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Conservation Genetics and Molecular Ecology 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 that 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 versus 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 or 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, whereas 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 that flank both sides of the targeted region of DNA. Once amplified to large quantities, the targeted region (usually between 100 and 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 22.1). More detailed and excellent 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, DeYoung and Honeycutt 2005).

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 applied, these proteins migrate differentially through a matrix based on their charge, 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 that, in turn, cause changes in the shape, net charge, and migration rate of proteins. Such changes can be revealed through electrophoresis and provide information showing variability among individuals, populations, or species. Although 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 necessary 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 or destructive sampling and is logistically difficult to manage in field situations.

Table 22.1. Applicability of common types of molecular markers for wildlife biologists. The number of Xs indicates the relative applicability (fair, good, very good, and excellent) of each technique to a specific question.

Type of marker[a]	Taxonomic delineations	Regional or subspecific population structure	Genetic diversity and subpopulation structure	Individual identity and paternity/maternity analysis
Allozymes	XXX	XXX	XXX	X
MtDNA sequences	XXXX	XXXX	XX	X
Microsatellites	X	XX	XXXX	XXXX
Minisatellites	X	X	XX	XXXX
AFLPs	X	X	XX	XXX
SNPs	XXX	XX	XX	X

Modified from Mace et al. (1996).

[a] AFLP = amplified fragment length polymorphism; mtDNA = mitochondrial DNA; RFLP = restriction fragment length polymorphism; SNP = single nucleotide polymorphism.

Fragment Analysis

Fragment analysis comprises various genetic techniques that explore nucleotide variation indirectly by comparing the size of DNA fragments electrophoretically. Although fragment analysis offers less resolution than does direct DNA sequencing, it is cost effective when examining many individuals and many different loci. Among fragment analysis techniques, some cut DNA in certain areas (e.g., **restriction fragment length polymorphisms** [**RFLPs**] and **minisatellite fingerprinting**), whereas others amplify many different loci (**amplified fragment length polymorphisms** [**AFLPs**] and **microsatellites**). With the exception of microsatellites, these techniques produce multiple fragments (bands) per individual (Fig. 22.1). 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 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 cut at specific locations in DNA sequences. Restriction enzymes cut 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 location 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.

Whereas RFLPs look for variation in a single targeted segment of DNA, other fragment-based methods examine

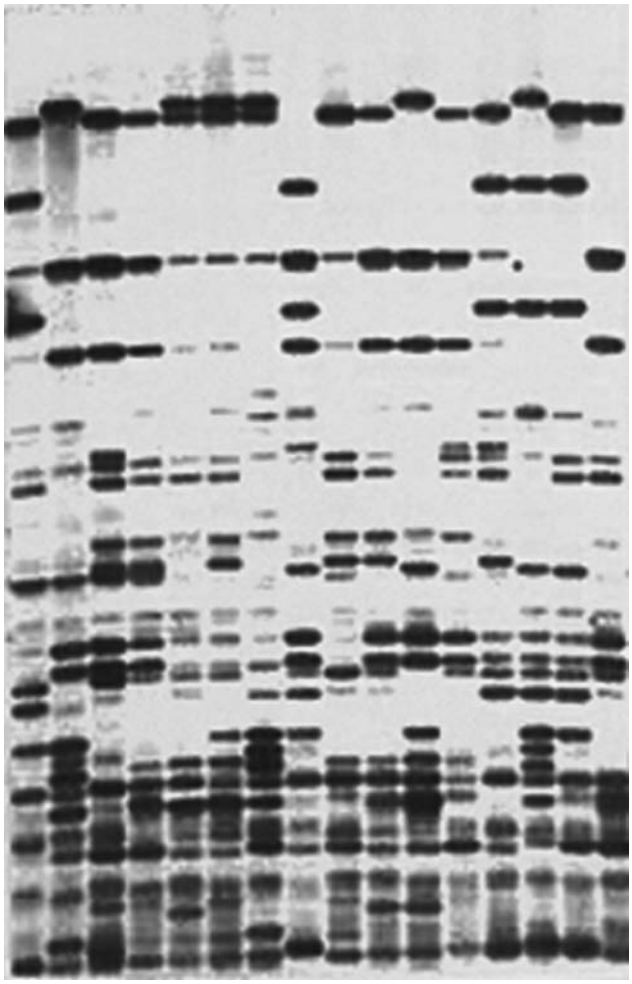


Fig. 22.1. Example of variation at multiple minisatellite loci. This illustration shows variation in and among families of pukeko (*Porphyrio porphyrio*) detected using markers pV47-2 and 3′HVR. *From Lambert et al. (1994).*

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.

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 short "adapters" of known sequence to the fragment ends. PCR is then used to selectively amplify subsets of these fragments. 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 also is a dominant marker system, but it has the advantage of amplifying several hundred markers using only a few selective PCRs (Mueller and Wolfenbarger 1999, Meudt and Clarke 2007).

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 also is typically high. Allelic variation 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 (or peaks) per individual (Fig. 22.2). 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 a particular 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.

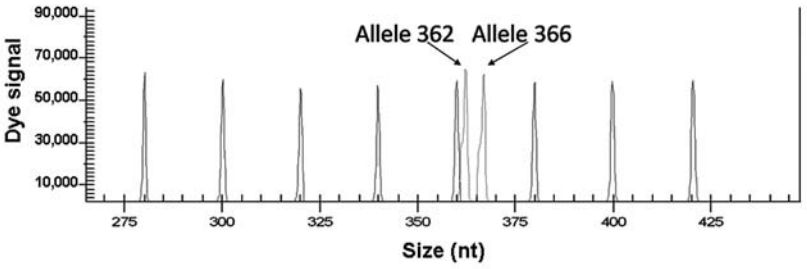


Fig. 22.2. An example of microsatellite data. This locus is heterozygous in this individual, with one allele of 362 base pairs and one allele of 366 base pairs.

