MOLECULAR APPROACHES TO THE STUDY OF PARENTAGE, RELATEDNESS, AND FITNESS: PRACTICAL APPLICATIONS FOR WILD ANIMALS

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Abstract: Historically, novel molecular techniques have been developed by the human genetics community, adapted for nonhuman animals by evolutionary biologists, and gradually adopted by the wildlife and fisheries communities. Today, evolutionary biologists routinely rely on molecules to assess mate choice, dispersal, parentage, sex ratios, and other population parameters. All in all, the use of molecular genetic markers has revolutionized population biology—human and otherwise. Prescient wildlife and fisheries biologists have recognized the importance of this revolution and are now using molecular genetic tools to evaluate captive or supplemental breeding programs, population dynamics, stocking strategies, and taxonomic issues. Herein, I explore the use of molecular genetic markers to address questions in wildlife biology and management. Specifically, I review how—among other topics—cannibalism, sex-ratios, dispersal, enumeration, genotoxicology, hybridization, and genetically modified organisms can be evaluated in the context of parentage, relatedness, and fitness. As science becomes more integrative and complex, it is easy to envision a future where collaborations between geneticists (who may not have the expertise to obtain the field samples) and wildlife biologists (who may not have the expertise and/or facilities to obtain the genotypes) are common and serve to answer both fundamental and applied questions.

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Novel molecular techniques usually arise within the human genetics community and slowly filter into ecology and evolutionary biology, wildlife management, and fisheries management. Evolutionary biologists have been using molecular (i.e., DNA) markers for over a decade, and recently the fisheries community has taken full advantage of the DNA tools at their disposal. For example, the ichthyology literature is now replete with molecular assessments of taxonomic status (reviewed in Turner 1999), genetic diversity and population structure (Carvalho and Hauser 1998), and biological parentage (DeWoody and Avise 2001, Avise et al. 2002). Further, fisheries biologists have carefully considered the genetics of founders prior to stocking (Hedrick et al. 2000, Page et al. 2004) or supplemental breeding (Barton and Scribner 2004, Fiurner et al. 2004). Wildlife biologists are faced with many of these same issues, and herein I focus on how molecular assessments of parentage, relatedness, and quantitative genetics can influence wildlife management.

The specialized terminology used in genetics (and many other disciplines) is often daunting to nonspecialists. Thus, I begin with a brief review of molecular markers and their potential roles in elucidating parentage and relatedness in wildlife populations. For a thorough treatise, see Avise (2004a). I follow with a discussion of other applications relevant to wildlife biology including dispersal, enumeration, genotoxicology, and hybridization.

TYPES OF MOLECULAR MARKERS

Before we consider the various molecules used in genetic assays, we must consider the various types of markers available to modern geneticists. Just as wildlife biologists have several means of marking individuals (e.g., ear tags, toe-clipping, etc.), geneticists use different methods of assaying specific loci—or points—within the genome. These genetic markers fall into 1 of several categories based on the type of assay employed (see categories of molecular markers below).

Most of the markers I discuss below generally behave as neutral or nearly neutral markers with respect to natural selection. This means that selection does not favor (or disfavor) particular alleles or genotypes at the locus or loci under consideration. Neutral markers like microsatellites (see Microsatellites section below) are those most germane to the assessment of parentage and relatedness in wildlife populations, but as databases like Gen-
Bank (an electronic repository of DNA sequences) grow, many studies will no doubt focus on protein-coding genes that are expressed. Typically, functional-coding genes that produce a protein product do not evolve in a strictly neutral fashion (i.e., they are nonneutral) because a mutant often decreases fitness and is less likely to be transmitted to the next generation.

Because of their differences, neutral markers and coding genes are often used to answer different sets of questions. For example, neutral markers and their associated molecular clocks (Bromham and Penny 2003) can be used to determine how long ago human and chimpanzees lineages split from each other. Also, neutral markers serve as reference points on maps of human and chimpanzee chromosomes.

In sharp contrast, coding genes are those that actually cause an embryo to develop into a human or a chimpanzee (e.g., Zhang et al. 2002). Most mutations that lead to new alleles at coding genes have a negative influence on reproductive fitness: familiar examples include albinism and hemophilia. On the other hand, beneficial alleles can be maintained via balancing selection. For instance, allelic variants at major histocompatibility complex (MHC) genes can help prevent infections (Edwards and Hedrick 1998, Penn et al. 2002). This is important to wildlife biologists because if MHC alleles are lost due to stochastic events (genetic drift), a population may become more prone to extinction because it will be unable to cope with future disease outbreaks (Hedrick et al. 2003).

The neutrality of molecular markers is often put forth as a null hypothesis in population genetics. Unless noted otherwise, most of the markers I discuss are neutral with respect to natural selection. Some exceptions to neutrality are explored in Gemmell et al. (2004) and Lim et al. (2004).

Allozymes.—The stalwarts of molecular biology, allozymes represent allelic (i.e., different) forms of enzymes, and they represent the products of protein-coding genes (Selander 1976). Allozymes were discovered in the 1960s and became widely used in the 1970s, particularly with respect to evaluating the genetic structure of populations (Wright 1978). In recent years, they have fallen out of favor for 2 reasons: allozyme assays typically require fresh tissue (often from the liver, kidney, or other internal organs), and they are relatively depauperate of genetic variation (i.e., they have low levels of polymorphism; generally only 1–4 alleles per locus). However, allozymes are probably the easiest markers to develop, and they work well for some assays, such as quantifying gene flow or delineating species. Further, allozymes are inexpensive yet still provide great insight into population genetic diversity.

