Ecology, 79(2), 1998, pp. 413–425

MOLECULAR CONTRIBUTIONS TO CONSERVATION

SUSAN M. HAIG

Forest and Rangeland Ecosystem Science Center, Biological Resources Division, United States Geological Survey, 3200 Southwest Jefferson Way, Corvallis Oregon 97331 USA

Abstract. Recent advances in molecular technology have opened a new chapter in species conservation efforts, as well as population biology. DNA sequencing, MHC (major histocompatibility complex), minisatellite, microsatellite, and RAPD (random amplified polymorphic DNA) procedures allow for identification of parentage, more distant relatives, founders to new populations, unidentified individuals, population structure, effective population size, population-specific markers, etc. PCR (polymerase chain reaction) amplification of mitochondrial DNA, nuclear DNA, ribosomal DNA, chloroplast DNA, and other systems provide for more sophisticated analyses of metapopulation structure, hybridization events, and delineation of species, subspecies, and races, all of which aid in setting species recovery priorities. Each technique can be powerful in its own right but is most credible when used in conjunction with other molecular techniques and, most importantly, with ecological and demographic data collected from the field. Surprisingly few taxa of concern have been assayed with any molecular technique. Thus, rather than showcasing exhaustive details from a few well-known examples, this paper attempts to present a broad range of cases in which molecular techniques have been used to provide insight into conservation efforts.

Key words: conservation genetics; genetic diversity; microsatellites; mitochondrial DNA; molecular ecology; PCR (polymerase chain reaction).

INTRODUCTION

In recent years, genetic issues have gone from relative obscurity to a significant emphasis in conservation research as modern molecular techniques revolutionize our ability to delineate relationships among individuals, populations, and species. However, some researchers have questioned the relative importance of genetic information, stating that ecological or demographic issues may be more pressing (e.g., Lande 1988, Schemske et al. 1994). As with any new field, the pendulum has swung widely as genetic techniques become part of a repertoire of tools needed to address our ever-increasing loss of biodiversity.

We have come to recognize that overlapping stochastic and deterministic demographic, environmental, and genetic factors contribute to population or species viability. Maintaining adequate levels of genetic diversity within and among populations is one critical aspect to consider, yet in some cases it may be futile to be concerned with long-term goals when habitat is being destroyed at a rate such that even short-term population survival cannot be assured. In these extreme situations, managing for high levels of genetic diversity may take a back seat to other concerns. However, molecular tools can be valuable as a means to plan for long-term genetic diversity and for clarifying demographic and ecological issues early in species recovery. For example, clarification of taxonomy can call greater or lesser attention to a particular taxon, resulting in reprioritization of conservation efforts. Defining the extent of hybridization can have a similar result. Understanding relative levels of within- and among-population differentiation can help focus efforts on specific populations in need of recovery. Finally, defining the structure of a population (e.g., founder relationships, rates of effective dispersal among subpopulations, and effective population size [N_e] leads to more accurate population management from the beginning of recovery efforts when options may be the most flexible. Thus, this paper will illustrate how molecular tools have contributed to resolving these issues in the past and the potential they hold for the future.

Historically, the field of conservation genetics has been portrayed through illustration of a few classic examples (e.g., felids, canids, and salmonids; see Avise and Hamrick 1995). These excellent studies provide a starting point for further studies of lesser known taxa and issues. In this paper, with full recognition of these pioneering efforts, I will illustrate work on some lesser known taxa in an effort to broaden our perspective on the contribution molecular genetics can make to a diversity of conservation issues. Further, I will show how molecular work is more meaningful when carried out

Manuscript received 15 July 1995; revised and accepted 15 February 1997; final version received 16 June 1997. For reprints of this Special Feature, see footnote 1, p. 359.

in conjunction with ecological and demographic studies.