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 also is 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 (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.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) are an emerging class of genetic markers that show great potential for wildlife applications. A SNP is a specific site in a DNA sequence in which a single nucleotide varies, resulting in different alleles (Primmer et al. 2002). Because DNA is comprised of **nucleotides** (A, C, T, or G) strung together to form DNA sequences, each SNP site consists of 4 possible nucleotide variants. Additional variation at a SNP site might include a deletion or insertion of a nucleotide. Once the allele at the SNP site is identified in an individual, it is compared to alleles from other individuals, thereby allowing for the examination of levels of genetic variation or similarity among individuals.

The occurrence of SNPs in the genome is quite common, as they have been documented to occur every 100–300 base pairs in humans (Brown 1999). They occur throughout the genome in both coding and noncoding regions of DNA and their **mode of inheritance** is thought to be well understood, following simple mutation models (Morin et al. 2004). Limitations for this type of marker include difficulty identifying alleles in individuals heterozygous at a particular SNP site and the tediousness and expense of identifying and characterizing SNP sites in non-model organisms (Brumfield et al. 2003, DeYoung and Honeycutt 2005).

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 tissues, 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 or DNA–DNA hybridization) whereas 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 on the species of interest. Some species are relatively easy to trap and sample, whereas 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 tissue. If an animal is killed (hunting) or found dead (road kill or disease), samples can easily be taken for genetic analysis. **Nondestructive 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 also can be gathered without having to handle the animal in question (e.g., feathers, hair, feces, and urine); see the section on noninvasive sampling for more details.

In most cases, genetic samples can be stored on ice, refrigerated, dessicated, collected into a preservative buffer, or frozen almost immediately after collection (Table 22.2). When fieldwork occurs in remote areas, sampling certain tissues (e.g., skin or feathers) may be more feasible than such tissues as blood. When working with blood, only a small amount is needed (5 drops) and should be mixed with a preservative, such as ethylenediaminetetraacetic acid (EDTA), or with a blood buffer storage solution, such as Longmire buffer, or stored dry on filter paper. Muscle tissue should be

Table 22.2. Sources of DNA and how samples should be collected

Tissue type	Amount	Quantity	Quality	Preservation method
Blood	5–10 drops	High	Good	EDTA[a] coated tubes Lysis buffer (Longmire) Filter paper
Muscle	Square 2 cm on a side	High	Good	Buffer
Feather	At least 1	Low	Good	Dry
Eggshell membranes	As much as 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
Buccal swab	Variable	Low	Good	Lysis buffer (Longmire)

[a] EDTA = ethylenediaminetetraacetic acid.

either placed in a preservation buffer 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 also can be a good source of DNA, as long as there is vascularization of the membrane. Each membrane should be stored dry in separate bags. Buccal swabs can be collected and stored at room temperature in buffer. Hair, bone, and teeth can be used as a DNA source if they are stored dry. For hair, only the follicle is needed. Scat also can 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/asc_usgs_samplingprotocols.pdf.

TAXONOMY

Species or Subspecies Identification

Taxonomists have been categorizing organisms into hierarchical groups ranging from kingdom and phylum levels to genus and species for hundreds of years. Past classifications have been defined using morphological and behavioral characteristics. Taxonomic delineations derived only from morphological characteristics can be erroneous (Avise 1989, Zink 2004), as they can either fail to recognize distant forms (Avise and Nelson 1989) or they can recognize forms that exhibit little evolutionary differentiation (Laerm et al. 1982). Classifications based on morphology and behavior have been acceptable in the past, yet the use of molecular genetic information can often help resolve discrepancies and refine taxonomic definitions. Although such neutral molecular markers provide important insight into historical and geographic patterns of variation in species, however, using them alone or elevating their significance relative to other forms of evidence, such as morphology or behavior, may, in some situations, mislead conservation efforts.

Although 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). The debate as to how best to classify organisms into species has been ongoing for >150 years (Darwin 1859, Mayr 1942, Wiley 1978, Cracraft 1983, de Quieroz 1998, Wheeler and Meier 2000). New species concepts are added almost continuously (Hey 2001) to address perceived failures of prior ones, and the debate continues as biologists attempt to place discrete boundaries on a continuous process (Winker et al. 2007). Because the **species definition** is integral to the Endangered Species Act (ESA; U.S. Fish and Wildlife Service 1973) and protection and management of many species, we briefly mention the 2 most commonly used: the **biological species concept** (Dobzhansky 1937) and the **phylogenetic species concept** (Cracraft 1983). The major difference between these 2 species concepts is the biological species concept emphasizes **reproductive isolation,** and the resultant limitation and/or preclusion of **gene flow,** whereas the phylogenetic species concept defines species using the criterion of reciprocal monophyly and typically relies solely on 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 also can 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 it 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 ability 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

or protein electrophoresis). Perhaps the most widely used and most applicable to questions in wildlife biology is analysis of the mtDNA sequence, although more and more studies augment such data with multiple loci from the nuclear genome. 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 (Avise 1994). It is maternally inherited and does not recombine, as does nuclear DNA. Although 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; Avise 1994) that 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. Additionally, many studies are moving toward using both mtDNA and nuclear DNA sequences to better resolve taxonomic issues (Barker et al. 2001, Barker 2004).

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 **percentage 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 or 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 also is important for recovery of the species or 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, sage-grouse (*Centrocercus* spp.) have recently been evaluated using behavioral, morphological, and genetic data, resulting in the recognition of a new species (Box 22.1). 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 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 mtDNA data (Rosenbaum et al. 2000). Finally, 2 subspecies of blue grouse (*Dendragapus obscurus obscurus* and *D. o. fuliginosus*) have been elevated to full species as a result of analysis of mtDNA (Barrowclough et al. 2004, Banks et al. 2006).

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, whereas introgression refers to the movement of genes between 2 genetically differentiated groups (Avise 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 it 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 that are locally adaptive) on a species due to a hybridization event can be quite negative. Because true hybrids are generally not protected by the ESA, hybridization provides interesting challenges for those charged with management and protection of species (O'Brien and Mayr 1991).

Molecular techniques provide an increasingly accurate estimation of taxonomic relationships and history of gene flow (Haig 1998). These techniques are being used to ad-

Box 22.1. 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 sage-grouse (*C. u. phaios*), 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 discovery led to description of a new species, the Gunnison sage-grouse (*C. minimus;* Young et al. 2000; see the figure).

