and DeSalle 1994, Crandall et al. 2000, Fraser and Bernatchez 2001). While definitions of an ESU are as highly debated and diverse (Fraser and Bernatchez 2001) as the species concepts, the ESU is useful if one is aware of the lack of agreement surrounding the best definition. Most genetic studies with applications to management use Moritz’s (1994a, b) definitions because they are well defined when using genetic data. Also, these definitions appear to be among the most well accepted and applied to date. These concepts have been applied to tiger quolls (*Dasyurus maculatus*) (Box 2), spotted owls (*Strix occidentalis*), koalas (*Phascolarctos cinereus*), and brown bears (*Ursus arctos*) (Firestone et al. 1999, Houlden et al. 1999, Waits et al. 2000, Haig et al. 2001).

CONSERVATION OF GENETIC DIVERSITY

A focus of conservation genetics has been preservation of genetic diversity within and between populations, especially in rare or endangered taxa. Genetic diversity can be estimated using molecular markers or morphological measurements. While studies have examined the underlying genetic variation and heritability of specific morphological traits in both captive and free-ranging wildlife

Box 2. Taxonomic redefinition of tiger quolls in Australia.

Firestone et al. (1999) defined Evolutionary Significant Units (ESUs) and Management Units (MUs) for tiger quolls that revised taxonomic classification and management plans for these carnivorous marsupials in Australia. Previously, 2 allopatric subspecies of tiger quoll have been recognized. The smaller subspecies, *Dasyurus maculatus gracilis* occurs only in northern Australia in northeastern Queensland. The larger subspecies, *D. m. maculatus* occurs in southeastern Australia and Tasmania. Each subspecies has been placed on the International Union for Conservation of Nature and Natural Resources list as either endangered or as vulnerable to extinction. Firestone et al. (1999) used both mtDNA sequencing and nuclear microsatellites to survey the genetic relatedness of tiger quolls. Their mtDNA sequencing results show reciprocal monophyly and significant differences in nuclear microsatellite allele frequencies between Tasmanian and all mainland tiger quolls. This suggests that, even though Tasmanian tiger quolls are recognized as the same subspecies as those in southeastern Australia, they are a separate ESU and that their taxonomic status should be revisited. The 2 subspecies on the mainland do not constitute different ESUs even though they are considered separate subspecies. Firestone et al. (1999) suggest that morphological differences between the 2 subspecies may reflect adaptation to climatic differences. Differences in microsatellite allele frequencies and mtDNA haplotypes exist between the 2 subspecies on the mainland suggesting they should be considered as distinct MUs. Thus, assessment of genetic data (Firestone et al. 1999) revealed differences between the 2 subspecies at the MU level in Australia, yet the classification in Tasmania should be reconsidered to recognize and preserve the unique genetic makeup and evolutionary path of tiger quolls.

(Williams et al. 1994, Merilä 1997, Kruuk et al. 2000, Réale and Festa-Bianchet 2000), intensive investigations are difficult to implement for many species. Frankham et al. (2002) reviewed using quantitative genetic approaches in a conservation context to study the effects of multiple genes and environmental variation on complex traits like morphology and behavior. Our primary focus is on what molecular markers tell us about the demography and genetics of a population and how that information can be applied to issues in wildlife conservation.

Genetic diversity and genetic variation are often used interchangeably to refer to a dizzying array of population characteristics. We use genetic diversity to refer to variation in frequencies of alleles at individual genes. It is difficult to quantify total genetic diversity in populations; most studies look at surrogates of this measure based on

variation at molecular markers. Four processes are generally thought to influence patterns of genetic diversity: mutation, gene flow, drift, and selection.

Mutation

The original source for most genetic differences we observe among individuals is mutation. The mutation rates of markers used by wildlife geneticists affects their information content. For example, if habitat fragmentation results in recent isolation of a number of small populations, there might be insufficient time for mutation to result in detectable levels of interpopulation divergence in mtDNA sequences. However, microsatellites, which have a high mutation rate and many alleles, lose many alleles when populations become small (Spencer et al. 2000). Thus, small populations in fragmented habitats may exhibit measurable differentiation for highly polymorphic markers. Alternately, if the objective of an investigation is to examine phylogenetic relationships between subspecies that may have been isolated for a long period, mtDNA sequences might be preferred over microsatellites, as rapid evolution in microsatellites could obscure phylogenetic relationships. An understanding of the mutational processes associated with different molecular markers can also be used to select the most appropriate statistical models for analyses of demographic bottlenecks and gene flow (Luikart and Cornuet 1998, Balloux and Lugon-Moulin 2002, Neigel 2002).

Normally mutation does not have a major role in management issues. One exception is the case of exposure of populations to environmental mutagens. Animals exposed to radioactive or chemical mutagens might be expected to have more genetic diversity because of an increased number of genetic mutations. This hypothesis was tested using bank voles (*Clethrionomys glareolus*) and barn swallows (*Hirundo rustica*) from the vicinity of the Chernobyl nuclear accident site (Matson et al. 2000). Microsatellite analysis provided evidence of increased mutation rates in the swallows (Ellegren et al. 1997). In the voles, higher levels of mtDNA variation were found near Chernobyl than at reference sites; however, it is difficult to attribute this increased genetic diversity to increased mutation, as immigration from nearby genetically differentiated populations provides another explanation (Baker et al. 2001). Other investigations have failed to detect any evidence of increased mutation in wildlife from contaminated sites (Johnson et al. 1999, Dahl et al. 2001, Stapleton et al. 2001). Given that mutations at specific genetic markers are relatively rare events, it is not surprising that it is difficult to detect increased mutation rates in the face of other powerful genetic forces such as gene flow and drift. Examinations of exposed populations for increased mutation rates will probably expand greatly in coming years as automated analysis have made it possible to screen large numbers of individuals and genes. There are also a growing number of assays for assessing DNA damage in wildlife populations (Matson et al. 2000, Theodorakis 2001).

Gene Flow

When organisms disperse to new populations and reproduce, they contribute genetic material to their new population. This process increases the genetic similarity of pop-

ulations exchanging individuals. Reductions in gene flow allow populations to diverge through processes of genetic drift, the accumulation and spread of different mutations, and selection for local conditions.

Gene flow differs from dispersal as typically measured by studies of animal movement. Radiotelemetry or tagging studies can often provide insight into the proportion of individuals that depart from their natal areas, but are inadequate for measuring the reproductive contribution of dispersing individuals to their new populations. While molecular tags are providing novel ways to track dispersing individuals, gene flow is typically measured through indirect methods using genetic markers (Slatkin 1985a).

One of most common approaches for estimating gene flow involves use of Wright's F_{ST} (1951). One common definition of F_{ST} is the proportion of the total variance in allele frequencies due to differences among populations. An attractive feature of this measure of genetic differentiation is that F_{ST} can be expressed as a function of the number of migrants per generation (Nm). Mills and Allendorf (1996) and Whitlock and McCauley (1999) discuss the assumptions necessary to use F_{ST} to estimate Nm and the difficulties of obtaining unbiased estimates of gene flow. Many estimators of F_{ST} have been developed and there is a large literature evaluating their merits and performance (reviewed by Slatkin 1985a and Neigel 1997, 2002). Other approaches to estimating gene flow include estimation of the frequencies of alleles that only occur in one of the populations in a comparison, i.e., the private alleles method (Slatkin 1985b). If alleles occur in one population at high frequencies but are absent from others, gene flow is probably low. A third set of methods involves using phylogenetic information from mtDNA sequences to estimate gene flow and dispersal distance (Slatkin and Maddison 1990, Neigel et al. 1991).