Use of Molecular Markers in Defining Species and Subspecies

Taxonomic definition

The U.S. Endangered Species Act and other endangered species programs around the world (e.g., IUCN [International Union for the Conservation of Nature and Natural Resources]) identify and protect endangered and threatened species, subspecies, and populations. While these designations can be controversial from an evolutionary perspective, they can ''make or break'' recovery of specific species or groups because resources allocated for their recovery often are prioritized based on their taxonomic status (e.g., Ryder 1986, O'Brien and Mayr 1991, Ball and Avise 1992). Conversely, recognition that a species, subspecies, or population is not distinct from nonthreatened, closely related taxa can free resources that could otherwise be spent on more critical needs. With new advances in molecular techniques, these assessments are now fairly straightforward and inexpensive and are often the only means of resolving taxonomic issues.

One case in point is determination of species status for the endangered Kemp's Ridley sea turtle (*Lepidochelys kempi*) and olive Ridley sea turtle (*L. olivacea*). Prior to mitochondrial DNA (mtDNA) analyses, hypotheses were put forth that either lumped the two as one species, split them into two species, made Kemp's Ridley a subspecies of olive Ridley, or classified Kemp's Ridley as a hybrid between loggerhead sea turtles (*Caretta caretta*) and either the hawksbill (*Eretmochelys imbricata*) or green turtle (*Chelonia mydas*). MtDNA analyses indicated that the two forms were distinct from populations of other sea turtles and the mtDNA separation was great enough for the two forms to be considered for separate protection under the U.S. Endangered Species Act (Bowen et al. 1991).

Similarly, resolution of potential species status of Hawaiian Honeycreepers has helped clarify and concentrate conservation efforts. DNA–DNA hybridization studies concluded that the ancestral form was the first passerine to inhabit the Hawaiian islands (Sibley and Ahlquist 1982). Subsequent mtDNA analyses of four members of the Amakihi complex (subspecies of *Hemignathus virens*) and two outgroup comparisons indicated that full species status should be granted to *H. v. chloris* and *H. v. stejnegeri* (Tarr and Fleischer 1993). This separation helped lend greater credence to current conservation efforts for these taxa.

In some situations, species clarification can result in lumping previously separate groups. On the South Island of New Zealand, two groups of rare, flightless, chafer beetles have been traditionally considered different species (*Prodontria modesta* and *P. bicolorata*) based on a color dimorphism (Emerson and Wallis 1994). Allozyme and morphological analyses indicated that strong geographic structuring occurred at the population level, but there was no evidence for speciation. While lumping these two groups into one species could potentially take away from conservation focus on each, threats from agricultural development to the single species remain critical; hence, efforts will continue as before.

A very practical use of species-specific markers is in identification of forensic material. Baker et al. (1996) used mtDNA and a portable laboratory to identify whale and dolphin species collected from markets and restaurants in Korea and Japan. They were able to identify 18 species of Odontates, a number of regional populations or subspecies of baleen whales, and a previously unrecognized subspecies of Bryde's whale (*Balaenoptera edeni*). These results have obvious law enforcement benefits as well as taxonomic implications.

Finally, while traditional phylogenetic analyses are helpful in understanding relationships among species or higher level comparisons, little more is usually made of the results. Krajewski (1994), however, proposed comparing levels of biodiversity among different groups using phylogenetic measures that weight species according to their contribution to overall genetic diversity of a clade. For example, using results from DNA–DNA hybridization for crane species in the family Gruidae, he determined that the greatest single contributor to family diversity was the Crowned Crane (*Balearica pavonina*) and the smallest contributor was the Common Crane (*Grus grus*). He concluded that these weightings should be taken into account when species are prioritized for conservation efforts.

Hybridization

Introgressive hybridization among closely related taxa can have serious conservation, political, and economic implications because it can have both positive (e.g., increased genetic diversity, fitness, and adaptability) and negative (e.g., loss of genetic diversity, genetic assimilation, outbreeding depression) consequences for population viability. However, development of new molecular techniques provides an increasingly accurate assessment of taxonomic relationships and the history of gene flow. Most noteworthy are issues associated with hybridization within salmonids (reviewed by Allendorf and Waples 1995, Ryman et al. 1995). In these species, there is extensive hybridization among closely related native and non-native species, subspecies, and races. There are also problems related to management of captive stock used to bolster wild populations. The conservation goal is to sort out this genetic mixing to maintain distinctness of species while assuring viable populations for the future. Management of salmonid populations influences human populations

as well. For example, the economy of local and native fishermen depends on these species, while industries such as power and timber companies feel the impacts of conservation policies. One species at the heart of the debate is the cutthroat trout (*Salmo clarki*). Allozyme and mtDNA data indicate that hybridization between native Cutthroat Trout and non-native species is one of the most critical factors contributing to loss of cutthroat populations. Genetically mongrelized populations leave remaining native populations isolated from each other, increasing the risk of extinction. Current conservation efforts focus on removing some introgressed populations so that native populations may interbreed and prosper (Allendorf and Leary 1988, Ryman et al. 1995).