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 mitochondrial DNA 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, areas 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).

dress 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 preserve 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 also can be used to identify the maternity and paternity of hybrids. Aldridge et al. (2001) described 2 sage-grouse × sharp-tailed grouse (*Centrocercus urophasianus* × *Tympanuchus phasianellus*) hybrids in Alberta, Canada. 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 (Árnason et al. 1991, Spilliaert et al. 1991). The expansion of barred owl (*Strix varia*) into the range of the endangered northern spotted owl (*S. occidentalis caurina*) led to a study investigating the potential for hybridization between the 2 species. Haig et al. (2004) used AFLP data and mtDNA sequence data to confirm instances of hybridization, which has important legal consequences under ESA.

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 (1994*b*:373) further defined ESU 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 (1994*a*) 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. Although 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). Although 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 (1994*a, 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 maculates;* Firestone et al. 1999; Box 22.2), spotted owls (*Strix occidentalis;* Haig et al. 2001), koalas (*Phascolarctos cinereus;* Houlden et al. 1999), and brown bears (*Ursus arctos;* Waits et al. 2000).

CONSERVATION OF GENETIC DIVERSITY

A focus of conservation genetics is preservation of genetic diversity in and among populations, especially in rare or endangered taxa. Genetic diversity can be estimated using molecular markers or morphological measurements. Although studies have examined the underlying genetic variation and heritability of specific morphological traits in both captive and free-ranging wildlife (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 the use of **quantitative genetic approaches** in a conservation context to study the effects of multiple genes and environmental variation on such complex traits as morphology and behavior; Ellegren and Sheldon (2008) discuss new approaches useful for studying natural populations. Our primary focus in this chapter 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 char-

Box 22.2. Taxonomic redefinition of tigor quolls in Australia

Firestone et al. (1999) defined evolutionary significant units (ESUs) and management units (MUs) for tiger quolls, which revised taxonomic classification and management plans for these carnivorous marsupials in Australia. Previously, 2 allopatric subspecies of tiger quoll had been recognized. The smaller subspecies, *Dasyurus maculatus gracilis,* occurs only in northern Australia in northeastern Queensland. The larger subspecies, *D. m. maculates,* occurs in southeastern Australia and Tasmania. Each subspecies has been placed on the International Union for Conservation of Nature list as either endangered or as vulnerable to extinction.

Firestone et al. (1999) used both mitochondrial DNA (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. These results suggest 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) suggested 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 that 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, and the classification in Tasmania should be reconsidered to recognize and preserve the unique genetic makeup and evolutionary path of tiger quolls.

acteristics. 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

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 in Ukraine (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, recent work indicates that it is difficult to attribute this increased genetic diversity to increased mutation (Meeks et al. 2007). Other investigations have failed to detect much evidence of increased mutation in wildlife from contaminated sites (Dahl et al. 2001, Stapleton et al. 2001, Berckmoes et al. 2005). Given that mutations 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 in coming years, as automated analyses have made it possible to screen large numbers of individuals and genes.

Gene Flow

When organisms disperse to new populations and reproduce, they contribute genetic material to their new populations. This process increases the genetic similarity of populations 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 they are inadequate for measuring the reproductive contribution of dispersing individuals to their new populations. Gene flow is typically measured through indirect methods using genetic markers (Slatkin 1985*a*). One of the 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 Neigel 1997, 2002). For other approaches to estimating gene flow, see Slatkin (1985*b*), Slatkin and Maddison (1990), and Neigel et al. (1991). Recently maximum likelihood and Bayesian approaches have been developed to estimate gene flow and other population parameters (Beerli and Felsenstein 2001; Wilson and Rannala 2003; Kuhner 2006, 2009). These methods can be quite powerful, but demand considerable computational resources.

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 (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. In cases of population growth or decline, it is assumed the change is constant over time (see Kuhner 2006). These conditions are not typical of many settings in which resource managers wish to estimate gene flow, such as in recently fragmented landscapes. Thus, although often useful in a relative sense, the absolute values of estimates of *Nm* should be regarded with some caution.

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. 22.3); a few successful individuals moving between populations each generation 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 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.

Sex-Specific Dispersal

Among wildlife species, there is considerable variation in the gender of dispersing individuals. Gender of dispersing individuals is typically beyond the control of wildlife biologists; however, it is important to understand that **breeding**

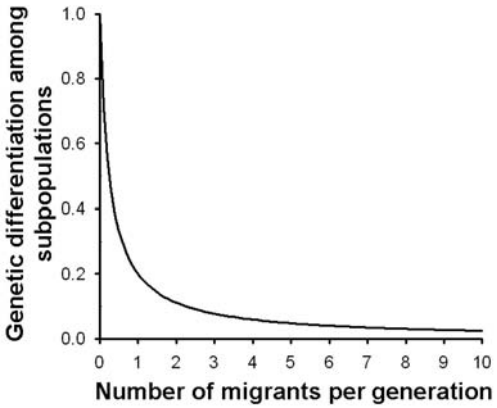


Fig. 22.3. Equilibrium relationship of genetic differentiation among subpopulations (as measured by the statistic F_{ST}) and number of migrants per generation. *Modified from Mills and Allendorf (1996).*

systems and **gender-biased dispersal** are likely to affect estimates of gene flow. Gender-biased dispersal and **social structure** in 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,** such as 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, suggesting that females, but not males, return to their natal beach (Karl et al. 1992).

Given the important contributions of mtDNA studies to understanding of female-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 from the Y chromosome have been 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 microsatellite loci and other easy-to-assay markers on the Y chromosome has enhanced the ability to characterize gene flow by males in several mammals (Handley and Perrin 2007, Yannic et al. 2008).

Studying male-specific markers in birds is more complicated than in mammals, because birds have **heterogametic (ZW) gender determination,** where the 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 sex-specific gene flow. Information about sex-specific movements also can be gained by comparing the spatial genetic structure of adult males and females (Lee et al. 2009).