For example, allozyme loci can be used in comparative assays of genetic diversity—to ask questions such as, "are more individuals heterozygous in population 1 than in population 2?" Recall that heterozygotes (AB) inherit nonidentical alleles from their mother and father, whereas homozygotes (AA) inherit the same alleles from both parents. Allozymes also can be used for relatedness, parentage, or kinship applications (Queller and Goodnight 1989), but they generally have been replaced by microsatellites, which tend to be more variable and hence more powerful markers. Also, because allozymes are functional proteins, they are prone to natural selection and thus may not serve as completely neutral markers (e.g., Koehn and Hilbish 1987).

Mitochondrial DNA.—Mitochondrial DNA, or mtDNA, was recognized as a potentially powerful genetic marker in the late 1970s. Before then, this cytoplasmic genome was thought to be an interesting evolutionary relict from a time when eukaryotic cells were first invaded by the bacteria-like ancestor of modern mitochondria (Margulis 1981). Multiple mtDNA molecules are found within each of hundreds of mitochondria located in the cytoplasm of eukaryotic cells; the more familiar nuclear genome—where all other genetic markers reside—is found within the nucleus. For many reasons, mtDNA is a valuable molecular marker; most notably, it is usually maternally inherited without recombination, and thus the molecular signals of genetic drift are particularly robust (Avis 2004a).

Unlike diploid nuclear loci such as allozymes or microsatellites, one cannot assess heterozygosity using mtDNA because there is no paternal contribution. In other words, mtDNA is haploid (1n) as opposed to diploid (2n); geneticists refer to mtDNA haplotypes instead of alleles to reinforce this distinction. mtDNA diversity differs from nuclear DNA diversity in that it is distributed among individuals rather than within and among individuals. This can be advantageous for those who want to address lineage-specific issues (e.g., taxonomy; McCluckie et al. 1999). mtDNA has been used widely for the study of population structure because it often shows the evolutionary footprint of differential dispersal between the sexes (Prugnolle and de Meeus 2002).
Microsatellites.—A decade after the development of mtDNA as a molecular marker, geneticists recognized that (chromosomal) repetitive elements in the nuclear genome could be used as genetic markers. Microsatellite loci are DNA sequences that contain short, repetitive elements whose copy number varies among alleles (Fig. 1). Because they are found thousands of times in vertebrate genomes and are not expressed, microsatellites generally are considered to be neutral markers—barring tight linkage to a functional gene. Microsatellite sequences can be replicated in vitro by means of the polymerase chain reaction (PCR; Erlich and Arnheim 1992). In Figure 1, allele 1 has 4 copies of a dinucleotide (AC) repeat, making the total length of this allele 50 nucleotides. Allele 2 has 7 copies of the same repeat motif, with a total size of 56 nucleotides. Alleles of different length can be distinguished via electrophoresis (Fig. 2). Genetic diversity, expressed as the number of alleles found in a population and the average heterozygosity within the population, is generally high at animal microsatellite loci. Some microsatellites exhibit >50 alleles per locus, and heterozygosities (i.e., the mean percentage of individuals heterozygous at a given locus) can approach 100% in randomly mating populations with large, long-term effective population sizes (DeWoody and Avise 2000). Of course, not all animals display this level of variability, but in general, microsatellites are the most informative molecular marker and thus are invaluable for the study of relatedness and parentage in natural populations.
Single Nucleotide Polymorphisms (SNPs).—At SNP loci, the nucleotide occupying a specific site in the genome may differ among chromosomes and individuals (Fig. 3). Single nucleotide polymorphisms are relatively new genetic markers that may or may not be selectively neutral depending on whether they are derived from a coding gene. The main advantage to using SNPs over microsatellites is that hundreds or thousands of markers can be assayed simultaneously (Kwok 2001). There are 2 primary disadvantages to using SNPs, at least as applied to most nonmodel organisms (e.g., wildlife). First, discovery of SNPs is not trivial and often requires large-scale sequencing efforts at the genomic level (Aitken et al. 2004). Second, individual SNPs typically display only 2 alleles; thus, on a per-locus basis most other molecular markers (e.g., microsatellites) are more informative (Glaubitz et al. 2003).

Sex-chromosome Markers.—In mammals, females bear 2 copies of the same sex chromosome (homogamic) and are designated XX; males are the heterogametic sex (XY). The X and Y chromosomes do not (for the most part) recombine with each other; thus, the Y chromosome reflects only the paternal history of a population. In birds, the situation is reversed, with females being heterogametic (ZW) and males being homogametic (ZZ). Microsatellites and SNPs can be isolated from sex-chromosomes and then used to serve as haploid analogues to mtDNA assays. In mammals, paternally inherited Y-chromosome markers can be used in conjunction with maternally inherited mtDNA to reveal surprising insights into population biology (e.g., Evans et al. 2001).

Marker Summary.—There are many cases in which molecular markers can be used to answer questions relevant to wildlife biologists (Table 1). Below, I emphasize applications associated with parentage, relatedness, and fitness data through the use of specific examples. For a thorough summary of the myriad methods involved in parentage analyses, see Jones and Arden (2003). Likewise, see Blouin (2003) for a recent review of the relatedness literature.