Hybridization has become a critical issue for canids as well (reviewed in Wayne 1995). In North America, debate stirred for years regarding the status of the red wolf (*Canis rufus*). The issue is whether the red wolf is a separate species or the result of hybridization between gray wolves (*C. lupus*) and coyotes (*C. latrans*). Red wolves went extinct in the wild in 1975 and ever since a tremendous effort has been placed in conservation efforts to restore wild populations. Both mtDNA and microsatellite data showed that red wolves are the result of extensive hybridization between coyotes and gray wolves (Wayne and Jenks 1991, Roy et al. 1994). Other instances of hybridization between these two species are detailed by Lehman et al. (1991), Wayne et al. (1992), and Garcia-Moreno et al. (1996). A similar situation exists for the simien jackal (*C. simensis*), the world's most endangered canid. MtDNA and microsatellites were used to determine that this Ethiopian wolf is a distinct species but that hybridization occurs with domestic dogs (Gottelli et al. 1994). Molecular markers have also been used to document additional cases of extensive canid hybridization in swift and kit foxes (*Vulpes velox* and *V. macrotis*; Mercure et al. 1993). In each of these cases, once hybridization is defined, difficult and controversial decisions must be made regarding whether hybrids should receive further attention and protection.

The case of the Florida panther (*Felis concolor*) is an unusual example in which molecular techniques helped point out that the potential to hybridize might result in increased population viability. Molecular analyses (allozymes, mtDNA, and minisatellites) suggest that Florida panthers have lower genetic variability than other pumas (Roelke et al. 1993) and have suffered from severe negative effects of inbreeding (O'Brien 1994). This has resulted in highly malformed sperm (95%) and a dramatically increasing incidence of cryptorchidism, a heritable defect causing undescended testicles in males (Barone et al. 1994). Since the current gene pool is clearly depauperate, plans are underway to introduce a Texas subspecies, *F. c. stanleyana*, to Florida in the hope that hybrids will be viable. This subspecies most likely interbred with Florida panthers in the 19th century (O'Brien 1994). While controversial, this plan would not only increase the gene pool but would contribute to improved demographic structure of the population by increasing reproduction.

Hybridization is common among avian species (Grant and Grant 1992), but few hybrid taxa have surfaced as significant conservation issues (although, see Avise and Nelson 1989, Zink and Kale 1995). Perhaps the most extensive example of avian hybridization occurs among Mallard-like ducks in the genus *Anas*. Here, allozyme and/or mtDNA results suggest extensive hybridization between Mallard Ducks (*A. platyrhynchos*) and Black Ducks (*A. rubripes*; Ankney et al. 1986, Avise et al. 1990), Hawaiian Ducks (*A. wyvilliana*; Browne et al. 1993) and New Zealand Grey Ducks (*A. superciliosa superciliosa*; Gillespie 1985, Rhymer et al. 1994). Swamping of the non-Mallard gene pool has occurred, in part, due to loss of habitat and behavioral dominance of male Mallards. In each case, the result has placed the integrity of the non-Mallard species in question.

One other recent avian example lies with the recent discovery of hybridization between Northern Spotted Owls (*Strix occidentalis*) and Barred Owls (*S. varia*) in the North American Pacific Northwest (Hamer et al. 1994). The situation leading to this hybridization is proposed to be the result of loss of old-growth habitat used by Spotted Owls and encroachment of Barred Owls into suboptimal Spotted Owl habitat. Currently, RAPD (random amplified polymorphic DNA) and microsatellite analyses are being used to assess the extent of this hybridization (S. M. Haig and T. D. Mullins, *unpublished manuscript*).