Population Structure and Fragmentation

Using F_{ST} to quantify **population structure** and gene flow assumes that sampling reflects the underlying population structure; however, choice of sampling locations is often based on other considerations, such as logistics or accessibility. Rather than assessing differences in gene frequencies among sampling locations, it is becoming more common to use **assignment methods** to determine the number of populations in a given sample and to assign individuals to those populations (reviewed in Manel et al. 2005). See Aspi et al. (2009) and Boessenkool et al. (2009) for recent examples of the use of these powerful assignment methods for assessing the structure of wildlife populations. Waples and Gaggiotti (2006) review different methods for identifying population structure based on genetic markers.

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. For example, gene flow was much lower in populations of Sitka deer (*Cervus nippon*) in areas of habitat fragmentation (Goodman et al. 2001). Sometimes even animals capable of long-distance movements, such as migratory songbirds (see Box 22.3) or black bears (*Ursus americanus;* Dixon et al. 2007), experience population subdivision due to habitat fragmentation, suggesting the capability for dispersal might poorly predict actual dispersal.

There is one caveat for most studies of the effects of recent habitat fragmentation on genetic differentiation: they lack temporal control. Although 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 are possible, given the ability to isolate **DNA from museum specimens.** For example, Martinez-Cruz and Godoy (2007) found that differentiation among fragmented populations of the Spanish imperial eagle (*Aquila adalberti*) is much greater today than it was in the past, when habitats were presumably more continuous.

Box 22.3. Assessing genetic diversity and structure in 2 endangered songbirds

The golden-cheeked warbler (GCWA; *Dendroica chrysoparia*) and black-capped vireo (BCVI; *Vireo atricapilla*) are migratory songbirds facing a variety of threats to their long-term viabilities (see figure). Both species have restricted breeding ranges (GCWA: central Texas; BCVI: northern Mexico, central Texas, and southern Oklahoma). Within these distributions, the populations have been highly fragmented by habitat loss due to urban, suburban, and agricultural development (Grzybowski 1995, Ladd and Glass 1999). Each species also has been heavily affected by brown-headed cowbird (*Molothrus ater*) nest parasitism.

Microsatellite loci were used to determine whether population fragments had experienced loss of genetic diversity and increased interpopulation differentiation due to bottlenecks and associated genetic drift (see Lindsay et al. [2008] and Barr et al. [2008] for details). Both species had levels of genetic diversity similar to other related songbirds, suggesting that neither had experienced species-wide loss of variation. There also was little evidence that individual population fragments had experienced recent bottlenecks, even though some of them were quite small. However, using both F_{ST} and assignment tests, there was more genetic structure among population fragments than would be expected for songbirds capable of flying hundreds of kilometers during migration. The influence of fragmentation on genetic structure also differed between the species. The greatest genetic differences among GCWA populations were between those separated by agricultural lands; this species depends on mature forests with little edge (Ladd and Glass 1999). The BCVI had greater differences among populations than did the GCWA, but these differences were not strongly influenced by intervening habitat. As a species dependent on early successional shrublands, differentiation may be the result of small numbers of birds colonizing habitats that are only temporarily available. These studies illustrate that habitat fragmentation can restrict gene flow even in species capable of moving great distances and that the habitat requirements of a species will influence its response to fragmentation (Leberg 1991).

Golden-cheeked warbler (left) and black-capped vireo (right). *Photos by K. Barr*

Gene Flow through Wildlife Translocations

One consequence of **translocation programs** is gene flow (Leberg 1990*a*). When such translocations might result in the loss of unique genetic characteristics of isolated populations, they should probably be avoided (Moritz 1999). However, when genetic differences have developed through habitat fragmentation resulting from land use, translocations can be used for **genetic restoration.** Many authors have discussed the possibility of using translocations to restore gene flow between populations isolated by habitat loss (Moritz 1999, Tallmon et al. 2004, Bouzat et al. 2009). Based on the relationship between gene flow and F_{ST} (Fig. 22.3), movement of only a few individuals per generation should be adequate 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.

Genetic markers can be used to assess whether translocated individuals reproduced and contributed genetic variation to their recipient populations (Mock et al. 2001, Olsson 2007). For example, Arrendal et al. (2004) found that translocated Eurasian otters (*Lutra lutra*) made genetic contributions to 1 of 2 populations into which they were released. These observations can be useful, because it is often difficult to know whether individuals released in augmentation ef-

forts successfully contributed genetic material to the recipient population.

The success of a translocation program also can be examined by assessing whether patterns of genetic similarity expected from natural dispersal have been disrupted (Latch and Rhodes 2005). For example, Leberg et al. (1994) assessed whether genetic structure of white-tailed deer (*Odocoileus virginianus*) populations in the southeastern United States had been influenced by extensive translocations. 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. (1994*a, 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 information prior to a translocation (Leberg 1999). Bouzat et al. (2009) provide an excellent example of the insights that can be gained by studying both the genetics and ecology of populations before and after a translocation to restore genetic diversity. They found that although translocations of greater prairie-chickens (*Tympanuchus cupido pinnatus*) restored genetic variation to historic levels and reduced inbreeding depression, the benefits of the effort were limited by habitat conditions.

Drift and Bottlenecks

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, but 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 multilocus heterozygosity) also is 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 between them and at least 1 of them is small enough to experience substantial genetic drift.

When a normally large population goes through a constriction in size, the event is referred to as a **genetic bottleneck.** During bottlenecks, drift is greatly accelerated. 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 that 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). But 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 through inbreeding 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 prebottleneck samples are often absent, samples from populations that may have experienced 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, Whitehouse and Harley 2001, Nichols et al. 2001; Box 22.3).

This comparative approach requires the assumption the populations had similar levels of genetic variation prior to the putative bottleneck event (Bouzat 2000). Use of preserved materials provides a more straightforward way to estimate prebottleneck levels of diversity. Matocq and Villablanca (2001) used museum specimens to show that low genetic variation in an endangered species was due to bottlenecks that occurred prior to a known recent reduction in population size. Conversely, museum specimens of greater prairie-chickens provided strong evidence that recent population reductions in Illinois resulted in reduced levels of genetic variation (Bouzat et al. 1998*b*). 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. Schwartz et al. (2007) discuss contributions that DNA from museum archived species have made to conservation biology and advocate for using genetic monitoring to better manage wildlife populations.