One problem with most indirect estimates of gene flow is that effects of recent gene flow on gene frequencies are often confounded with historical gene flow. If isolation is recent, populations might appear to have high gene flow even if they are completely isolated because molecular differences have not had time to accumulate (Slatkin 1985a; Neigel 1997, 2002). Many estimators of gene flow are based on populations being in an "equilibrium" condition where population size and number of successful migrants have not changed dramatically for many generations. These conditions are not typical of many settings in which resource managers wish to estimate gene flow, such as in fragmented landscapes. Thus, estimates of gene flow, while useful in a relative sense, should be regarded with some caution. One widely used approach to distinguish between the effects of current and historical gene flow, as well as other factors, on patterns of population differentiation is nested clade analysis (Templeton 2001). Nested clade analysis tests a number of hypotheses associated with the spatial distribution of sequence variation. Knowles and Maddison (2002) cautioned that this approach does not estimate error rates and performs poorly in computer simulations.

The greater the exchange of individuals between populations the more that genetic similarity of the populations will increase. However, the relationship between gene flow and genetic similarity is not linear (Fig. 3); a few successful individuals moving between populations each gen-

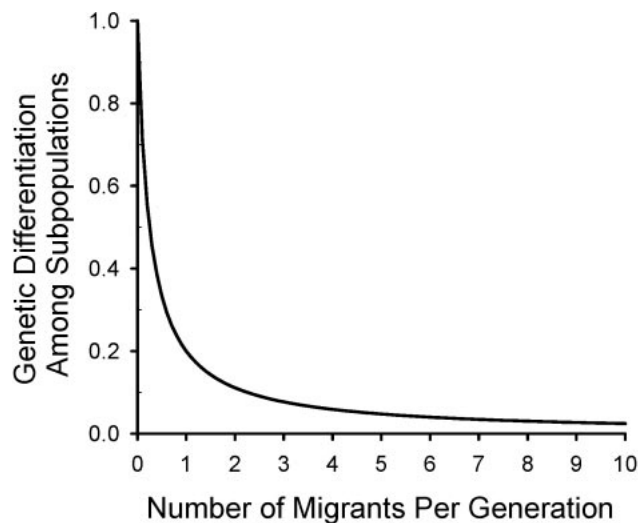


Fig. 3. Equilibrium relationship of genetic differentiation among subpopulations (as measured by the statistic F_{ST}) and number of migrants per generation (modified from Mills and Allendorf 1996).

eration is often sufficient to retard the effects of genetic drift on the similarity of gene frequencies. A consequence of the nonlinear relationship of gene flow and genetic differentiation is that once gene flow is sufficiently high to erase most genetic differences between populations, estimates of F_{ST} approach zero and it is difficult to estimate the number of migrants per generation (Waples 1998). However, knowledge that gene flow is high enough to minimize F_{ST} should be sufficient for most management decisions; a precise estimate of Nm is not needed.

Dispersal and Population Structure

Among wildlife species, there is considerable variation in the gender of dispersing individuals. Gender of individuals moving between populations, as well as a species' social structure, can have a large effect on partitioning of genetic variation within and among populations (Chesser 1991, Chesser and Baker 1996). Gender of dispersing individuals is typically beyond the control of wildlife biologists, however it is important to understand that breeding systems and gender-biased dispersal are likely to affect estimates of gene flow. Gender-biased dispersal and social structure within populations can alter effective population size, influencing rates of loss of genetic diversity (Chesser et al. 1993).

Gender-biased dispersal will have large consequences for data generated by maternally inherited markers like mtDNA. There is a large set of literature, reviewed by Avise (1994), documenting differences in spatial distribution of biparental nuclear markers (e.g., allozymes and microsatellites) and mtDNA. For example, frequencies of mtDNA genotypes of green turtle (*Chelonia mydas*) differ dramatically among some nesting beaches (Bowen et al. 1992). However, nuclear DNA in this species exhibits much less spatial subdivision than does mtDNA (Karl et al. 1992). This pattern suggests males may be responsible for most of the gene flow among beaches with females generally returning to a beach near their birthplace. Among the many applications using combinations of nuclear genes and mtDNA to characterize gender-specific gene flow and breeding systems in wildlife are studies of macaques

(*Macaca* spp.), African wild dogs (*Lycaon pictus*), snow geese (*Chen caerulescens*), and red grouse (*Lagopus lagopus scoticus*) (Avise et al. 1992, Melnick and Hoelzer 1992, Girman et al. 2001, Piertney et al. 2000).

Given the important contributions of mtDNA studies to understanding of gender-specific gene flow, it is clear that markers tracing gene flow in males would be of great value. In mammals, an obvious choice for a paternally inherited marker would be the Y chromosome, which is only passed from males to their sons. Sequences on the Y, as well as other markers, such as Y-linked microsatellites have contributed to the study of human gene flow and show promise in investigations of free-ranging mammals (Hurles and Jobling 2001). In one of the few wildlife studies published to date, sequences from the Y-chromosome were used to show there was little male mediated gene flow between a recently colonized population of wolves and more established populations (Sundqvist et al. 2001). Identification of new microsatellite loci as well as other easy-to-assay markers on the Y chromosome would greatly enhance the ability to characterize gene flow by males in many mammals (Hurles and Jobling 2001, Sundqvist et al. 2001).

The process of studying male-specific markers in birds is more complicated because in heterogametic (ZW) gender identification, females are heterogametic. Thus, there are no paternally inherited genetic markers similar to the Y chromosome in mammals. Scribner et al. (2001) provide an example of how information from different types of molecular markers, together with theoretical models, can be used to estimate male and female specific gene flow in birds. In their study of spectacled eider (*Somateria fisheri*), they used mtDNA, a gender-linked Z-specific microsatellite locus, and biparentally inherited microsatellites to document large differences in gender-specific gene flow.

If gene flow is limited for many generations by natural barriers to dispersal, populations on opposite sides of the barrier can exhibit striking levels of differentiation. Fragmentation of habitat should, given sufficient time, also result in genetic differentiation among recently isolated populations, especially if sizes of populations inhabiting the habitat remnants are small. Leberg (1991) found that genetic differentiation among populations of wild turkeys (*Meleagris gallopavo*) stocked onto semi-isolated areas was much higher than in native populations in continuous habitat. Gene flow, assayed with microsatellites, was much lower in populations of Sitka deer (*Cervus nippon*) in areas of habitat fragmentation (Goodman et al. 2001). Similar associations between recent habitat fragmentation and low gene flow have been made in the Greater rhea (*Rhea americana*), bank voles, collared lizards (*Crotaphytus collaris*), and moor frogs (*Rana arvalis*) (Gerlach and Musolf 2000, Bouzat 2001, Templeton et al. 2001, Vos et al. 2001). There is one caveat for most studies of the effects of recent habitat fragmentation on genetic differentiation; they lack temporal control. While it might be true that recent fragmentation of continuous habitat reduced gene flow, it is often difficult to eliminate the possibility that observed patterns of genetic differentiation are due to events that occurred long before any human activities affected the populations. Analyses of genetic differentiation before and after a fragmentation event should be possible given the ability to isolate DNA from speci-

mens preserved in museums (Roy et al. 1996, Mundy et al. 1997, Bouzat et al. 1998a, Iudica et al. 2001, Whitehouse and Harley 2001).

Gene Flow Through Translocations

One consequence of translocation programs is gene flow (Leberg 1990a). Sometimes this aspect of translocations is intentional. For example, many authors have explored the possibility of using translocations to restore gene flow between populations isolated by habitat loss (Triggs et al. 1989, Leberg 1990a, Haig et al. 1996, Moritz 1999, Storfer 1999). There has been little direct assessment of the genetic effects of such activities. Based on the relationship between gene flow and F_{ST} (Fig. 3), movement of only a few individuals per generation should be necessary to minimize the tendency of isolated populations to genetically differentiate. Mills and Allendorf (1996) provide an excellent review of factors to be considered when designing programs to restore genetic connectivity of populations. It might be necessary to release more individuals than genetic considerations might suggest, because released individuals often don't survive to reproduce (Leberg 1990b).