GENETIC PARENTAGE
Parentage and Breeding Success

Who breeds with whom? This question has important implications not only for sexual selection theory, but also for applied wildlife management. For example, age-structured harvests have been considered or implemented in many species from large-mouthed bass (Micropterus salmoides; slot-limits) to white-tailed deer (Odocoileus virginianus; quality deer management). Such management schemes often make assumptions (usually implicitly) about the relative reproductive success of var-
ious age classes. Only rarely, however, has breeding success been evaluated empirically for various age classes (Coltman et al. 2003). With modern molecular markers, breeding success can be assessed in astonishing detail. Consider the example of a small, freshwater fish, the mottled sculpin (Cottus bairdi). Fiumera and colleagues (2002) sampled 455 adults and/or subadults and offspring from 22 nests in a closed, local population. Their collections represented nearly the entire reproductive output of the population for that breeding season. By genotyping each adult and/or subadult and over 1,200 embryos at 5 microsatellite loci, they were able to estimate the reproductive success of each adult. They also were able to make broad conclusions about the mean number of mates per male (2.8) and per female (1.0), the effective number of breeders (54), and using a capture–mark–recapture framework described below, the number of potentially breeding adults (570). These techniques could become an important tool for managers to conduct intensive breeding assessments for many wildlife species.

**Monogamy, Promiscuity, and Cuckoldry**

Appearances can be deceiving. So learned ornithologists near the end of the twentieth century when geneticists revealed that 90% of bird species are sexually promiscuous despite the prevailing notion that polyandry (i.e., multiple mating by females) was virtually nonexistent. These molecular genetic data overturned decades of dogma, and ultimately caused a true paradigm shift with regard to mating system theory. Griffith et al. (2002) reviewed >100 genetic studies of avian taxa and found that even among socially monogamous species >10% of the offspring are the product of extra-pair paternity (i.e., cuckoldry). Despite scores of detailed behavioral studies by dedicated ethologists, most birds manage to hide their infidelity not only from their mates, but also from biologists. This is somewhat ironic, as in many respects birds are the most conspicuous vertebrates; breeding adults are often colorful, noisy, and diurnal. If biologists are fooled by birds, what about other more secretive animals?

As might be expected, the disparity between social (observed) and genetic (actual) mating systems is not restricted to birds; sunfish (DeWoody and Avise 2001), lizards (Bull et al. 1998), and primates (Schülke et al. 2004) also cuckold their mates. Social mating system aside, most vertebrates are genetically promiscuous to some degree (Birkhead 2000). Wildlife examples abound: deer (DeYoung et al. 2002, Sorin 2004), mice (Baker et al. 1999b), bats (Rosset et al. 2000), birds (Griffith et al. 2002), alligators (Davis et al. 2001), lizards (Zamudio and Sinervo 2000), and newts (Jones et al. 2002) all mate multiply (Table 2).

The evolutionary forces which drive multiple mating are hotly debated (Birkhead 2000). Multiple mating is clearly advantageous to males; their reproductive success is expected to increase as their
number of mates rises. It is not quite so easy to explain why females mate multiply, but potential reasons include enhanced genetic diversity of offspring, nuptial gifts offered by courting males, and fertility assurance (Stockley et al. 1993). Some females mate multiply because they are coerced; they apparently incur no evolutionary benefit in terms of increased offspring survivorship and seem to merely tolerate multiple matings (Lee and Hays 2004).

As with evolutionary causes, the physiological causes underlying mating behavior are seldom known. However, recent evidence suggests that a single gene may be responsible for monogamy vs. promiscuity in voles of the genus Microtus. Lim et al. (2004) showed the vasopressin V1aR gene regulates pair-bond formation in Microtus. In particular, the gene is highly expressed in monogamous prairie and pine voles but not in promiscuous meadow or montane voles. The difference in expression is attributed to an expanded microsatellite in the gene regulatory region of the monogamous voles relative to the promiscuous species. It certainly is premature to suggest the V1aR gene dictates mating behavior in other species, but gene function often is widely conserved across taxonomic boundaries, suggesting that this gene might also influence mating behavior in other species. The work of Lim et al. (2004) is a beautiful example of how molecular genetics can illuminate organismal biology.

Sexual Serendipity

Occasionally, genetic appraisals of mating systems can lend insights into previously unrecognized aspects of organismal behavior. For instance, Zamudio and Sinervo (2000) found that many juvenile lizards were sired posthumously (i.e., their fathers died long before they were born) and that 1 phenotypic group of males used this strategy more effectively than the other 2 phenotypic groups, perhaps due to highly competitive sperm or to cryptic female choice. In another example, microsatellite assays of large-mouthed bass (an exceptionally well-studied species) revealed that they are often genetically monogamous, much to the surprise of many fisheries biologists (DeWoody et al. 2000a). Finally, during a genetic parentage analysis of the knobbed whelk (Busycon carica), Avise et al. (2004) found that these whelks are actually genetically dioecious (separate sexes) although they were previously thought to be sequential hermaphrodites.

Sex-ratio Adjustment

In bird species where one sex is more costly to raise than the other (e.g., most sexually dimorphic species), breeding adults may evolve the ability to manipulate the sex ratio in their clutches in order to increase their net reproductive output. For example, female common grackles (Quiscalus quiscula) weigh considerably less than males of similar age and thus require less food; presumably, harsh environmental conditions favor clutches skewed towards females because mortality is higher in males (Howe 1977). Several other (competing) hypotheses addressed the general trend of male-biased mortality, but critical tests were lacking, in part because most chicks cannot be sexed based upon morphology alone. In this context, Sheldon
et al. (1998) used gender-specific DNA markers to sex collared flycatcher (Ficedula albicollis) chicks and determined that differential nutritional requirements probably are responsible for male-biased mortality—a post-ovulatory sex-ratio adjustment mechanism. Interestingly, pre-ovulatory mechanisms of sex-ratio adjustment also have been identified with the help of DNA markers. Female Seychelles warblers (Acrocephalus sechellensis) produce sex-biased clutches depending on the quality of their territory. Male-producing eggs are disproportionately found in low-quality territories, whereas female-producing eggs are found in high-quality territories (Komdeur et al. 1997, Komdeur 2003). These and other molecular studies of gender in hatchlings are possible because sex determination is known to be genetic in birds.