The commonly used genetic indices of bottlenecks differ in their sensitivities to population contractions. Loss of allelic diversity is much more sensitive to short population bottlenecks than is heterozygosity (Leberg 1992, Spencer et al. 2000). 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** also is 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 also is 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 whether a population has experienced a loss of genetic variation. These methods are based on expectations of heterozygosity, distributions of frequencies of alleles, or **distributions of allele sizes** for populations that have not experienced bottlenecks (Cornuet and Luikart 1996, Luikart and Cornuet 1998, Luikart et al. 1998, Garza and Williamson 2001). 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 these approaches provide reasonable indices of a population's history of bottlenecks (Luikart and Cornuet 1998, Spencer et al. 2000). However, Larsson et al. (2008) showed these methods where not able to detect a bottleneck of black grouse (*Tetrao tetrix*) that was detectable by comparing contemporary and historical samples. Williamson-Natesan (2005) provides a comparison of these methods and a discussion of their merits.

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 (*Oryctolagus cuniculus*) population 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). Gene flow also will make it very difficult to detect the effects of even severe bottlenecks (Busch et al. 2007).

Effective versus 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, N_c**). At times, many individuals in a population are not reproductively active because of age or social constraints, and 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. 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 one where all individuals have an equal chance of producing any progeny making up the next generation. The list of possible factors that can could cause $N_e < N_c$ is large (Crow and Kimura 1970, Hedrick 2000, Leberg 2005). 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 a strong influence on the effective size of wildlife populations (Frankham 1995*a*). 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 N_e (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 represented by a few individuals, then $N_e << N_c$. In species with nonoverlapping generations, highly polygamous mating systems also can 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 influences of sex ratio and mating system on N_e are modified by **generation length** in complex ways (Nunney 1993). Methods assuming nonoverlapping generations should be applied with caution when attempting to estimate N_e of wildlife populations (Leberg 2005).

There are several genetic techniques for estimating N_e. One common approach is to quantify genetic changes through time by taking ≥2 temporal samples (Waples 1989, Jorde and Ryman 1995). If insufficient time is available to obtain samples separated by several generations, the genetic characteristics of contemporary populations can be compared to those of museum specimens (Bouzat et al. 1998*b*, Schwartz et al. 2007, Pertoldi et al. 2008). Recently, there have been a series of powerful methodologies developed to estimate N_e based on this temporal method (Wang and Whitlock 2003, Wang 2005). There are a number of other approaches to estimation of N_e based on genetic data from a single sample (e.g., Tallmon et al. 2008); see Leberg (2005) for a review of issues related to estimating N_e in wildlife populations. In addition to genetic approaches, demographic data can be used to estimate N_e (Harris and Allendorf 1989, Nunney 1993, Engen et al. 2007). 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, Leberg 2005).

Drift and Bottlenecks from Human Activities

Reduced levels of genetic variation have been documented in large numbers of threatened species or populations (Rossiter et al. 2000, Rico et al. 2008, Gebremedhin et al. 2009). 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 (Bouzat 2001, Goodman et al. 2001). Creation of corridors between these fragments or the imposition of gene flow through **translocations** has been suggested as strategies for prevention of loss of diversity in fragmented populations (Hedrick 1995, Mills and Allendorf 1996, Epps et al. 2007).

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 (Stockwell and Leberg 2002, Mock et al. 2004, Sigg 2006). For example, Fitzsimmons et al. (1997) found that populations established with translocated bighorn sheep (*Ovis canadensis*) often had reduced genetic diversity compared to the source of the released individuals. 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 (*Cervus canadensis;* Williams et al. 2002*b*).

Not surprisingly, **allele frequencies** of translocated populations often differ from those of their sources (Scribner 1993, Fitzsimmons et al. 1997, Stephen et al. 2005). However, caution should be used when interpreting differences in allele frequencies among translocated populations and their sources. Although differences might be the result of the founder event, they also could have occurred through drift after the translocated population became established (Williams et al. 2000*b,* Stephen et al. 2005). Vonholdt et al. (2008) provide an example of using genetic markers to monitor the genetic composition and social structure of a reintroduced population of wolves.

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 1990*a*). 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.

Harvest programs should have little effect on genetic variation, because loss of variation due to drift is small if the population is large. However, harvests 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 (Ryman et al. 1981, Laikre and Ryman 1996). For example, harvest regulations and hunter preferences resulting in greater harvests of males can have large effects on the N_e of ungulate populations (Ryman et al. 1981, Coltman 2008). Harris et al. (2002), Coltman (2008), and Allendorf et al. (2008) review the possible effects of game harvests on genetic diversity and effective population size.

Selection

Many 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. Although this assumption is violated occasionally, most 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 such phenomena 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 lack of direct concordance often observed between patterns of variation at molecular markers and complex morphological, behavioral, or life history traits has lead to calls for conservation geneticists to more critically evaluate whether molecular data are sufficient for designating conservation priorities (Pearman 2001, Reed and Frankham 2001).

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 selection in maintaining marker variation in wildlife populations. Initial surveys of natural populations detected higher levels of allozyme variation than expected. This observation 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, Reed and Frankham 2003). However, there also have been studies that found no relationship between heterozygosity and traits related to fitness (Britten 1996). In red deer (*Cervus elaphus*), antler growth was actually lower in heterozygotes for some allozymes (Hartl et al. 1995). Furthermore, there is little direct evidence that it is the loci themselves 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). 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 microsatellite heterozygosity have been detected for several wildlife species (Coulson et al. 1998, Hansson et al. 2001, Höglund et al. 2002). Given that most microsatellite loci do not directly affect phenotype, such associations probably reflect the relatedness of an individual's parents or the physically proximity of assayed microsatellites to other loci affecting the traits of interest. In a recent review of the relationship of heterozygosity to traits related to fitness, Chapman et al. (2009) found that although there are many examples of such correlations, the amount of variance in traits explained by heterozygosity is generally small.

Recently, there has been considerable interest given to examining relationships between **individual viability** and loci in the **major histocompatiblity complex.** These genes are involved in **immune responses,** and there is some evidence that selection maintains variation in 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 also have 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. Although 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 little 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 also have 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).