When translocations are used to establish new or augment existing populations, gene flow is expected to increase the similarity of populations at release sites to that of the population that served as the source of the released individuals. For example, Taylor et al. (1997) found that frequencies of mtDNA genotypes of recipient populations of koala were more similar to those of the source population than to other populations. These observations can be useful, because it is often difficult to know whether individuals released in augmentation efforts successfully contributed genetic material to the recipient population. This approach was used to assess the relative reproductive contributions of different subspecies to populations of northern bobwhite (*Colinus virginianus*) and wild turkey established by translocations from multiple sources (Nedbal et al. 1997, Mock et al. 2001).

The success of a translocation program can also be examined by assessing whether patterns of genetic similarity expected from natural dispersal have been disrupted (Leberg 1999). For example, Leberg et al. (1994) assessed whether genetic structure of wild turkey and white-tailed deer (*Odocoileus virginianus*) populations in the southeastern United States had been influenced by extensive translocations of these species. They found populations connected by translocations to be more genetically similar than populations that had not had individuals transferred between them. With similar data, Ellsworth et al. (1994a,b) concluded that releases of white-tailed deer had little effect on the genetics of native populations arguing that most translocated deer had not made genetic contributions to their recipient populations. Additional analysis led Leberg and Ellsworth (1999) to conclude that translocated individuals did contribute to the recovery of recipient populations; however, genetic contributions of the released individuals were restricted to the populations into which they were released. This set of studies illustrates the complexities of understanding the genetic and demographic consequences of translocations when only samples collected after the translocation event are available for analysis. Considerably more information about translocations could be discerned by obtaining genetic profiles of translocated

individuals prior to release so information could be used to track post-release reproductive success (Leberg 1999).

Drift and Bottlenecks

The genetic composition of a wildlife population is not constant. As a result of chance differences in reproductive success and survival among individuals with different genotypes, allele frequencies will change from one generation to another. Random change in the frequencies of alleles is referred to as genetic drift. The effect of drift on a population is expected to be small when population sizes are large. In large populations, small random changes in allele frequencies will occasionally cause an allele to be lost; however, this loss is mitigated by formation of new alleles through mutation. However, when populations are small and isolated from other populations, gene frequencies can drift dramatically. A population that is maintained at a small size for several generations has different genetic characteristics than it had prior to the reduction in size. Because of large random changes in allele frequencies, alleles will be lost in a small population faster than they are replaced through mutation, reducing allelic diversity. The average number of genes at which an individual is heterozygous (mean multi-locus heterozygosity) is also expected to decrease if a population remains small because matings between relatives will become unavoidable. Another consequence of drift associated with small population size is increased genetic differentiation. Genetic differences, based on neutral, molecular markers, between 2 populations will increase rapidly if there is no gene flow among them and at least one is small enough to experience substantial genetic drift.

When a normally large population goes through a constriction in size, it is referred to as a genetic bottleneck. During bottlenecks, drift is greatly accelerated. Bottlenecks can occur in wildlife populations for any reason that might result in a reduction in population size such as overharvest, disease, pollution exposure, or habitat loss (Leberg 1991, Ellegren et al. 1993, Van Hooft et al. 2000, Bouzat 2001, Van der Walt et al. 2001, Whitehouse and Harley 2001). Bottlenecks often occur at the establishment of a new population. This type of bottleneck is referred to as a founder event. Founder events are often severe bottlenecks, as only a few individuals may establish a population; however, they tend to be of short duration.

The duration and size of the bottleneck have large effects on loss of genetic diversity. During severe bottlenecks of short duration, theory (Nei et al. 1975) and experiments (Spencer et al. 2000) indicate many alleles will be lost. However, because most alleles are relatively rare in populations, there is no large loss of heterozygosity (Leberg 1992, Spencer et al. 2000). However, if the bottleneck is of long duration, relatedness of individuals will increase along with associated loss of heterozygosity (Nei et al. 1975). Thus, population growth rate can have a large effect on levels of genetic diversity following a reduction in population size.

Detecting Bottlenecks and Drift

A severe reduction in population size will lead to loss of heterozygosity, reduced allelic diversity, and drift of allele frequencies. Because pre-bottleneck samples are often absent, samples from populations that may have experi-

enced a bottleneck are often compared to populations of the same or related species that are believed to have levels of genetic variation not affected by small population sizes (Leberg 1991, Ellegren et al. 1993, Scribner 1993, Ardern and Lambert 1997, Pertoldi et al. 2000, Williams et al. 2000, Whitehouse and Harley 2001, Nichols et al. 2001). This approach requires the assumption the populations had similar levels of genetic variation prior to the putative bottleneck event (Bouzat 2000). Use of preserved materials would provide a more straightforward way to estimate pre-bottleneck levels of diversity. Matocq and Villablanca (2001) provide examples of the use of museum specimens to show that low genetic variation in endangered species was due to bottlenecks that occurred prior to a known recent reduction in population size. Conversely, museum specimens of greater prairie-chickens (*Tympanuchus cupido*) provided evidence that recent population reductions in Illinois resulted in reduced levels of genetic variation (Bouzat et al. 1998a). Unfortunately, sizes of museum collections from localities of interest are often insufficient to make strong statistical comparisons with contemporary populations. For populations that are likely to be of management concern, it would be appropriate to establish baseline genetic characteristics and preserve DNA samples for monitoring future changes in population size.

The commonly used genetic indices of bottlenecks differ in their sensitivity to population contractions. A series of experiments producing known bottlenecks indicate that loss of allelic diversity is much more sensitive to population bottlenecks than is heterozygosity (Leberg 1992, Spencer et al. 2000). This observation is supported by theory and studies of natural bottlenecks (Nei et al. 1975, Stockwell et al. 1996). Not surprisingly, it is easier to detect loss of alleles when using loci with many alleles, such as microsatellites, than with less polymorphic allozyme markers (Spencer et al. 2000). Both simulations and experiments indicate that temporal change in allele frequencies is also a much better index of bottleneck severity when drift is estimated with highly polymorphic loci (Richards and Leberg 1996, Luikart et al. 1999, Spencer et al. 2000). Although allelic richness is strongly influenced by past size of a population, this parameter is also sensitive to sample size. Thus, when comparing allelic richness among samples, estimates should be adjusted to the smallest sample size of any population used in comparison (Leberg 2002).

Several approaches have been developed to alleviate the need to compare a sample of interest to a reference sample to see if a population has experienced a loss of genetic variation. When a population experiences a bottleneck, the proportion of alleles that are present at low frequencies is reduced. Thus, it is possible to compare the distribution of alleles with different frequencies to the null distribution expected in the absence of a bottleneck; deviation from the null distribution is evidence that the population has experienced a contraction in size (Luikart et al. 1998). It is also possible to compare the heterozygosity in a population, based on Hardy-Weinberg expectations and the observed number of alleles, to the heterozygosity obtained from a null distribution (Cornuet and Luikart 1996, Luikart and Cornuet 1998). A similar approach examines the ratio of the number of alleles to the range of allele sizes of microsatellite loci (Garza and Williamson 2001); this ratio

decreases when a population is reduced in size. These approaches are dependent on selection of the correct model of mutation used to generate the null distributions. An examination of populations that had experienced known reductions in population size suggests they provide reasonable indices of a population's history of bottlenecks (Luikart and Cornuet 1998, Spencer et al. 2000).

When considering the effects of bottlenecks on genetic variation, it is critical to realize that not all population reductions will result in measurable losses of genetic variation. Population sizes often have to be quite small for several generations to produce a substantial loss of variation. Thus, a 90% reduction in size of a European rabbit population (*Oryctolagus cuniculus*) was insufficient to produce measurable genetic response, because the remnant population was not reduced below approximately 50 individuals and recovered rapidly (Queney et al. 2000). Likewise, experimental populations reduced to 16 individuals for one generation exhibited almost no loss of variation when they rapidly recovered to a large size (Spencer et al. 2000).