Outside of the vertebrates, hybridization is rarely identified as a conservation issue. It is likely that more examples will arise as the use of molecular techniques becomes more common and as other taxa are taken more seriously in conservation assessments. In plants, there are a few examples where genetic analyses have been carried out to resolve conservation issues (reviewed in Rieseberg 1991). For example, introduction of domestic herbivores on Santa Catalina Island, California, caused significant decline in the Catalina mahogony (*Cercocarpus traskiae*), California's rarest tree. The population, which has declined from 40 to <7 individuals, is now also threatened with genetic assimilation via hybridization with more common species: *C. betuloides* Nutt. ex T. and G. var. *blancheae* (C. K. Schneid) Little. Allozymes indicated low genetic diversity in *C. traskiae* and extensive hybridization (Rieseberg et al. 1989). Current conservation efforts include destruction of hybrids, establishment of pure *C. traskiae* in several locations, and removal of non-native herbivores.

Use of Molecular Markers in Defining Population Viability

Within- and among-population genetic diversity

The partitioning of genetic diversity within and among populations is a major contribution that conservation geneticists can make towards evaluating population viability. Evaluation of gene flow, or lack thereof, provides an assessment of populations that are most genetically depauperate, most fragmented, and closest or most distant to others. This translates into identification of populations in need of new individuals, populations that could safely donate individuals to more vulnerable populations, or populations in need of further demographic or environmental consideration. These factors are used administratively in assessment of recovery needs, progress towards recovery, and allocation of funds relative to other species or populations. Further, while combining genetic results with extensive field data is the best way to evaluate population viability, at times field data collection may be too expensive or time-consuming within a defined timeframe. In this situation, molecular analyses can provide at least a rough evaluation of some of the issues at hand in a timely fashion.

There have been numerous conservation efforts requiring quantification of gene flow (Avise et al. 1995), with some of the most complete work carried out on endangered desert fishes of the southwest United States (Vrijenhoek 1995), sea turtles (Bowen and Avise 1995), and whales (Baker and Palumbi 1995). Much of the work has dealt with species that, until the development of molecular techniques, were untrackable in the wild. Efforts are now focused on surveying additional species or taxa and on using several molecular techniques to provide a more comprehensive picture of their population biology and status.

In recent studies of vulnerable plant species, allozymes have been used most often in describing population structure (e.g., Godt et al. 1996, Hall et al. 1996, Sun 1996, Young and Brown 1996). For example, within- and among-population genetic diversity was low for 19 populations of the threatened wetland herb, *Helonias bullata* ($H_{es} = 0.05$ for the species; $H_{ep} = 0.03$ within populations; Godt et al. 1995). Ironically, some of the smallest populations had the highest genetic diversity. This result was attributed to their status as relictual populations. However, since the overall result was low genetic diversity and habitat was limited and isolated, habitat conservation measures are now being taken to preserve the species. Similarly, a rare Hawaiian fern (*Adenophorus periens*), known only from one population on the island of Hawaii, had extremely high levels of allozyme diversity relative to two more widespread congeneric species (Ranker 1994). In this case, more pressing concerns such as adverse effects from volcanic emissions were considered proximate threats to the species, regardless of their genetic status. Plans are now underway to transplant some individuals away from danger.

No allozyme diversity was found in the rare northwest North American aquatic plant *Howellia aquaticus* (Campanulaceae; Lesica et al. 1988) or in *Lactoris fernandeziana* Phil., a plant found on the Juan Fernandez Islands of Chile and the only member of the family Lactoridaceae (Crawford et al. 1994). A more genetically comprehensive study of the endangered, endemic Santa Cruz Island bush mallow [*Malacothamnus fasciculatus* (Nutt.) Greene var. *nesioticus*] utilized allozymes, RAPDs, and ribosomal DNA to evaluate its status relative to other varieties within the species (Swensen et al. 1995). The two populations had unique RAPD and allozyme alleles, rendering them different from the other varieties. Further, the only genetic diversity within either population was detected by rDNA analyses. Based on this low genetic diversity and ecological threats to their limited habitat, plans are now proceeding to protect the variety by fencing individuals and implementing controlled burns to allow resprouting from root stocks.