There are promising applications for the use of molecular markers to elucidate variation in fitness traits, and thus improve our understanding of selective pressures faced by wildlife. For example, Slate et al. (2002) used a large number of maps to identify specific genes, referred to as quantitative trait loci for birth weight in wild population of red deer. Ellegren and Sheldon (2008) review using gene mapping and other genomic approaches to understand the genetic basis for variation in fitness traits in wild populations.

Genetic approaches can be used to better understand the implications of **selective harvesting** of wildlife based on hunter preferences or harvest regulations (Allendorf et al. 2008, Coltman 2008). For example, Coltman et al. (2003) used a partly genetically reconstructed pedigree to provide evidence that trophy hunting of bighorn sheep might be selecting for slower horn growth. Because genetic traits are often correlated, selection for one trait, such as horn size, might well affect others, such as body mass or fecundity (Coltman 2008, Sasaki et al. 2009). We are just beginning to understand the effects of selective harvests on wildlife, but at least in some cases, increasing the relative mortality of individu-

als with larger body sizes—or bigger antlers, tusks, or horns—is likely to reduce not only the sizes of those traits, but also influence other correlated traits that might affect population viability (Allendorf et al. 2008, Coltman 2008, Allendorf and Hard 2009).

Genetic Diversity and Population Viability

Observations of **inbreeding depression** in captive (Lacy et al. 1996) and field populations (Jiménez 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 (Leberg 1990*b*, 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. 1998*a*).

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 (see Chapter 35, Volume II). These concerns about effects of inbreeding on demography occur on a time scale relevant to management activities (e.g., Westemeier et al. 1998, Johnson and Dunn 2006, Ewing et al. 2008). 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 1995*b*).

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 2–4 times as large.

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); however, predicting future inbreeding depression based on population history and environment is difficult (Leberg and Firmin 2008). 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, Edmands 2007). Additionally, other mechanisms besides 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, Jaquiery et al. 2009). Reviews of the mechanisms through which genetic diversity can affect population viability can be found in Soulé (1986), Frankham et al. (2002), Leberg and Firmin (2008), and Chapter 35, Volume 2.

Captive Breeding Programs

When populations decline drastically and only a few 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 that individuals can be released into the wild. Because most captive populations are limited in size, they are subject to inbreeding and drift. 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).

Once a captive breeding program has been established, **pedigrees** can be used to avoid matings between close relatives or the over- or underrepresentation of the genes of individual founders in the captive population (Lacy et al. 1995). However, because number of individuals brought into captivity is usually small, inbreeding can be a serious problem if the **founding population** includes related individuals. In such cases, it can be important to consider the genetic identity of animals bred in captivity, so that net genetic variability is maximized and inbreeding is minimized. Molecular genetic techniques have proven to be valuable for inferring relatedness and promise to be useful for examining relatedness of founders (Haig et al. 1994, 1995). Jones et al. (2002*b*) used microsatellite data to augment wild and captive pedigree information on whooping cranes (*Grus americana*), revealing unknown shared genotype information for founders. Rudnick and Lacy (2008) have shown that improvements in inbreeding avoidance obtained by supplementing information from pedigree relationships with genetic analysis of founder relatedness will be small, unless the founder population included close relatives.

Another issue in captive breeding is **adaptation** to captive conditions. Adaptations like extended reproduction periods and tameness can occur very rapidly and may make it difficult to successfully reintroduce captive bred individuals back into the wild. See Frankham (2008) for a discussion of this problem and possible solutions.

MOLECULAR ECOLOGY

In addition to addressing the traditional concerns of conservation genetics, genetic markers have increasingly provided insight into the ecology of populations. These applications are sometimes referred to as **molecular ecology,** a field that is interwoven with conservation genetics, but that includes applications extending beyond the conservation of genetic variation. As reviewed by Waits and Paetkau (2005), some of these approaches are quite relevant to investigations of wildlife populations.

Noninvasive Sampling

Many wildlife investigators attempt to determine **population size, survival rates,** and **movement patterns** (see Chapters 11 and 20, This Volume). **Mark–recapture methods** are often used to achieve these goals. These approaches usually require capturing individuals and marking them in some way that would allow for their identification if they are ever recaptured. Although these techniques work well for many species, there are others for which this type of study does not. Species that are dangerous and expensive to catch (e.g., bears) and those that are highly elusive (e.g., felids) do not lend themselves to conventional mark and recapture techniques.

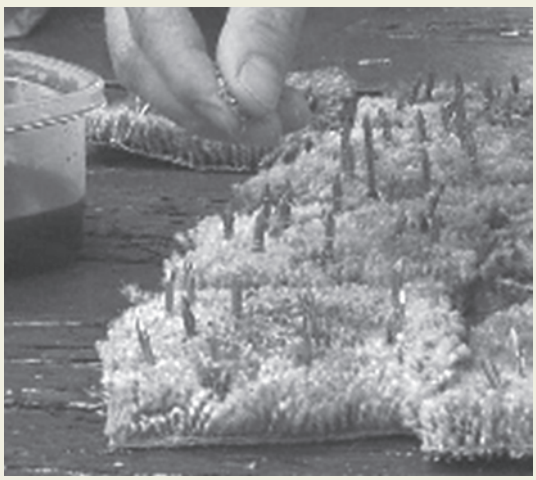
Because DNA can be obtained from hair, feathers, shed skin, feces, and urine (Table 22.2), biologists have **noninvasive** ways to obtain genetic information (Waits and Paetkau 2005). Each individual animal has a **unique molecular fingerprint,** so it is possible to use this genetic fingerprint in the same way a biologist might a tag or band in a traditional mark and recapture study. One advantage of this **genetic tag** is that it remains with the individual throughout its lifetime and can even be used to associate the individual with its parents and offspring.