Effective vs. Census Population Size

One goal of conservation genetics is to understand how much genetic diversity would be lost from a population reduction or management activity. Genetic diversity is often lost more rapidly than would be predicted from the number of individuals in the population (referred to as the census population size or N_c). At times many individuals in a population are not reproductively active because of age or social constraints. Some individuals are vastly more successful than others in transmitting their genes to the next generation. When individuals differ in their ability to successfully reproduce, genetic diversity will be lost more rapidly than expected on the basis of N_c . One way of understanding these issues is to estimate the effective population size, N_e . N_e is the number of individuals in an ideal population that would lose genetic variation at the same rate as the actual population being studied. An ideal population is a one where all individuals have an equal chance of producing any progeny making up the next generation. The list of possible factors that can cause $N_e < N_c$ is large (Crow and Kimura 1970, Nunney 1999, Hedrick 2000). We discuss only those factors likely to have a large effect in wildlife populations with emphasis on those that might fall under the control of managers.

Temporal variation in population size can have large effects on loss of genetic variation (Crow and Kimura 1970, Vucetich et al. 1997), and may have strong influence on the effective size of wildlife populations (Frankham 1995a). A normally large population that occasionally experiences a large decline in numbers, may lose considerable genetic variation during those periods when it is small; this variation is not immediately recovered when the population returns to a large size. Kalinowski and Waples (2002) provide a framework for examining the relationship between N_e and N_c over multiple generations when population size is not stable.

Unequal sex ratios reduce effective population size (Wright 1931). If one gender is much more common than the other, members of the more rare gender will disproportionately contribute genes to the next generation. If sex ratios are highly skewed and the rare gender is only repre-

sented by a few individuals then $N_e \ll N_c$. In species with nonoverlapping generations, highly polygamous mating systems can also result in small estimates of N_e (Nunney 1993).

The age structure of a population can complicate efforts to estimate effective population size in wildlife species. Most wildlife populations have overlapping generations; simple formulations of the effects of sex ratio and temporal variation of effective size assume nonoverlapping generations. In some age-structured populations, fairly large numbers of individuals might be too young or too old to reproduce. To make the issue even more confusing, the influence of sex ratio and mating system on N_e are modified by generation length in complex ways (Nunney 1993). Equations developed for populations with nonoverlapping generations should be applied with caution when attempting to estimate effective size of wildlife populations.

There are several genetic techniques for estimating effective population size. One approach is to quantify genetic changes through time by taking 2 or more temporal samples (Waples 1989, Jorde and Ryman 1995). If insufficient time is available to obtain 2 samples separated by several generations, it might be possible to compare the genetic characteristics of contemporary populations with those of museum specimens (Bouzat et al. 1998a, Glenn et al. 1999, Rosenbaum et al. 2000b, Whitehouse and Harley 2001). Other approaches include assessing linkage disequilibrium between physically unlinked loci (Hill 1981, Bartley et al. 1992) and the amount of heterozygote excess in progeny (Pudovkin et al. 1996, Luikart and Cornuet 1999). Finally, there is a set of approaches based on expectations of patterns and levels of variation resulting from mutations (Slatkin and Hudson 1991, Hedrick 1996, Jensen-Seaman and Kidd 2001). Such approaches require knowledge of mutational processes and are more appropriate for estimates of effective population size over longer time periods than those that are the focus of management. In addition to genetic approaches, demographic data can be used to estimate N_e (Harris and Allendorf 1989, Nunney 1993). When using any of these approaches, it is important to realize that "effective size" can refer to several population genetic parameters and, thus, measure loss of different components of genetic diversity (see Crow and Kimura 1970, Schwartz et al. 1998).

Drift and Bottlenecks From Human Activities

Reduced levels of genetic variation have been documented in large numbers of threatened species or populations (Arden and Lambert 1997, Hoelzel et al. 1999, Rossiter et al. 2000). Reductions of genetic diversity are often symptomatic of small populations that have become endangered through loss of habitat and other causes.

Even in abundant species, individual populations can lose genetic variation when they become isolated in fragments of habitat incapable of supporting large populations (Sarre 1995, Haavie et al. 2000, Pertoldi et al. 2000, Bouzat 2001, Goodman et al. 2001, Lee et al. 2001). Creation of corridors between these fragments or the imposition of gene flow through translocations have been suggested as strategies for prevention of loss of diversity within fragmented populations (Hedrick 1995, Mills and Allendorf 1996, Mech and Hallett 2001).

By definition, reintroduction programs create founder

events. Populations established through releases will often have less genetic diversity than those that are the source of released individuals; this loss is related to the number of individuals released (Grobler and Van de Bank 1994, Tarr et al. 1998, Le Page et al. 2000, Stockwell and Leberg 2002). For example, Fitzsimmons et al. (1997) found that populations established with translocated mountain sheep (*Ovis canadensis*) often had low levels of genetic diversity compared to the source of the released individuals. These releases were often between 8 and 69 individuals per population. In releases that involved large numbers of eggs, Rowe et al. (1998) found no loss of variation in populations of Natterjack toad (*Bufo calamita*) created through translocations. Scribner and Stuwe (1994) found the amount of genetic drift experienced by populations of Alpine ibex (*Capra ibex*) was related to the number and sex ratio of individuals used to establish a population as well as by subsequent population growth. Slow population growth following translocation appears to be responsible for a loss of heterozygosity in a population of elk (Williams et al. 2002).

Not surprisingly, allele frequencies of translocated populations often differ from those of their sources (Scribner 1993, Fitzsimmons et al. 1997, Rowe et al. 1998, Tarr et al. 1998). Founder events associated with translocations can also create large differences among translocated populations from the same sources (Leberg 1991, Scribner 1993, Tarr et al. 1998). However, caution should be used when interpreting differences in allele frequencies among translocated populations and their sources. While differences might be the result of the founder event, they could also have occurred through drift after the translocated population became established (Williams et al. 2000).

Reintroduction strategies that may make sense based on the species' ecology might have the unintended consequence of reducing the effective population size of the newly established population (Leberg 1990b). The benefits of such strategies, such as faster initial population growth by releasing more females than males, or of reduced dispersal through release of family groups, should be evaluated in light of their genetic consequences. For example, if it makes sense to release family groups to reduce post-release dispersal, it would be best to release as many groups as possible to avoid inbreeding and loss of genetic variation.

Most harvest programs should have little effect on genetic variation because loss of variation due to drift is small when population size is large. However, harvest strategies can reduce effective population size far below the census population size, creating the potential for rates of drift that might be surprising if only the total population size is considered (Laikre and Ryman 1996). Ryman et al. (1981) evaluated a range of harvest strategies for moose (*Alces alces*) and found the largest relative differences between N_e and N_c occurred when harvest mortality was higher for adults than for calves and when harvests skewed sex ratios. Harris et al. (2002) reviewed the possible effects of game harvests on genetic diversity.

Selection

Many of the genetic markers used by conservation geneticists are thought to be selectively neutral. Thus, the specific genotypes associated with these marker systems

have little or no effect on the survival or reproduction of individuals. This assumption is most likely violated from time to time but most of the genetic variation examined using many types of markers probably has little consequence for the fitness of individuals (Hedrick 2000). Because marker systems are unlikely to be under direct selection, they are useful for measuring phenomena such as gene flow, inbreeding, and drift that tend to affect variation throughout the genome and, thus, result in genetic signatures that are detectable with molecular markers.

Although the neutrality of molecular markers aids in their usefulness for studying many population processes, it also means the linkage of molecular markers and genetic traits of concern to the well being of individual organisms is at best indirect. The relationship between levels of molecular variation within and among populations with genetic variation affecting traits related to individual fitness may often be weak (Morgan et al. 2001, Reed and Frankham 2001). The lack of direct concordance often observed between patterns of variation at molecular markers and complex traits has led to calls for conservation geneticists to more critically evaluate whether molecular data are sufficient for designating conservation priorities (Pearman 2001, Reed and Frankham 2001).