However, not all species have genetic sex-determination mechanisms. For example, environmental conditions determine sex of some reptile eggs (e.g., temperature-dependent sex determination in turtles) and some fishes (e.g., behavior dependent sex determination in some ciclids). Despite decades of research in this arena (Bull 1983), the mode of sex determination is still unknown for many, if not most, species. Avise et al. (2004) used molecular markers in whelk to first prove that sex determination was indeed genetic and then to estimate the sex ratio in breeding adults as well as in embryos.

Collectively, these studies illustrate the power of molecular markers in addressing a fundamental population parameter, the sex ratio. In many cases, the sex ratio is impossible to estimate morphologically.

Immune System and Mate Choice

Recent molecular work suggests that many animals, including humans, mice, lizards, and salmon, choose their mates based partly on their MHC genotype (Potts et al. 1991, Wedekind and Füri 1997, Landry et al. 2001, Olsson et al. 2003). The idea is that animals prefer mates with dissimilar MHC types. Why? Many MHC genes code for proteins that are integral to the immune system; they help animals differentiate between self and nonself. Natural selection favors MHC heterozygotes over homozygotes because heterozygotes can recognize a wider variety of pathogens and then initiate a specific immune response. Mate choice based on MHC is thought to operate through negative assortative mating (opposites attract), and is presumably maintained because MHC-diverse progeny have greater evolutionary fitness than MHC-similar progeny. For example, male white-tailed deer can afford to expend the energy necessary to grow large antlers because their MHC diversity makes them less prone to infections than their MHC homozygous brethren. In other words, antlers serve as an honest indicator of genetic quality and thus play an important role in mate choice (Ditchkoff et al. 2001).

Cannibalism and Predation

Surprisingly, genetic parentage analyses also can be informative with regards to cannibalism. Cannibalism is common in many organismal groups (Elgar and Crespi 1992), including fishes (Lindström 2000), amphibians (Pfenning 1999), reptiles (Rootes and Chabreck 1993), birds (Margalida et al. 2004), and mammals (Mattson et al. 1992). However, the evolutionary benefits to cannibalism vary depending on the circumstances. In the context of this review, an obvious question arises: do animals cannibalize relatives? Behavioral experiments in the laboratory suggest that some amphibians do (Pfenning and Collins 1993, Walls and Blaustein 1995), but genetic markers are required to answer this question in wild animals. DeWoody et al. (2001) collected cannibalized embryos from the stomachs of guardian male fishes and genotyped them using microsatellite markers. Even when unrelated embryos were available as a food source, the cannibal often ate his own genetic offspring, thus clearly documenting the occurrence of filial cannibalism in nature—presumably because sires cannot detect olfactory cues from encapsulated embryos. American alligators (Alligator mississippiensis) are also cannibalistic, and in some populations cannibalism is responsible for most juvenile (50.2%) and adult (63.7%) mortalities (Rootes and Chabreck 1993). Molecular assays of remains retrieved from the cannibal's stomachs (sensu DeWoody et al. 2001) could determine if alligators also prey upon close relatives. In theory, filial cannibalism should be rare or nonexistent unless the benefits (e.g., potential increased reproductive output in the future) outweigh the costs (e.g., decreased contemporary reproductive output).

Of course, molecular analyses are not restricted to cannibalism per se. Many authors have recently employed genetic techniques to assess diets because, in the words of Scribner and Bowman (1998), "the highly degraded state of many prey samples from gastrointestinal tracts often precludes unambiguous identification." Those authors used microsatellites to identify waterfowl species preyed upon by glaucous gulls (Larus hyperboreus) and
found that goslings of several goose species fall prey to gulls, but eider offspring rarely do. For a thorough review of cannibalism and predation as assessed by molecules, see Symondson (2002).

EXTENDED KINSHIP AND RELATEDNESS

Relatedness, or the identical fraction of 2 genomes, is not merely of academic interest. Many animals (including humans) actively avoid mating with close relatives, because those sexual unions lead to inbred progeny. How do wild animals gauge the relatedness of potential mates? No one knows for certain, but 1 school of thought suggests the same MHC molecules involved in disease resistance play a role via olfaction. In this context, animals choose MHC-dissimilar mates not because of the specific benefits associated with high MHC diversity, but because potential mates that have dissimilar MHC genes probably have many unlike genes (i.e., mean relatedness between potential mates is low). They also choose dissimilar mates because their progeny enjoy general benefits conferred by greater overall heterozygosity. In other words, the MHC may serve as a proxy for overall genome-wide relatedness (Grob et al. 1998).

Genome-wide relatedness (r) between 2 individuals ranges from zero to 1 depending on the coancestry between individuals x and y (Lynch and Ritland 1999). For completely unrelated individuals whose genomes contain no alleles that are identical by descent, r = 0. Conversely, r = 1.0 only for clonemates or identical twins. In theory, r = 0.5 between first-order relatives (e.g., parent-offspring or full-siblings) and r = 0.25 between second-order relatives (e.g., half-sibs or grandparent-grandchild). In practice, first- and second-order relatives can be distinguished from one another (and from unrelated individuals) using molecular markers, but third-order relatives such as cousins (where expected r = 0.125) often cannot be distinguished from any other relationship category because the error (sampling variance) associated with the estimate usually exceeds r itself (Blouin et al. 1996, Glaubitz et al. 2003).