A similar approach involving RAPDs has been used to evaluate translocation strategies for Corrigan grevillea (*Grevillea scapigera* A. S. George), a woody perennial from western Australia (Rossetto et al. 1995). Pushed to the brink of extinction due to massive habitat loss, the species has been reduced to four populations with a total of 27 plants. RAPD data indicated little difference among populations, thus it was concluded that translocations would not result in negative genetic effects. RAPD data were also used to identify variable individuals to be used for recovery efforts.

Minisatellite DNA, RAPDs and allozymes have also been used to evaluate translocation options for the endangered Red-cockaded Woodpecker (*Picoides borealis*), a species suffering from habitat fragmentation of old-growth pine in the southeastern United States Minisatellites were used to identify population structure in a small South Carolina population in need of translocation so that a subsequent population viability analysis could estimate the number of individuals needed to maintain a stable population (Haig et al. 1993*a*, *b*). Choosing potential donor populations was carried out using RAPDs and allozymes. Results from both techniques, sampling similar populations throughout the species range, did not detect significant differentiation among local populations (Stangel et al. 1992, Haig et al. 1994*a*). However, significant population differentiation was apparent (high F_{ST}) when distant populations were compared (Haig et al. 1996). Thus, it was recommended that birds could be moved among local populations if donor populations could withstand the loss and habitat conditions were similar between the

two populations. Movements between regions were discouraged due to greater genetic differences among distant populations.

The threatened black rat snake (*Elaphe o. obsoleta*) and eastern Massasauga rattlesnake (*Sistrurus c. catenatus*) have both suffered extreme population fragmentation in Canada in recent years due to habitat loss. However, estimates of nucleotide diversity within populations and sequence divergence between populations were derived from RAPD data and suggested that populations were not genetically differentiated in either species (Gibbs et al. 1994). Since population fragmentation has been recent, genetic factors may not be important to address at this time and future efforts can focus on other factors affecting species viability.

Similarly, amphibian populations are suffering decline in many parts of the world (Blaustein et al. 1994). Concern over the status of isolated northern leopard frog (*Rana pipiens*) populations in Utah and Arizona led Kimberling et al. (1996) to investigate genetic distance among populations using RAPDs. They found significant population differentiation and reduced genetic diversity in several of these populations. These populations are now under consideration for better protection.

Mitochondrial DNA (mtDNA) variation has also been used as a means of quantifying within- and among-population diversity. The utility of these markers is somewhat controversial due to maternal inheritance and other factors (reviewed in Moritz 1994, Avise 1995), yet mtDNA appears suitable in situations where there is distinct philopatry along matrilineal lines or where populations are somewhat sedentary. One helpful example is the case of the endangered eastern barred bandicoot (*Perameles gunnii*), a species confined to Tasmania and a city park in Hamilton, Australia. No genetic diversity was resolved within or among these populations using allozymes (Sherwin et al. 1991). RFLP (restriction fragment length polymorphism) analyses of mtDNA indicated high variability within the Hamilton population and none in the Tasmanian population (Robinson 1995). RFLPs also distinguished animals from east and west Hamilton, as well as north and south Tasmania. MtDNA sequence divergence between the Tasmanian and Hamilton populations was high (8.21%). Thus, recommendations for recovery of the Hamilton population did not include introduction of Tasmanian animals and strategies were designed to reduce inbreeding.

The future in defining population structure clearly lies in further development and use of microsatellites (see Parker et al. 1998). One of the first microsatellite studies to address interpopulation differences was carried out on black bears (*Ursus americanus*) at three national parks in Canada (Paetkau and Strobek 1994). While allozyme studies of black bears had concluded that there was low genetic diversity and effective population size (Wathen et al. 1985), two of the three populations sampled with microsatellites had heterozygosity values that were an order of magnitude higher than the highest values reported from allozyme work. A similar approach was used for population studies of polar bears (*U. maritimus*). Use of microsatellites revealed differentiation among populations as well as populations with limited gene flow (Paetkau et al. 1995). In both cases, the increased number of variable loci found in microsatellites greatly increased the detail with which populations could be described and differentiated, thus providing better resolution for identifying genetically depauperate populations.