If a specific trait such as body size, disease resistance, or fecundity was the target of management activities; it is unlikely that assays of genetic variation with molecular markers would be informative. At times the distributions of traits of adaptive importance correspond with patterns of other genetic markers; however, strong selection on a trait can obscure the influences of gene flow and drift measured with neutral markers. One exception to the general disconnect between studies of molecular markers and variation in fitness traits is the recent application of large numbers of markers to identify specific genes, referred to as Quantitative Trait Loci (or QTLs), responsible for variation in complex morphological or behavioral traits. The work of Slate et al. (2002) identifying QTLs for birth weight in red deer (*Cervus elaphus*) may be the first application of this approach in an unmanipulated population of a wildlife species.

In spite of the general assumption that much of the variation characterized by molecular markers is neutral, there is a large body of work attempting to understand the role of natural selection in maintaining variation in molecular markers in wildlife populations. Initial surveys of natural populations detected higher levels of allozyme variation than expected. This generated interest in examining whether individuals that were heterozygous for allozyme loci might have high fitness; such selection would promote high levels of variation (Allendorf and Leary 1986).

Many studies have found that individuals that are heterozygous for one or more allozyme loci have traits that might enhance fitness such as high growth, increased survival and fecundity, or developmental stability (Allendorf and Leary 1986, Reed and Frankham 2003). Examples of fitness-related traits of wildlife associated with specific allozyme genotypes at single or with multiple locus heterozygosity include spur length in wild turkeys (Leberg 1994), horn growth in mountain sheep (Fitzsimmons et al. 1995), developmental stability in the brown hare (*Lepus europaeus*) (Hartl et al. 1995a) and survival in marbled salamanders (*Ambystoma opacum*) and red deer

(Pemberton et al. 1988, Chazal et al. 1996). In white-tailed deer, single or multiple locus heterozygosity has been associated with many traits (Smith et al. 2001b) including fetal and adult growth (Cothran et al. 1983), fat accumulation (Cothran et al. 1987), and antler size (Scribner et al. 1989). However, there have also been studies that found no relationship between heterozygosity and traits related to fitness (Britten 1996). In red deer, antler growth was actually lower in heterozygotes for some allozymes (Hartl et al. 1995b). Furthermore, there is little direct evidence that it is the allozyme loci under examination that are producing variation in fitness components. The allozymes might be physically linked, through proximity on chromosomes, to genes producing the effect, or alternatively, high heterozygosity might indicate that an individual's parents were not closely related (Leberg et al. 1990). Arguments can be found to favor or discredit any explanations of allozyme heterozygosity-fitness correlations.

Understanding relationships between heterozygosity and fitness is being enhanced by examining similar relationships using molecular markers that are probably not under selection. Associations between fitness traits and several indices of heterozygosity or outbreeding, based on microsatellite loci, have been detected for several wildlife species (Coulson et al. 1998, Slate et al. 2000, Hansson et al. 2001, Hoglund et al. 2002, Slate and Pemberton 2002). For example, survival was positively associated with a measure of outbreeding in greater horseshoe bats (*Rhinolophus ferrumequinum*) (Rossiter et al. 2001). Because microsatellites are typically found in regions of the genome that are not transcribed, it seems likely that heterozygosity-fitness correlations are not due to these loci. It is more likely that such associations reflect the relatedness of an individual's parents or the physical proximity of assayed microsatellites to other loci affecting the traits of interest.

Recently there has been considerable interest in examining relationships between individual viability and loci in the major histocompatibility complex. These genes are involved in immune responses and there is some evidence that selection maintains variation within populations (Hughes 1991, Hughes and Yeager 1998, Richman et al. 2001). For example, Ditchkoff et al. (2001) found that specific genotypes of the major histocompatibility complex were associated with antler development, body mass, and serum testosterone in white-tailed deer. It is possible that such associations are due to variation in pathogen resistance of different major histocompatibility complex genotypes. Studies have also suggested the major histocompatibility complex might influence mate choice in mammals (Potts et al. 1991, Brown 1998, Penn 2002).

Although examination of correlations between genotypes at molecular markers and traits related to individual fitness has been a focus of wildlife genetics, there have been few attempts to apply knowledge in this area directly to management. Any program designed to increase abundance of certain genotypes would be difficult to implement in a natural setting and might be ill advised. While it has been argued that breeding programs in captive populations should emphasize maintenance of allozyme or major histocompatibility complex diversity because these loci may influence individual survival or fecundity (Wayne et al. 1986, Hughes 1991), selective breeding schemes to favor

variation at a few molecular markers could result in an increase in the rate of loss of genetic variation at all loci (Hedrick et al. 1986, Vrijenhoek and Leberg 1991, Miller 1995, Lacy 2000). Because there is a poor understanding of how different genes interact to affect individual well being, most captive breeding programs advocate maintenance of overall genetic variation and reduction of relatedness. Models have also shown that selection of individuals, on the basis of marker genotype, to be used in reintroduction programs can result in an overall reduction in genetic variation in newly established populations (Haig et al. 1990).

Genetic Diversity and Population Viability

Observations of inbreeding depression in captive (Lacy et al. 1996) and field populations (Jimenez et al. 1994, Keller et al. 1994, Keller and Waller 2002), and studies of heterozygosity-fitness relationships (Reed and Frankham 2003) have led to the realization that loss of genetic variation could affect population viability (Gilpin and Soulé 1986, Lacy 1997). Simulation models (Mills and Smouse 1994, Robert et al. 2002) and laboratory studies (Ayala 1968, Spielman and Frankham 1992, Frankham 1999, Reed and Bryant 2000) have demonstrated decreased population growth and increased extinction rates with loss of genetic variation. Furthermore, observations of wildlife populations that have experienced loss of genetic variation due to bottlenecks also support the conclusion that such losses can affect population productivity (Bouzat et al. 1998b).

Practices that lead to reduced genetic variation, such as establishing populations with only a few individuals or allowing populations to remain small and fragmented, might have serious consequences for population viability. These concerns about effects of inbreeding on demography occur on a time scale relevant to management activities (e.g., Westemeier et al. 1998). On a longer time scale, managers must be concerned about loss of allelic variation that can affect the ability of populations to adapt to new environmental challenges (Allendorf and Leary 1986, Frankham 1995b).

Most conservation geneticists promote maintaining large effective sizes of populations to prevent loss of genetic variation and possible associated reductions in population viability. Recommendations concerning population sizes necessary to prevent adverse genetic consequences vary considerably; there is no general agreement on what appropriate minimum numbers are acceptable for long-term management goals (Gilpin and Soulé 1986, Simberloff 1988, Hedrick and Kalinowski 2000, Reed and Bryant 2000). Most published recommendations of minimum population size are in terms of minimum effective size; the number of breeding age individuals in most populations should be at least twice as large. Maintaining large populations can be especially difficult in captive breeding programs and several strategies have been developed to maintain genetic diversity in captivity.

The relationship between loss of genetic diversity and population viability is not as straightforward as the discussion above might suggest. A population with a history of inbreeding might suffer from future inbreeding less than other populations (Fu et al. 1998), and inbreeding depression may be influenced by environmental conditions

(Bijlsma et al. 1999). Furthermore, matings of individuals from genetically differentiated populations, as might occur through translocation, could under some circumstances increase genetic variation in a population while causing a decrease in individual viability (Templeton 1986, Leberg 1993). Additionally, other mechanisms beside inbreeding and the loss of genetic variants, such as slow accumulation of mutations with slight deleterious effects, may affect the long-term consequences for small populations (Lande 1995). Reviews of the mechanisms through which genetic diversity can affect population viability can be found in Soule (1986) and Frankham et al. (2002).