Nevertheless, within appropriate bounds this quantitative framework allows critical tests of myriad hypotheses regarding mate choice (Rudnick et al. 2005), kin selection (Richardson et al. 2002a), and inbreeding (Keller and Waller 2002).

Most biologists are at least vaguely aware of the detrimental effects of inbreeding (termed inbreeding depression), but recent studies drive home the point; offspring derived from related parents have higher mortality rates and lower fecundity (Amos et al. 2001, Bean et al. 2004). This is of interest to wildlife biologists because reintroductions using animals translocated from a single, local population probably consist of at least some relatives and thus, the mortality of their offspring could be elevated. For example, elk (Cervus elaphus) harems often consist of related females, and thus reintroduction efforts that rely on translocating wild elk from a donor population should attempt to capture individuals from different harems so as to reduce overall relatedness (Williams et al. 2002).

Many species have a social structure that is less pronounced than that of elk (e.g., bears); thus, genetic structure and relatedness may not be obvious. In those cases, molecular genetic data can be especially informative. Consider a situation where bears are to be translocated from a donor population to a recipient population. Using noninvasive sampling (Taberlet et al. 1999), one could collect hair follicles from baited sampling stations that are visited by individual bears. These hair samples could be used to generate DNA fingerprints, and those data compared among all bears sampled to estimate relatedness. Such data could reveal that the bears visiting stations A and B are actually first-degree relatives (e.g., full-siblings); thus, the translocation efforts might want to exclude 1 of these 2 individuals.

The power to estimate relatedness using DNA markers is positively correlated with (1) the genetic variability of the markers employed and (2) the number of markers employed. With regards to the former, microsatellites are clearly superior to all other existing molecular markers. On the other hand, SNPs are much more numerous and (in theory) can be genotyped at literally thousands of loci throughout the genome. All things considered, microsatellites are probably the best available markers for estimating relatedness in wildlife species (Blouin et al. 1996, Glaubitz et al. 2003).

Social Structure

Genetic structure (i.e., the partitioning of genetic variance within and among populations) and social structure are inextricably linked. For example, the eusocial African naked mole-rat (Heterocephalus glaber) forms societies unique among mammals; they form underground colonies containing scores of nonbreeding workers and a single reproductive queen (Honeycutt 1992). In this regard, they are not unlike the Hymenopteran insects (ants, wasps, bees). Molecular genetic analyses have revealed that naked mole-rats are extraordinarily inbred because of their (eu)social structure (Reeve et al. 1990).
One can think of many wildlife species where social structure is known (at least superficially), but little is known about the relatedness of individuals within social groups. Consider quail coves or pescary sounders. Do these social groups consist of random draws from the local gene pool, or are they composed of close relatives? And how dynamic are the social groups? Do individuals flow from 1 group into another, or are they truly discrete entities? Molecular markers can address these issues if they are used in an appropriate experimental design that considers: (1) the intensity of the sampling regime (are all the pescaries in a social group sampled, or only a fraction?); (2) seasonal changes in the composition of social groups (quail coves in the early summer may consist largely of siblings, but coves sampled in the fall may be admixed); and (3) population-specific issues (social groups in 1 population may consist of relatives because habitat condition is good and juvenile dispersal is low, but poor habitat in a second population could force juvenile dispersal).

Both empirical and theoretical studies of genetic structure have long been considered in population biology, but the empirical relationship between social structure and genetic structure is less well-explored (although see Dobson et al. 1997 or Comer et al. 2005). Pearse and Crandall (2004) review the plethora of computer programs available to assess genetic structure.

Dispersal

Dispersal and gene flow are critical parameters to most population genetic and demographic models. Indeed, the entire capture-mark-recapture framework depends in large part on the assumption of closed or open populations (Williams et al. 2002). Closure violations are often due to dispersers, but unfortunately dispersal can be as difficult to estimate as population census size, and for the same reason: both require some sort of tag or mark. Even when detailed demographic data are available, studies of dispersal can be greatly enhanced with molecular markers. Consider banded-tailed kangaroo rats (Dipodomys spectabilis) where ecological studies suggest both sexes are philopatric, and thus the potential for inbreeding is high. Genetic studies indicate that inbreeding is actually low because of long breeding forays that, "cause genes to move further than individuals disperse" (Winters and Waser 2003). Various genetic approaches are available to detect dispersers, and 1 of the most powerful is the assignment test. In this test, one uses conventional population genetic data and the probability of a multilocus genotype to assign individuals to populations (Waser and Strobeck 1998, Berry et al. 2004).

Alternatively, dispersal can be inferred directly from parentage analyses. The assumptions associated with parentage-inferred dispersal are that multilocus genotypes (DNA fingerprints) are unique and that the mode of inheritance is known and consistent (i.e., follows Mendel's rules). In practice, these assumptions are met in most microsatellite-based parentage studies; the uniqueness of a DNA fingerprint can be evaluated statistically via the probability of identity (Paetkau et al. 1995, Waits et al. 2001), and virtually all DNA markers follow Mendel's rules (Avise 2004a). An added advantage to parentage-based approaches is that one is actually considering gene flow as opposed to ecological or demographic dispersal. Recall that from a genetic perspective, dispersal is unimportant unless the disperser's gametes are transmitted to the next generation. For instance, Jones et al. (1998b) used parentage analyses to document—without ever sampling the fish directly—that female sticklebacks deposit their eggs in multiple nests and may travel over 1 kilometer to do so. In this case, the mother's presence was inferred by her gametes alone.