A more detailed microsatellite study involves identifying genetic diversity following a severe bottleneck in koalas (*Phascolarctos cinereus*) in Australia (Houlden et al. 1996). Some populations had much lower genetic diversity than others. These animals have been translocated to a number of sites since the 1920s when the bottleneck occurred, but often the new populations were founded by only a few individuals. This has resulted in some populations having low genetic diversity. Management strategies will have to be altered to reduce this trend.

Finally, one avenue of research that has not been well explored is the idea proposed by Avise (1992) regarding ecosystem-level analyses of the effect of habitat fragmentation on a number of populations for a number of species and taxa. His work in the southeastern United States has illustrated how patterns of habitat fragmentation can have similar effects on populations from widely varied taxa. Current efforts in the Pacific Northwest to address similar questions raised by President Clinton's Northwest Forest Management Plan are underway, starting with amphibians and birds (S. M. Haig, R. S. Wagner, and T. D. Mullins, *unpublished data*). In the Northwest, this approach represents not only an implementation of the Southeastern model but also an attempt to characterize many species of concern listed in the Northwest Forest Plan. Because traditional field work yields little information regarding population structure and status for many of the more cryptic, aquatic, or subterranean species, they would most likely go unrecognized without some genetic sampling to address these issues.

Population and race-specific markers

One often overlooked contribution molecular techniques can make in conservation efforts is the potential to identify population- or race-specific markers. While these markers do not confer information directly regarding population status or viability, the ability to track movements of genes or individuals over great geographic distances and throughout the annual cycle can be critical for understanding migration patterns and

habitat needs, dispersal patterns, metapopulation structure, and regulatory issues such as illegal harvesting. Some of the best examples to date are from marine species where data on annual migration patterns have allowed more comprehensive habitat protection measures to be implemented.

The humpback whale (*Megaptera novaeangliae*) has a worldwide distribution but has plummeting populations due to overharvesting. Each year, individuals from six subpopulations or stocks migrate over 10 000 km round-trip from summer feeding areas in high latitudes to winter breeding and calving grounds in low-latitude waters (Baker et al. 1994, Baker and Palumbi 1995). Variation in mtDNA restriction types among these subpopulations was used to identify distinct subpopulations, migration movements, and mixing of genetically distinct subpopulations in feeding areas (Baker et al. 1990). This potential subpopulation mixing contributes to our understanding of gene flow and effective population size for the species, as well as strategies for habitat conservation.

The green turtle is another endangered marine species whose movements were unknown until molecular markers shed some light on population structure and dispersal (reviewed in Bowen and Avise 1995). Their long life span, long time to sexual maturation, long migration distance, and the difficulty of monitoring individuals left biologists in a quandary over population structure and status. Further, green turtles, like other marine turtles, use nesting beaches that are often thousands of kilometers from their feeding areas, making it difficult to determine their breeding distributions. MtDNA genotypes have recently been shown to be population specific when populations are defined by their breeding beaches (Norman et al. 1994). These markers were not individual specific, but were specific enough to natal populations to provide an understanding of the genetic origins of individuals at feeding sites in the nonbreeding season.

Identification of run-specific markers in chinook salmon (*Oncorhynchus tshawytscha*) from the Sacramento–San Joaquin River Basin in the Central Valley of California presents an interesting conservation dilemma (Nielsen et al. 1994): fish in winter runs are protected as endangered species, but some of the races in the spawning runs are not. MtDNA markers were established for the spawning runs to facilitate identification of fish from winter runs. Now that markers are available and winter population structure can be assessed, further spawning runs may be protected. Recent analyses of single-locus minisatellite markers have further distinguished among chinook populations in British Columbia (Heath et al. 1995). These markers may be applicable to other chinook populations, as well.