USING DNA AS AN INDIVIDUAL MARKER

Estimating Population Size/Survival

Wildlife biologists are often interested in estimating population size and survival rates of individuals within a population. Traditional wildlife studies typically use some mark and recapture methods to reach these goals. The methodologies behind these techniques have been well established (Jolly 1965, Seber 1965, Otis et al. 1978, Nichols et al. 1981, Pollock 1981, White et al. 1982, Lebreton et al. 1992) and are widely used (Lancia et al. 2004). These techniques involve capturing a sample of the population, giving each animal a unique tag and then releasing the animal into the population. In subsequent time periods, additional samples are taken and the proportion of the population that is marked is estimated and used to estimate population size and survival. Sophisticated modeling of capture probabilities and model selection techniques exist and are widely used.

While traditional mark and recapture techniques work well for many species, there are others for which this type of analysis does not work well. Species that are dangerous and expensive to catch (e.g., grizzly bears, *Ursus arctos horribilis*) and those that are highly elusive do not lend themselves to conventional mark and recapture techniques. Because DNA can be obtained from hair (Foran et al. 1997, Taberlet et al. 1997, Woods et al. 1999, Sloane et al. 2000), feathers (Ellegren 1991), feces (Reed et al. 1997, Flagstad et al. 1999, Ernest et al. 2000), and even frozen urine (Valiere and Taberlet 2000) biologists have non-invasive ways (through hair snags or from collecting feathers, feces, or frozen urine) to sample individuals that have been difficult to sample in the past. Because each individual animal has a unique molecular fingerprint that remains with them throughout their lifetime, it is reasonable to believe this fingerprint (or a portion of it) may be used in an analogous way to the unique "mark" assigned by a biologist in a traditional mark and recapture study. As a result, biologists are beginning to use molecular approaches to estimate population size.

For mark and recapture methods to work using DNA as an unique "mark", molecular biologists need to use a genetic marker (or series of markers) that are variable enough that no 2 individuals will have the same 'molecular tag'. This means that an adequate number of molecular markers that are sufficiently polymorphic need to be used. These markers need to be biparentally inherited, dominant, and highly polymorphic. Microsatellites are currently the best marker for this application; with each individual's "molecular tag" based on its genotype for a

number of highly polymorphic microsatellite loci. Once sufficient microsatellite loci are examined to uniquely identify an individual, traditional mark and recapture methods can be used. Using DNA to identify individuals, scientists have been able to estimate population size of humpback whales (*Megaptera novaeangliae*) (Palsbøll et al. 1997), mountain lions (*Puma concolor*) (Ernest et al. 2000), coyotes (Kohn et al. 1999), and Pyrenean brown bears (*U. a. pyrenensis*) (Taberlet et al. 1997) although in most cases more sophisticated mark and recapture methodologies could have been used. Currently, use of DNA as a unique molecular tag is being added to traditional methodologies and is being integrated into the mark and recapture software (K. P. Burnham, personal communication).

This technique has excellent potential for estimating population size (and potentially survival rates) of species that are difficult to trap. However, it has several limitations. The first is the quantity and quality of DNA that is extracted from hair, feathers, feces, and frozen urine. Typically, only small amounts of DNA (often in the range of picograms) can be extracted from such samples (Taberlet et al. 1999). The DNA that is successfully extracted often is degraded and chopped into smaller pieces and may contain PCR inhibitors. With low quantity DNA, contamination becomes a serious issue, as does a phenomenon known as allelic dropout (Taberlet et al. 1999). Allelic dropout occurs when pipetting small amounts of DNA and only 1 of 2 alleles of template DNA gets amplified by PCR. The consequences are that only one allele of a heterozygous genotype is amplified resulting in incorrect assignment of that individual as a homozygote instead of a heterozygote. Low quality DNA (severed into many short fragments) is undesirable because it becomes difficult to amplify a microsatellite allele if the template DNA of a certain microsatellite is severed in that region. Failure to address possible genotyping errors can result in large overestimates of population size (Waits and Leberg 2000). These issues can be handled by using strict extraction protocols to avoid contamination, adopting a multi-tube approach to prevent allelic dropout, and using only short microsatellite loci to avoid problems with degraded DNA (Taberlet et al. 1999).

The second issue deals with the assumption the method used can uniquely identify individuals. For this type of analysis, a sufficient number of highly polymorphic microsatellite loci are needed so that no 2 individuals will share the same molecular tag. The difficulty lies in knowing how many microsatellite loci are sufficient to assure unique molecular tags (Mills et al. 2000b, Waits and Leberg 2000). Using simulations, Waits and Leberg (2000) demonstrated that population estimates tended to be biased downward as the probability that 2 or more individuals shared a molecular tag increased. Mills et al. (2000b) found similar results using Lincoln-Peterson estimators and multiple-recapture estimators in program CAPTURE. The probability of sampling identical genotypes can be estimated using theoretical equations (Paetkau and Strobeck 1994) although these estimators assume random associations between alleles within and among loci, which is likely inaccurate for populations with substructure (Waits et al. 2001). Waits et al. (2001) introduced a probability of identity estimator that can be used as a conservative upper bound for the probability of observing identical

genotypes for multiple individuals that may be related from the same population. Application of molecular genetics to mark and recapture methodology is relatively new. While methodologies and logistics of this application are still being examined, it is believed that it will become a widely applied and accepted use of molecular genetics in wildlife studies.

Tracking Individual Movements

Because individuals can be identified with highly polymorphic markers and sampled through collections of scat or hair, it is possible to obtain information concerning their movements (Kohn and Wayne 1997). In these studies, movement data are obtained by "recapturing" individuals as a result of multiple collections of their DNA at different locations and times. This method has been applied to a number of mammalian carnivores (Taberlet et al. 1997, Kohn et al. 1999, Ernest et al. 2000, Lucchini et al. 2002). Information obtained is often limited by sampling protocols; if sampling is confined to roads or paths, an incomplete picture of an individual's use of space will be obtained. Use of specially trained dogs to find scat provides one approach for detecting scat in areas off roads and paths (Smith et al. 2001a). Using DNA from skin samples, Palsbøll et al. (1997) studied long distance migration of individual humpback whales.

At times it is not necessary to identify "recaptured" individuals to obtain information on movements. If breeding populations differ in genetic composition it is possible to identify the origin of dispersing or migrating individuals. Genetic stock identification allows estimates of the proportion of a sample of individuals that originated from different source populations (Smouse et al. 1990, Xu et al. 1994, Pearce et al. 2000). Assignment tests estimate the probability that a specific individual was a member of the different source populations in the sample (Cornuet et al. 1999, Manel et al. 2002). Variations on these approaches have been used to gain insight into migratory patterns of Canada geese (*Branta canadensis*) (Pearce et al. 2000), noctule bats (*Nyctalus noctula*) (Petit and Mayer 2000), and shorebirds (Haig 1998, Wennerberg 2001). Stock identification has proven useful in assigning samples of loggerhead turtles (*Caretta caretta*) collected in foraging areas to their nesting beaches (Bowen et al. 1995, Bass and Witzell 2000), and by identifying which populations are most affected by incidental captures associated with commercial fisheries (Laurent et al. 1998). Genetic tests have been used to identify which of several populations of wolves and rock wallabies (*Petrogale lateralis*) was the source of dispersing individuals that established new populations (Eldridge et al. 2001, Lucchini et al. 2002). In another example, Blanchong et al. (2002) were able to ascertain whether individual white-tailed deer were likely to have been harvested from a specific management unit. Assignment tests might also improve the ability to detect poaching when populations are governed by different harvest regulations (Manel et al. 2002). Precise estimates from either technique require the genetic composition of possible source populations are well characterized by a large number of genetic markers and individuals; sampling requirements decrease as genetic differences among populations increase. Although stock identification and assignment tests can be powerful, levels of genetic differ-

entiation in many species, such as northern pintails (*Anas acuta*) and double-crested cormorants (*Phalacrocorax auritus*) (Cronin et al. 1996, Waits et al. 2003), is sufficiently small making identification of natal or source populations impractical.