ENUMERATION

Genetic markers have been a tremendous boon to biologists who need to know the size of a particular population. Capture-mark–recapture (CMR) theory has been around for decades, but many species are not amenable to techniques that require physical marks because they cannot be caught in the first place. Fortunately, individual DNA fingerprints (marks) can be generated from DNA samples and then used in a CMR context. There are 2 distinct categories of DNA-based, CMR analyses. The first relies on biological samples collected from specific individuals; often, these samples are collected noninvasively. Indeed, CMR enumeration based upon noninvasive DNA samples is rapidly evolving into a discipline of its own (McKelvey and Schwartz 2004, Paetkau 2004, Triant et al. 2005). Another category of DNA-based, CMR analyses relies on genetic parentage analyses to estimate the number of breeding adults in a population.

ESTIMATING POPULATION SIZE VIA GENETIC PARENTAGE ANALYSES

Interestingly, genetic parentage analysis affords the possibility of capturing and marking individuals
Table 3. Hypothetical reconstruction of an (unsampled) sire’s genotype using the maternal genotype and a full-sib progeny array. If the inferred genotype of the sire is sufficiently rare (i.e., the probability of identity is low enough), this genetic tag is unique. In most microsatellite studies of parentage, the mean probability of identity is less than 1 × 10^-5, suggesting that census population sizes should normally exceed 100,000 individuals before 2 unrelated individuals would share a multilocus genotype. Most local populations are not this large, and multilocus genotypes are very rarely shared (i.e., they serve as unique marks).

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<th>Individual</th>
<th>Locus 1</th>
<th>Locus 2</th>
<th>Locus 3</th>
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<tr>
<td>Known mother</td>
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<td>160/160</td>
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<tr>
<td>Juvenile no. 1</td>
<td>120/134</td>
<td>160/162</td>
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<td>Juvenile no. 2</td>
<td>120/136</td>
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<td>Juvenile no. 4</td>
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<td>Juvenile no. 5</td>
<td>122/134</td>
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<td>Inferred sire</td>
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</table>

that have never been sampled—not even noninvasively! For example, female American alligators (Alligator mississipiensis) guard nests containing dozens of eggs. Females and offspring can be captured for genetic analyses, but the sires are more wary. Sires can, however, be sampled indirectly; if one has samples from the mother and her eggs, the sire’s genotype can be inferred with great accuracy (Jones et al. 1998a, Davis et al. 2001). If most or all of the clutches in a local population are assayed in this manner, one can produce robust estimates of the number of breeding adults in a population. This is especially important in extremely polygynous species where most breeding males do not have access to females; the ratio of breeding adults to the total number of adults has major implications for the maintenance of genetic diversity.

These types of data can be considered in a CMR framework (Pearse et al. 2001). By sampling mothers and their associated hatchlings, one can infer the genotype of the sire using the principles of Mendelian inheritance (Table 3). If these genotypes, or tags, are used as the original marks, the recaptures come when: (1) a male with a matching genotype is physically captured or (2) the same male’s gametes are sampled in a subsequent breeding bout. Conversely, one could sample the males prior to the parentage analyses, in which case the inferred sire genotypes become the recaptures.

There are several caveats associated with parentage-based enumeration. First, reconstructing paternal genotypes from progeny arrays assumes that several offspring from each clutch are sampled from each sire. If only 2 offspring are sampled (e.g., 2 white-tailed deer fawns) and they are both AB heterozygotes and their mother is an AA homozygote, the father(s) could be a BB homozygote or he could be heterozygous for the B allele and any other allele in the population. In the latter case, the second allele went unsampled in the progeny. The point is that the stochasticity associated with Mendelian inheritance precludes the definitive assignment of a genotype unless progeny arrays are large.

A second caveat associated with parentage-based enumeration is necessary when a full-sib progeny array is sampled in the absence of genetic data on either parent. In those cases, single-locus genotypes can be inferred, but multilocus genotypes (required for individual identification) cannot be reconstructed reliably (DeWoody et al. 2000a). Notably, the genotypes of both parents can be inferred from very large half-sib progeny arrays.

**Mutation Rates and Genotoxicology**

Molecular markers are often used to study the influence of environmental pollutants on DNA (genotoxicology; Bickham et al. 2000), but here I focus on unexpected variation in pedigrees (microsatellites) and in cells (mtDNA). Although many bioassays have been developed for the study of contaminated populations, DNA is especially powerful because one can directly compare observations to expectations. In other words, genetics has a strong theoretical foundation (e.g., Mendelian inheritance) against which empirical data can be tested.

**Microsatellites**

Diploid, sexual individuals faithfully inherit 1 allele from each parent, so juveniles are genetic chimeras of their parents. Rarely, novel mutations arise in parental germines (eggs and sperm) and are transmitted to the next generation. In such cases, the mutant allele can be identified because it does not match the alleles carried by either parent (Yauk 1998). At vertebrate microsatellite loci, a mean of perhaps 0.1% of the gametes are mutants with a range of 0.001% to 3.6% (Primmer et al. 1996, Brohede et al. 2002). These rates suggest that spontaneous mutations should be encountered in surveys of just a few hundred meioses, and that is indeed the case (DeWoody et al. 1998, Jones et al. 1999, Davis et al. 2001). These baseline mutation rates can be elevated if organisms live in contaminated environments, as evidenced by barn swallows from Chernobyl (Ellegren et al. 1997) and by mice exposed to severe air pollution (Somers et al. 2004).
Theoretically, elevated mutation rates can be estimated via parent–offspring pairs if one surveys either enough loci or enough offspring. For example, 400 microsatellites are now available for the fox (Vulpes vulpes; Kukekova et al. 2004). If enough mother–offspring sets were available, the baseline mutation rate in control foxes could be estimated and compared to the mutation rate in foxes from contaminated environments. The thought of genotyping hundreds of loci in hundreds of families is daunting, but it is done routinely in humans. One can imagine the day when technology will allow similar assays in wildlife species, and then ecotoxicologists will have a new method of evaluating wildlife health.