Migratory birds present a problem similar to marine species with regard to understanding annual movements and population mixing. Most avian conservation efforts focus on protection of breeding sites, but recognition that birds may spend up to 80% of their time at migration and winter sites has led to renewed interest in identifying and protecting nonbreeding areas. For endangered species, identification of specific winter sites for specific breeding populations can further focus conservation efforts for specific breeding populations. For example, monitoring movements of the endangered Peregrine Falcon (*Falco peregrinus*) has been critical to planning conservation efforts. Decline of the species was largely attributed to exposure to DDT (dichloro diphenyltrichloroethane) and other hazardous chemicals, and understanding population-specific movements aided in determining where toxins were picked up. Initially, a tandem repeat was cloned from Merlin (*F. columbarius*) genomic DNA that resulted in highly polymorphic restriction patterns in Peregrines. This lead to development of population-specific markers that differentiated Greenland and Argentina populations (Longmire et al. 1988). Then the minisatellite probe M13 was used to identify fragments only present in female Peregrines. One of the sex-linked fragments also proved to separate individuals from *F. p. tundrius* (breeds in Alaska) and *F. p. anatum* (breeds in northern Mexico) when the subpecies mixed on migration (Longmire et al. 1991). Further work on these markers will provide a more complete picture of falcon movements.

In a broader sense, RAPD markers are being used to track movements of shorebird (Order: *Charadriiformes*, Family: *Charadriidae*) populations throughout the annual cycle (Haig et al. 1997). Because conventional bind-banding has limited applicability when so few birds are resighted, development of molecular markers could be a far more effective means of tracking populations throughout the annual cycle. In a survey of nine species, population-specific RAPD markers were found in Dunlin (*Calidris alpina*), Hudsonian Godwit (*Limosa haemastica*), and Semipalmated Sandpipers (*C. semipalmatus*). Markers identified for Hudsonian Godwits strongly suggested either a new population or migration route. Given the tenuous status of this species, these results are now being investigated by field workers. Of additional interest, however, was calculation of a relative probability of assignment of individual birds to population sampled. These probabilities were most helpful in assigning population origin in Hudsonian Godwits, Semipalmated Plovers (*Charadrius semipalmatus*), Pectoral Sandpipers (*Calidris melanotos*), and Dunlin. They were least informative in Red-necked Phalaropes (*Phalaropus lobatus*). Not surprisingly, these results mirrored levels of population differentiation and dispersal tendencies. This work is now being carried out on the endangered Piping Plover (*Charadrius melodus*), for which some

breeding populations continue to decline in areas that should be stable. However, because many winter sites are declining or of poor quality, it may not be a breeding site problem. Thus, if markers can identify the breeding origin of winter birds, their success in winter can be traced to breeding population status and vice versa. This might change the focus of habitat protection activities to include winter sites.

Identification of population structure

Over 20 yr ago, we became aware of the potential role molecular markers could play in assessing population structure (i.e., genetic diversity, effective size, inbreeding, etc.) and status when Bonnell and Selander (1974) used allozymes to examine genetic diversity in breeding colonies of northern elephant seals (*Mirounga angustirostris*). In their landmark paper, they reported no polymorphisms were found in the 24 loci examined (reconfirmed by Hoezel et al. 1993) and concluded that this lack of heterozygosity might be attributed to population reduction due to years of hunting in the 1800s. Little attention was paid to these issues until 5 yr later when Ralls et al. (1979) began investigating the potential detrimental effects of inbreeding in small populations of captive ungulates. Subsequently, O'Brien et al.'s (1983) use of molecular markers pointed to lack of genetic diversity and subsequent decline in the cheetah (*Acinonyx jubatus*). From this point on, conservation biologists have been quick to recognize the utility of molecular techniques in quantifying and evaluating the interrelated factors that may affect population viability.

Traditionally, allozymes have been used to assess population structure (conservation applications reviewed in Avise et al. 1995). While these techniques are straightforward, often little variability is detected (particularly in birds—Barrowclough 1983). For some organisms, however, allozymes can still render critical information in a simple fashion, especially when linked with ecological or demographic information. In Wyoming, for example, bighorn sheep (*Ovis canadensis*) have declined from over 150 000 individuals to $<$7000 in 70 yr. Allozyme sampling of this population indicated that horn growth was significantly higher (up to 13%) in more heterozygous adult rams than in less heterozygous adult rams (FitzSimmons et al. 1995). Since hunting these sheep involves selective removal of large-horned rams, status of the population may be further threatened if these animals continue to be removed.