Collecting **noninvasive samples,** such as scat or feathers, often involves searches of locations the organism is expected to use, such as trails, tree rubs, nests, roosts, or den sites (Pearce et al. 1997, Kohn et al. 1999, Piggott et al. 2006). Collection of scat can be aided with the use of specially trained dogs (see Chapter 5, This Volume; Long et al. 2007*a, b*), some of which can distinguish between the feces of target and nontarget species (Smith et al. 2005). Hair snares have been used to sample a variety of mammals. Hair from bears has been sampled by placing barbed wire around an attractant (Triant et al. 2004, Kendall et al. 2008); a similar approach may work for white-tailed deer (Belant et al. 2007). Hair from felids and other carnivores has been collected from scented hair snares that elicit rubbing behavior (Weaver et al. 2005, Castro-Arellano et al. 2008; Box 22.4). Barbed snares or glue pads for small or medium-sized carnivores have been placed at den entrances (Scheppers et al. 2007) or at the entrance of baited enclosures (Belant 2003, Williams et al. 2009). Snares have even been propelled by a blowgun to collect hair samples from primates (Amendola-Pimenta et al. 2009).

Often **systematic** or **random sampling** is needed for estimating population size or other demographic parameters. Dogs trained for scat detection can be used to sample transects (Smith et al. 2005, Long et al. 2007*a, b*). Hair snares can be placed along transects, on a sampling grid, or at ran-

Box 22.4. Documenting the presence of lynx using molecular techniques

When Canada lynx (*Lynx canadensis*) 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 (see figure), 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 (approx. 900 base pairs) 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 polymerase chain reactions. Restriction enzymes were then used to create DNA fragments, and hairs of lynx were distinguished from other samples by banding patterns (Mills et al. 2000*a*).



Baiting a hair snare with catnip.

dom points throughout a study area (Mowat and Paetkau 2002, Castro-Arellano 2008, Williams et al. 2009).

Another advantage of some **noninvasive sampling** is that it does not require handling the organism. Thus, an individual can be sampled repeatedly without influencing the individual's behavior, making it less prone to being sampled. Furthermore, when samples are collected without the aid of attractants, little or no behavior alteration is expected from noninvasive sampling. Behavioral changes following capture and tagging with traditional approaches have the potential for influencing estimates of demographic parameters (see Chapter 11, This Volume).

Estimating Population Size and Survival

For **mark and recapture methods** based on DNA, molecular biologists need to use a genetic marker (or series of markers) that is variable enough so that no 2 individuals will have the same **molecular tag.** Microsatellites are currently the most commonly used marker for this application: each individual's "molecular tag" is based on its genotype for a number of highly polymorphic loci. Using DNA to identify individuals, scientists have been able to estimate population size for a number of species, including humpback whales (*Megaptera novaeangliae;* Palsbøll et al. 1997), fishers (*Martes pennanti*) and American martens (*M. Americana;* Williams et al. 2009), eastern imperial eagles (*Aquila heliaca;* Rudnick et al. 2008), mountain lions (*Puma concolor;* Ernest et al. 2000), lesser horseshoe bats (*Rhinolophus hipposideros;* Puechmaille and Petit 2007), and brush-tailed rock-wallaby (*Petrogale penicillata;* Piggott et al. 2006). Estimates of survival rates, as well as population sizes, have been obtained for Arctic fox (*Alopex lagopus;* Meijer et al. 2008) and grizzly bears (*Ursus arctos horribilis;* Boulanger et al. 2004).

Methods for estimating population size and survival using molecular tags have been reviewed by Lukacs and Burnham (2005*a*). Software has been designed specifically to analyze capture–recapture data based on genetic tags incorporating features like **identification error** (Lukacs and Burnham 2005*b,* Knapp et al. 2009). Other approaches address the possibility of sampling an individual multiple times in the sample collection period and allowing population estimates from only a single period of sample collection (Miller et al. 2005, Petit and Valiere 2006, Puechmaille and Petit 2007). Robinson et al. (2009) found that such models performed better than did multiple occasion capture–recapture estimators. The ability to obtain a population estimate for a single intensive sampling occasion is advantageous when sampling remote study areas that would be difficult to visit multiple times.

Molecular tags have excellent potential for estimating population size (and potentially survival rates) of species that are difficult to trap; however, there are 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 can be extracted from such samples, and the DNA is often **degraded** (Taberlet et al. 1999; Table 22.2). 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 only 1 of 2 alleles of template DNA is amplified by PCR. The consequences are that only 1 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. **Genotyping errors** and **amplification failure** of DNA from collected scat are influenced both by climate and age of the feces (Piggott 2004, Murphy et al. 2007). Such genotyping errors can result in large overestimates of population size (Waits and Leberg 2000). These issues can be addressed by using strict extraction protocols to avoid contamination, adopting repeated PCR amplifications to identify cases of allelic dropout, and using only short microsatellite loci to avoid problems with degraded DNA (Taberlet et al. 1999, Bonin et al. 2004). Alternately, there are statistical approaches to identify genotyping errors (Miller et al. 2002, McKelvey and Schwartz 2004); these approaches could prove useful in reducing costs associated with multiple PCR amplifications and when DNA is limited. Roon et al. (2005*a*) evaluated several approaches to identifying genotyping errors and noted that statistical approaches for filtering errors would provide inadequate resolution, unless genotyping error rates are very low.

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. If too few loci are used to identify an individual, it is possible that multiple individuals will have the same molecular tag, resulting in underestimates of population size (Mills et al. 2000*b,* Waits and Leberg 2000). Of course, limited amounts of DNA and increased expense make it undesirable to analyze more loci than necessary to assign unique tags to individuals. There are several approaches for estimating the numbers of loci that should be examined in studies using noninvasive DNA samples (Waits et al. 2001, Hoyle et al. 2005).

Finally, noninvasive samples have the potential to include DNA from multiple individuals. This problem would be most common in cases where several individuals might use a common latrine or leave hair on the same hare snare. For example, Scheppers et al. (2007) found multiple genotypes of badgers at hair snares located at den entrances and along trails; they advocate using DNA from single hairs to identify individuals. This source of bias was examined by Roon et al. (2005*b*), who provide suggestions for addressing the issue of multiple genotypes in a sample.