Note that disparities between suspected parentage and true parentage normally will be revealed at multiple genetic loci (Fig. 4). Because the probability of independent mutations at multiple loci is the product of their individual probabilities, we can evaluate the likelihood of parentage exclusion vs. multiple mutations. For example, if we assume the frequency of a microsatellite mutation is 0.0001, the probability of detecting 2 mutations in the same individual at independent loci is 1 in 100 million. In most such cases, parentage exclusion is more parsimonious than multiple independent mutations.

Mitochondrial DNA

Microsatellites are not the only molecular markers that can be used for genotoxicological assays in the context of parentage analysis. Mitochondrial DNA has 2 features that make it particularly attractive for use in this regard. First, its replication machinery does not have proofreading functions that exist for nuclear DNA, leading to an approx. 10X increase in the nucleotide substitution rate in mtDNA relative to nuclear DNA. Second, the population of mtDNA molecules within an individual is usually homogenous or homoplasmic (Avise 2004a). Because ionizing radiation can cause point mutations in mtDNA molecules, and these mutations are not repaired as in the nucleus, one can look for an increased level of heteroplasmy (heterogeneity) within animals from contaminated environments (Baker et al. 1999a, Wickliffe et al. 2002).

HYBRIDIZATION

Intraspecific Hybridization with Genetically Modified Organisms

For better or worse, genetically modified (GM) organisms are part of life in the twenty-first century (Fig. 5; Avise 2004b). Fortunately, most biologists now realize that the potential benefits of GM (or transgenic) organisms must be considered alongside the potential risks. Although most geneticists are generally satisfied that the health risks associated with eating GM animals are nil, there is a substantial risk associated with GM organisms promulgating their transgenes to wild conspecifics. Agronomists as well as aquaculturists have been forced to consider this unpleasant possibility (Hallerman and Kapuscinski 1995, Muir and Howard 2002, Ellstrand 2003), and wildlife biologists should take heed as the production of transgenic game species may be inevitable.

Consider the lessons of our production-oriented colleagues; GM organisms can pose a serious threat to natural populations (Hedrick 2001). For instance, growth hormone transgenic coho salmon (Oncorhynchus kisutch) consistently outgrow (by a factor of 7) nontransgenic fish. When food becomes scarce, transgenic fish establish behavioral dominance and may resort to cannibalism of nontransgenic cohorts (Devlin et al. 2004). Additionally, large transgenic fish with a mating advantage but a viability disadvantage (e.g., low-quality gametes) can drive a wild population to extinction (Fig. 6; Howard et al. 2004).

These worrisome examples highlight negative consequences associated with gene flow from GM animals to wild animals. Theoretical transgene-invasion models (Muir and Howard 1999, Hedrick 2001, Howard et al. 2004) have not yet been evaluated in terrestrial vertebrates, but it seems likely that under certain circumstances, a transgene could rapidly spread through a wildlife population—perhaps with devastating results. For example, several artiodactyl species already have been cloned for agricultural purposes. One can imagine a deer farmer willing to release a GM animal(s) into an alleged closed herd that is contained by a high fence, only to find that escapees threaten the very existence of the wild herd. If GM wildlife species are produced, biologists will be forced to contend not only with exotic invasive species, but also with exotic invasive genes propagated by native species. Somewhat ironically, wildlife biologists faced with such scenarios may employ DNA fingerprinting techniques (themselves rooted in molecular biology) to evaluate the flow of artificial transgenes from GM strains into wild populations.

Interspecific Hybridization among Wild Species

Courtship rituals and mate choice evolved to prevent animals from copulating with undesirable intraspecific- and interspecific-mates. Nevertheless, hy-
Fig. 4. Mutation vs. misassigned parentage. Each band in a column represents the allelic profile of an individual; thus, homozygotes exhibit a single band (2 identical copies of an allele), and heterozygotes exhibit 2 bands (2 nonidentical alleles). The 2 individuals on the left are the putative mother and father of 10 juveniles. Each individual is genotyped at 3 independent (i.e., unlinked) loci. Juvenile no. 4 is genetically consistent with both putative parents at locus 2 and at locus 3 but not at locus 1. Thus, the extra band in juvenile 4 is probably a de novo mutation (see DeWoody et al. 2000b and Ibaruguchi et al. 2004 for appropriate statistical frameworks). Juvenile no. 8 is not compatible with the putative father at locus 2, and the other 2 loci also suggest that paternity in this case is attributable to a different (i.e., unsampled) male.
Hybridization is common between many species (Arnold 1997, Scribner et al. 2000), and genes often flow from one gene pool into another through the process of introgression (e.g., Beaumont et al. 2001). The role of hybridization and introgression in conservation policy is reviewed in Allendorf et al. (2001), and here I merely highlight a few case studies that are particularly relevant to wildlife biologists.

Interspecific hybridization threatens the integrity of many wildlife species, and animals often hybridize because of indirect human influences. In other words, shifts in a species’ range because of habitat changes may increase the incidence of hybridization over time. For example, Mank et al. (2004) used microsatellite data from historical museum samples to document gene flow between mallards (Anas platyrhynchos) and black ducks (A. rubripes). In little more than 50 years, the unabated introgression of mallard genes into the black duck genome reduced genetic differentiation between the species from 0.146 to 0.008. These and other data suggest the essence of black ducks may soon be lost in a sea of mallard genes.