Species Identification

Wildlife biologists often find “signs” of wildlife such as feces, tufts of hair, feathers, blood, and even frozen urine and need to know what species (or individual of a known species) left that sign. This is particularly important for programs monitoring status of regulated or protected species. DNA can be extracted from these materials and either sequenced or used in a fragment analysis (such as microsatellites) to identify which species left the “sign.” If a species has uniquely identifiable populations or regions, this technique may also be applied to identify to which population or region a sample belongs.

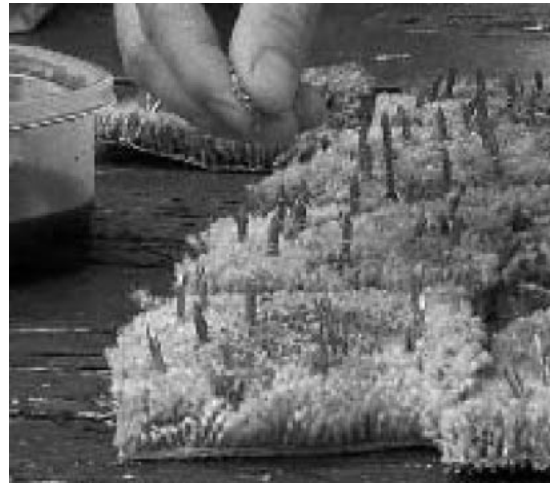
To perform molecular species identification, one must first find an area in the genome (usually in the mitochondrial genome) that is not polymorphic between members of the same species but is polymorphic among members of different species. Typically, there will be certain areas within the sequence that are diagnostic of particular species. To differentiate martin (*Martes americana*), fisher (*M. pennanti*), wolverine (*Gulo gulo*), and lynx (*Lynx canadensis*), for example, Foran et al. (1997) compared DNA sequences (approximately 600 base pairs) of the cytochrome b and D-loop region of the mitochondrial genome from the 4 different species. After amplifying the 600 base pair region, they cut that region with a series of restriction enzymes, which resulted in a variable restriction pattern that was diagnostic to each species (Foran et al. 1997). Valiere and Taberlet (2000) were able to distinguish different canid species using frozen urine. Similarly, Ernest et al. (2000) used a panel of 12 microsatellite loci to distinguish mountain lion and bobcat (*Lynx rufus*) DNA from feces. This type of analysis can be done at the population level if there is a diagnostic region that defines different populations. A further application of this approach to a management issue is the National Canada Lynx Survey (Box 3).

Dietary Analysis

Molecular probes can be used to examine food habits in the absence of recognizable remnants of plant and animal parts such as hair or seeds (Symondson 2002). Possible sources of dietary information useful for such analyses include stomach contents, mammalian scat, and bird regurgitant (Symondson 2002). For example, Scribner and Bowman (1998) used microsatellite analysis to distinguish between several species of juvenile waterfowl in stomachs of glaucous gulls (*Larus hyperboreus*). Taberlet and Fumagalli (1996) demonstrated that it was possible to identify the species of bones found in owl pellets. In such an analyses, care must be used to select genetic markers with an appropriate level of resolution. If markers only work on a small number of species, some prey will not be identified. However, using approaches that can identify a wide range of species might also detect non-dietary items. For example, while attempting to identify large felids from scat, Farrell et al. (2000) detected dipterian DNA that could be the result of flies visiting the feces. Molecular

Box 3. Documenting the presence of lynx using molecular techniques.

When lynx populations declined in the contiguous United States, the federal government implemented a survey based partially on DNA approaches. The survey was designed to learn where lynx did or did not occur. Across the potential range of the species south of Canada, transects were established and hair snares, designed to snag samples of hair, and attractant were used to collect samples (McDaniel et al. 2000). The technique of Foran et al. (1997) could not be used because it required amplification of a long fragment of DNA (~900 bp) that could not be amplified using degraded DNA from hair samples. Instead, a shorter fragment was used and sequences of that fragment from hairs were amplified with PCR. Restriction enzymes were then used to create DNA fragments and hairs of lynx were distinguished from other samples by banding patterns (Mills et al. 2000a).



Baiting a hair snare with catnip.

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approaches allow not only the analysis of contemporary scat samples, but also from prehistoric materials such as coprolites of the extinct ground sloth (*Nothrotheriops shastensis*) (Hofreiter et al. 2000). Symondson (2002) reviewed several recent studies of dietary analysis of mammal and bird excrement; considerable effort and expense would be required to apply this approach to a large number of samples.

CAPTIVE BREEDING PROGRAMS

Pedigree Analysis

When populations decline drastically and only a small number of individuals remain, biologists often capture some of the remaining individuals in attempts to establish a captive population. These animals are bred to expand the captive population so individuals can be released into the wild. It has been shown that sound management of the

genetic aspects of breeding programs is needed to be successful (Ralls and Ballou 1986, Foose and Ballou 1988, Hedrick and Miller 1992). Because number of individuals brought into captivity is usually small, inbreeding is a serious problem. Thus, it is important to consider the genetic identity of animals bred in captivity so that net genetic variability is maximized and inbreeding is minimized. After several generations are mated, pedigrees are developed to document matings from all previous generations.

The methodology for maximizing genetic diversity and minimizing mean kinship in captive bred populations is well established (Ballou and Lacy 1995). There are other methods of quantifying genetic structure in pedigrees such as founder equivalents (Hedrick and Miller 1994), although minimizing mean kinship appears to be superior (Miller 1995). Some biologists have suggested that instead of maximizing genetic variation at the genome level, selective breeding of individuals with certain alleles at specific loci (e.g., those containing alleles with particular selective benefits) should be implemented (Allendorf 1986, Hughes 1991). It has been advocated (Hughes 1991) that breeding programs maximize diversity at the Major Histocompatibility Complex. Others believe that by selecting for rare alleles at a particular locus, the overall genomic diversity is reduced more quickly (Gilpin and Wills 1991, Miller and Hedrick 1991, Vrijenhoek and Leberg 1991). Miller (1995) used Przewalski's horse (*Equus caballus*) and California condor (*Gymnogyps californianus*) pedigrees to evaluate using "rare kinship" as a management strategy. He found this strategy increased population mean kinship and reduced gene diversity compared to a "no management" control and was inferior to a strategy based on minimizing mean kinship (Miller 1995).

The founding individuals are assumed to be unrelated in most captive breeding programs (Lacy et al. 1995). All subsequent analyses calculate relatedness as a measure of common ancestry relative to the founding population (Lacy 1995). It is important to understand the relatedness of the founding individuals to maximize the remaining genetic diversity and minimize inbreeding in captive breeding programs. Molecular genetic techniques are an effective method to assess relatedness of founders of captive breeding programs. The relatedness of founders of several captive bred species has been examined using multilocus DNA fingerprinting (Brock and White 1992; Geyer et al. 1993; Haig et al. 1994, 1995). More recently, microsatellite analyses have proven to be valuable for inferring relatedness (Queller et al. 1993, Haig 1998) and promise to be useful for examining relatedness of founders. Jones et al. (2002) used microsatellite data to augment wild and captive pedigree information on whooping cranes (*Grus americana*). Microsatellite data revealed unknown shared genotype information for founders and allowed them to develop a pedigree based on DNA that will help in the genetic management of the species.

MATING SYSTEMS

Study of mating systems is not new and has been broadly characterized in many species. Biologists have been interested not only in the type of mating system (monogamy, polygamy, polyandry, etc.) but also in how such systems evolved. This often involves examination of

parentage and mating success. Historically, mating systems have been studied through direct behavioral observations (Young 2004). Biologists spend hours observing mating rituals and copulations to characterize different mating behavior. This characterization is particularly difficult where females mate with more than one male outside a socially bonded pair, and where females store sperm for long periods of time making it difficult to discern the true biological father. As a result, true patterns of gametic exchange may differ substantially from the apparent mating systems characterized by behavioral observations. Inaccurate estimation of parentage can be a major obstacle in understanding selection and evolution of mating systems (Mock 1983, Gyllensten et al. 1990). An accurate documentation of reproductive success reduces the possibility of inaccurately equating mating prowess or other reproductive features with actual transfer of genes across generations (Avice 1994). Because effective population size, N_e relies heavily on an accurate depiction of the mating system (Chesser 1991, Nunney 1993), confirmation of mating systems using genetic techniques is crucial.