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). 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 (Kohn et al. 1999, Ernest et al. 2000, Lucchini et al. 2002). Walker et al. (2008) used DNA from scats to study individual movements and social interactions of the southern hairy-nosed wombat (*Lasiorhinus latifrons*). Smith et al. (2006*a*) present estimates of movements and home range sizes of kit foxes (*Vulpes macrotis*), based on DNA from scat, and discuss issues associated with using scat as a DNA source for tracking individual movements. 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. 2001*a*). 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, 2005). Variations on these approaches have been used to gain insight into migratory patterns of noctule bats (*Nyctalus noctula;* Petit and Mayer 2000); Wink (2006) reviews the use of DNA markers to study bird migration. Stock identification has proven useful in assigning samples of loggerhead turtles (*Caretta caretta*) collected in foraging areas to their nesting beaches (Bass and Witzell 2000), and in identifying which populations are most affected by incidental captures associated with commercial fisheries (Laurent et al. 1998). Using shed feathers, Rudnick et al. (2008) were able to quantify the degree of natal dispersal and movement in a population of eagles. Gardner-Santana et al. (2009) used assignment tests to examine movements of wild Norway rats (*Rattus norvegicus*) among sites and were able to identify individuals that had dispersed. This approach also has been used to document low amounts of individual dispersal by black bears among habitat fragments (Dixon et al. 2007). 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. These approaches require the genetic composition of possible source populations to be 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 differentiation in many species, such as northern pintails (*Anas acuta;* Cronin et al. 1996) and double-crested cormorants (*Phalacrocorax auritus;* Green et al. 2006), are sufficiently small to make identification of breeding populations impractical.

Another approach useful for identifying dispersing individuals is **parentage analysis,** a special case of assignment testing (Manel et al. 2005). By determining parent offspring relationships through intensive genetic sampling, it is possible to determine which individuals have dispersed from natal sites (Nutt 2008). Waser et al. (2006) show how estimation of parentage can improve on estimates of natal dispersal rates and distances, even in organisms that can be readily captured and tagged.

Species Identification and Detection

Although accurate individual identification can sometimes be challenging with some noninvasive samples, **species identification** is less problematic (see Foran et al. [1997] for an early example). 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 information is particularly important for programs monitoring status of regulated or protected species. DNA extracted from these materials can provide such identification. If a species has **uniquely identifiable populations,** this technique also may be applied to identify which population is the source of a sample.

Species identification can be used to sample for the presence of a rare species, such as in the National Canada Lynx Survey (Box 22.4). Other examples of surveys of the occurrence of a species at a sample site using noninvasive DNA include Dalen et al. (2004), Bidlack et al. (2007), and Ruell and Crooks (2007). A range wide survey of DNA from rabbit droppings was used to determine the current distribution of New England cottontails (*Sylvilagus transitionalis*), a species that has been declining for several decades (Litvaitis et al. 2006).

Other applications of species identification include investigations related to the **illegal harvest** of wildlife (Baker 2008). Cassidy and Gonzales (2005) discuss the need for careful standards when processing samples that might be used in criminal cases. Wasser et al. (2008) discuss the use of assignment tests to help trace poached ivory back to its population of origin.

DNA-based species identification can assist in a variety of other wildlife investigations. Smith et al. (2006*b*) gained information on habitat use by kit foxes based on the distri-

bution of their scat. Analysis of salvia from bite wounds to sheep was used to determine the predator was a dog rather than a wolf (Sundqvist et al. 2008). Onorato et al. (2006) showed that analysis of scat and hair from the vicinity of ungulate carcasses sites greatly enhanced the ability to determine which predators had visited the site or were involved in the depredation. DNA analysis of tissue remnants has been valuable in identifying which species are involved in bird collisions with aircraft (Dove et al. 2008).

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, Waits and Paetkau 2005, Tollit et al. 2009). Possible sources of dietary information useful for such analyses include stomach contents, mammalian scat, and bird regurgitant. For example, Scribner and Bowman (1998) used microsatellite analysis to distinguish among several species of juvenile waterfowl in stomachs of glaucous gulls (*Larus hyperboreous*). Analysis of scat was used to verify predation by dingos (*Canis familiaris dingo*) on an endangered wombat (*Lasiorhinus krefftii;* Banks et al. 2003). In such 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 also might detect nondietary 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. Although biases from DNA degradation may result, causing some food types to be over- or underrepresented in molecular analyses of scats, such errors may not be greater than those observed in conventional diet studies (Deagle and Tollit 2007). Clare et al. (2009) give an interesting example of how molecular analyses of bat feces can provide new insights into foraging ecology of species that are difficult to study using traditional analyses of food habits.

Another significant advantage of DNA-based **analysis of scat** is the ability to trace multiple samples back to single individuals. This makes it possible to determine whether individual predators of the same species differ in their **food habits.** For example, Fedriani and Kohn (2001) found that groups of coyotes, and even coyotes within groups, differed in their diets. In a similar study, Prugh et al. (2008) found that spatial and temporal variations in prey availability could explain some of the diet variation among individual coyotes.

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, or hair). Forensic scientists can use **DNA-based identification approaches** when gender of a tissue sample or blood strain might indicate a violation of wildlife harvest regulations (Gilson et al. 1998, Wilson and White 1998, An et al. 2007).

Mammals

Gender can be identified from DNA samples for many groups of mammals, including wombats, rabbits, ungulates, carnivores, seals, primates, and whales (Aasem 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). There are 2 main strategies for detecting gender in mammals using molecular techniques. The first approach is to use PCR to amplify a region specific to the **Y chromosome,** such as the SRY locus, to identify males. If the marker is not detected, the sample is assumed to be from 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). A second approach is to amplify **homologous fragments** of the **X and Y chromosomes,** such as the amelogenin gene (Sullivan et al. 1993, Brinkman and Hundertmark 2009) or zinc finger proteins (Shaw et al. 2003). This approach produces 2 different sized bands, thereby alleviating the need for additional amplification controls. 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 for which the gender is known. Care also must 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. This issue can be circumvented in felids by using primers designed for the zinc-finger and amelogenin regions, where deletions in Y-chromosome regions are absent in a wide range of prey species, thus minimizing potential contamination from prey DNA (Pilgrim et al. 2005).

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 chromosomes followed by a restriction digest that allows for differentiation 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*) in 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*) using feathers (Dinsmore et al. 2002) 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 provided 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.