A similar, albeit less extreme, example is found in Cervids. In western Texas, the range of white-tailed deer is spreading due to habitat changes associated with fire prevention and historical grazing practices. Cathey et al. (1998) used Y-chromosome sequences to assess the frequency of hybridization between white-tailed deer and mule deer (O. hemionus) in a contact zone. Those authors found that roughly 7.5% of the sympatric deer in a local population were hybrids and that gene flow was largely unidirectional (from male white-tailed deer into female mule deer).

Habitat changes and other indirect causes aside, humans may directly move species via introductions. These exotics may hybridize and compromise the gene pool of native species (Perry et al. 2001).

Hybridization between Wild and Domesticated Animals

Human domestication of wildlife species began 5,000-10,000 years ago and continues today. Hybridization between wild animals and their domes-
tic relatives can be a major problem (Gottelli et al. 1994), and the potential for such problems increases as the number of domesticated species grows. Deer farms are common in many parts of the world, from the United States to New Zealand. Likewise, many game birds are raised domestically and released for put-and-take hunting operations. Ring-necked pheasant (*Phasianus colchicus*) provide a convenient example of a species that has adapted to captivity to the point where domesticated birds rarely survive in nature; when they do, they could potentially pass many of their domestic traits onto wild birds and reduce the fitness of the entire population (cf. Howard et al. 2004). The dangers associated with hybridization between domestic and wild stocks have been realized by our fisheries colleagues (Garant et al. 2003, Brannon et al. 2004), and molecular analyses of parentage and relatedness can quantify the incidence of such events (McGinnity et al. 2003).

**QUANTITATIVE GENETICS AND FITNESS**

Heritability ($h^2$) is generally considered that portion of a phenotype attributable to additive genetic effects as opposed to the environment. Given that phenotypes play an important role in resource management (e.g., length limits in fisheries), it follows that quantitative studies of $h^2$ may impact management recommendations. Quantitative genetic parameters like heritability are best estimated with pedigrees, but this rarely is possible for truly wild animals. Nevertheless, molecular markers can be used to estimate genealogies required for heritability research (Ritland 2000). For example, King et al. (2001) estimated the heritability of: (1) scatulation (number of ventral and subcaudal scales) and (2) behavior (latency to move and propensity to strike) in garter snakes (*Thamnophis sirtalis*). King et al. (2001) were able make these estimates because multiple paternity is common in this species, and microsatellite analyses allowed them to identify multiple, full-sibling sireships within single litters. Similarly, Sinervo and Zamudio (2001) performed regression analyses in a quantitative genetics framework to estimate heritability of traits (throat color) associated with alternative reproductive strategies in side-blotched lizards (*Uta stansburiana*).

Unfortunately, conventional quantitative genetic models (e.g., parent–offspring regression) often make numerous assumptions about the mating system, the degree of inbreeding/outbreeding, and selection intensity. Such approaches may soon be supplanted entirely by mixed models that relax many of these assumptions while accommodating complex, multigenerational pedigrees. A comprehensive review of mixed models is beyond the scope of this paper, but Kruuk (2004) provides a thorough overview of the “animal model.”

Animal models can have great utility in studies of wild populations. For instance, Fitzsimmons et al. (1995) found that artificial selection mediated by sport hunting can have a substantial influence on the phenotype of bighorn sheep (*Ovis canadensis*). In an ingenious use of the animal model, Colman et al. (2003) quantified the intensity of harvest-mediated selection under a bighorn sheep management plan. They did so by using microsatellites to augment a partial pedigree that was based on behavioral observations of maternity. With an accurate pedigree in hand, the authors used an animal model to show that rams who would be the best breeders (in terms of horn production, the trait associated with sport hunting) are more likely to be shot at a young age. Furthermore, there was a negative association between breeding potential and lifetime reproductive success. This has resulted in a dramatic decline in the mean size of ram horns over the last decade because, “hunters have selectively targeted rams of high genetic quality before their reproductive peak, depleting the genes that confer rapid early body and horn growth” (italics added; Colman et al. 2003).

Molecular markers have now been developed for virtually every game species. Thus, wildlife biologists have the tools at their disposal to evaluate the influence of virtually any management practice on the phenotype.

**MANAGEMENT IMPLICATIONS**

Genetic markers can be used to study countless aspects of organismal biology associated with parentage, relatedness, and/or fitness. Indeed, many of the parameters listed in Table 1 can be addressed only through the use of molecular genetic markers.

Up until this point, I have focused on positive attributes of molecular markers. Of course, there are some negatives associated with genetics: (1) The requisite equipment is very expensive. Outfitting a new molecular lab costs upwards of $100,000 U.S. dollars. However, wildlife biologists can avoid this expense by finding a collaborator in academia or by contracting services to private industry. (2) The assays themselves are expensive, ranging from $2–$10 per individual (depending on the type and number of markers). While substantial, the expenses associated with genetic assays are on par with that of modern demographic techniques such as Global Positioning System.
collars. (3) Practitioners must be trained in genetics, molecular biology, and statistics. Most wildlife biologists already have rudimentary training in genetics and a strong background in statistics; the major hurdle is molecular biology, and a good collaborator can provide this training.

Much as statistics revolutionized ecology and wildlife management in the early part of the twentieth century, genetics will continue to play a prominent role in the twenty-first century. The potential of genetics to describe previously unexplained ecological phenomena is great. In particular, DNA assays of parentage and relatedness can empower wildlife biologists with data that will allow them to make more informed management decisions.

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