Maternity and Paternity Analysis

With advent of more advanced molecular genetic techniques, it has become possible to accurately assign parentage. This allows biologists to accurately assess mating success and to re-evaluate characterizations of mating systems. Many different molecular techniques can be used to assess different questions of parentage. Quinn et al. (1987, 1989) used Restriction Fragment Length Polymorphisms to examine whether snow goose goslings in a brood were the offspring of the nest attendants and apparent parents. They documented intraspecific brood parasitism where not all goslings in the brood had the same mother, likely extra-pair fertilization where not all goslings had the same father, and where neither nest attendant was the parent. Burke et al. (1989) used multilocus fingerprinting to show that dunnoek sparrow (*Prunella modularis*) broods could have multiple fathers. Previously, Kemp's ridley sea turtles were thought to have little or no multiple paternity. Kichler et al. (1999) used microsatellite analysis to show they are polyandrous. Richardson and Burke (1999) used single-locus minisatellite DNA profiling to examine the relationship between the age and instance of extra-pair fertilization in male Bullock's orioles (*Icterus bullockii*). Amplified Fragment Length Polymorphisms were used by Questiau et al. (1999) to document frequency of extra-pair parentage in a population of bluethroat (*Luscinia svecica*).

Mating Success

Molecular genetic techniques simplify studies of mating success of males and social organization. Murphy (1998) used microsatellite analysis to investigate correlations between phenotypic and behavioral traits and reproductive success in mountain lions in the Northern Yellowstone ecosystem (Box 4). Pemberton et al. (1992) were able to assess the mating success of red deer using DNA fingerprinting. They found that behavioral methods used to estimate male reproductive success underestimated true success of successful males and overestimated success of unsuccessful males. Similarly, Semple et al. (2001) showed that sage-grouse broods had multiple fathers suggesting that dominant males may not be as reproductively

Box 4. Documentation of reproductive success of mountain lions using paternity analysis.

Reproductive success is important in an evolutionary sense because it relates an individual's phenotypic traits to overall fitness. Variation in reproductive success is typically associated with variation in traits such as differences in age, mate access, survival of young, and longevity of adults. Alternatively, reproductive success may be the result of environmental variation or purely by chance. Murphy (1998) examined variation in reproductive success of male and female mountain lions in the Northern Yellowstone Ecosystem and its relation to spacing patterns and mating systems.

Because male mountain lions spend little or no time with young, it is difficult to identify the father of litters without extensive monitoring of male behavior. Therefore, Murphy (1998) used a panel of 18 polymorphic microsatellite loci to identify the paternity of 23 litters. He used the number of young produced by females and the number of litters fathered by males to describe short-term variation in reproductive success for mountain lions. He then tested whether phenotypic and behavioral traits were correlated with reproductive success. Murphy (1998) found that a single male sired each litter and that reproductive success was positively correlated with age of the male and the number of females in his territory. Neither age of the female nor the size of the male territory was correlated with reproductive success. This study confirmed that the mating system of mountain lions is classically polygynous.



Mountain Lion.

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successful as once thought. The complex social structure of the cooperatively breeding acorn woodpecker (*Melanerpes formicivorus*) has been clarified (Haycock et al. 2001) and, similarly, insight into the social organization of the brown long-eared bat (*Plecotus auritus*) has been established (Burland et al. 2001).

GENDER IDENTIFICATION

Wildlife biologists studying animals in the field typically need to know the gender of individuals to examine differences between males and females. For example, studies of population dynamics often compare survival rates between males and females. In sexually dimorphic wildlife species it is straightforward to differentiate males from females. However, for some species it is difficult to accurately assign gender to an individual without invasive procedures. The same problem arises with gender identification from wildlife "signs" such as feces, urine, feathers, or hair. Molecular genetic techniques can be used on a variety of different species to assign gender to individuals using only a small sample (e.g., blood, feathers, feces, urine, hair). Forensic scientists can use DNA-based identification approaches when gender of a tissue sample or bloodstain might indicate a violation of wildlife harvest regulations (Gilson et al. 1998, Wilson and White 1998).

Mammals

Gender can be identified from DNA samples for many groups of mammals including wombats, rabbits, ungulates, carnivores, seals, primates, and whales (Aasen and Medrano 1990, Griffiths and Tiwari 1993, Reed et al. 1997, Taberlet et al. 1997, Sloane et al. 2000, Wallner et al. 2001, Ensminger and Hoffman 2002, Huber et al. 2002). Most protocols call for detection of genetic markers, such as the SRY locus, that is associated with the Y chromosome to identify males. If the marker is not detected the sample is assumed to be a female. However, because degraded DNA or inhibitory compounds found in some samples can prevent detection of a locus (Kohn and Wayne 1997), it is necessary to have controls with other markers to verify there is nothing about the sample that would prevent correct gender identification (Taberlet et al. 1997, Wilson and White 1998). Generally, genetic methods of gender identification have proven to be quite reliable for mammals. However, an approach that works for one set of species, might not work for others (Ensminger and Hoffman 2002). Thus, the reliability of any protocol should be verified with samples where the gender is known. Care must also be taken when using DNA markers from scat to identify the gender of carnivores. Ernest et al. (2000) found that scat from 3 of 4 female mountain lions contained male genotypes. They hypothesized the male genotype might be the result of DNA from male prey since the SRY marker is not species-specific.

Birds

Gender of birds is typically difficult to assign as the majority of the world's bird species have males that look identical to females (Griffiths et al. 1998). To address this issue, Griffiths et al. (1998) designed primers around homologous regions in the chromo-helicase-DNA-binding (CHD) gene on sex chromosomes W and Z in birds. This technique takes advantage of the fact that chromosomes W and Z evolve at different rates. Homologous regions on sex chromosomes typically are different sizes due to mutations involving insertions and deletions of DNA nucleotides. Their method simultaneously amplifies homologous regions on the W and Z chromosome followed by a restriction digest, which allows for differentia-

tion of males (ZZ-1 band) and females (ZW-2 bands) in many species of birds with the possible exception of Struthioniformes. Ellegren (1996) developed PCR primers for collared flycatchers (*Ficedula albicollis*) within the CHD gene that resulted in gender identification of closely related species without the restriction digest step. Kahn et al. (1998) designed a different set of primers in a more conserved region of the CHD gene that works in most avian species. Bello and Sanchez (1999) further modified this technique to allow for gender identification in ostriches (*Struthio camelus*). This technique has been used to identify gender of many species including mountain plover (*Charadrius montanus*) (Dinsmore et al. 2000) using feathers and kakapo (*Strigops habroptilus*) from feces (Robertson et al. 1999).

SUMMARY

Molecular genetic techniques represent a relatively new and powerful set of tools that can address both research and management issues in wildlife science. These approaches have shown their utility in wildlife management by helping identify species and appropriate units for conservation. Knowledge gained about the factors affecting distribution and loss of genetic variants has led to refinements in population management such as maintaining effective population sizes and connectivity between reserves. More recently, the introduction of PCR has allowed noninvasive collection of genetic material from a variety of sources such as hair, feathers, and feces. Together with the ability to examine highly polymorphic loci and gender-specific markers, noninvasive sampling has allowed genetic assays to contribute to ecological studies of sex ratios, food habits, population size, and mating systems. In this chapter we provide general theory of population genetics and have identified those techniques and applications currently used in wildlife studies. This body of literature is expanding rapidly and readers are referred to more detailed accounts of population genetic theory, techniques, and applications. With rapid development of DNA-based technologies, it is likely that currently unforeseen applications of genetic approaches will soon be available to assist wildlife scientists addressing a wide variety of problems.

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