Several recent papers have illustrated that interpretation of heterozygosity and allelic diversity resulting from allozyme studies needs further attention (Leberg 1992). In cotton-top tamarins (*Saguinus oedipus*), an endangered primate from Colombia, a survey of 21 loci in 106 captive individuals resulted in average heterozygosity of 1% (Cheverud et al. 1994). However, body mass was found to be significantly heritable (h^2 = 35%). Thus, low levels of genetic diversity based on molecular markers did not correspond to a lack of quantitative genetic variation in this important character. It is likely that most important life history traits associated with fitness are also polygenic, quantitative traits (Storfer 1996). For these tamarins, results suggest there might be sufficient genetic variation that would provide for adaptation to wild conditions when a reintroduction program is initiated despite their low protein polymorphism. Additional molecular studies using microsatellites may provide greater insight into this issue.

Concern over interpretation of allozyme data as it relates to population structure, particularly in conservation situations, led Hartl and Pucek (1994) to more closely examine the relationship between heterozygosity (H) and percentage of polymorphic loci (P) in their study of a severely bottlenecked population of European bison (*Bison bonasus bonasus*) in Poland's Bialowieza Primeval Forest. They examined these two factors in their population and for similarly bottlenecked Artiodactyla species or populations. Their allozyme survey of 69 presumptive bison loci not only indicated low average heterozygosity (H = 1.2%), but this value was three times lower than results from a previous survey of 20 loci (H = 3.5%), which had concluded their status was not of concern (Gebczynski and Tomaszewska-Guszkiewicz 1987). Data from other Artiodactyla indicated that the percentage of polymorphic loci was the only factor that appeared significantly reduced in genetically bottlenecked species/populations. Further, heterozygosity was only useful as an indicator of overall genetic variability in nonbottlenecked species/populations. These results led them to suggest that genetic depletion should be considered by examining the ratio of H:P and variance of H among a number of loci.

Recent isolation of hypervariable DNA loci, such as microsatellites or minisatellites, has revolutionized our ability to identify population structure. For example, DNA fingerprints can be helpful indicators of changes in genetic diversity over short periods of time. In Guam Rails (*Rallus owsoni*), flightless birds formerly endemic to the Pacific island of Guam, DNA fingerprints, allozymes, and pedigree analyses were used to examine potential loss of genetic diversity after the birds had been extirpated from the wild and were maintained in captivity (Haig and Ballou 1995). Using the pedigree as a guide, fingerprinting indicated a 6% increase in DNA similarity (i.e., decrease in diversity) between the 21 founders of the captive population and the captive population 4 yr later (n = 105). Interestingly, allozyme data also indicated a 6% loss of heterozygosity in a similar comparison. Further, DNA similarity was high for the founders (S = 0.62) and subsequent population

($S = 0.64$), while allozyme heterozygosity was average (0.03) relative to other avian species. Thus, both techniques measured similar levels of change between subsequent generations but differed in levels of variability measured. Population managers interpreted these results to indicate the captive population structure needed to be carefully managed so as not to lose further diversity.

Estimating levels of DNA similarity in highly social alpine marmots (*Marmota marmota*) led to discovery of a past population bottleneck (Rassmann et al. 1994). Low levels of DNA fingerprint polymorphisms were detected in the Bavarian Alp population at Berchtesgaden National Park. Subsequent analyses did not indicate abnormally low mutation rates. Further, population estimates were fairly high and dispersal widespread. An explanation for these results was finally proposed when heterozygosity and effective population size were estimated from population and DNA fingerprint data. Effective population size was at least an order of magnitude lower than what was predicted from other populations. After considering the possibility of a social system leading to inbreeding, Rassman et al. (1994) concluded that, rather, the population had undergone a drastic reduction in number of breeding pairs in the past. This reduction was attributed to significant losses during harsh winters.

More specific assessments of effective population size can be made once the mating system is identified. In the endangered and cooperatively breeding Red-cockaded Woodpecker, DNA profiles were used to identify parents in several populations (Haig et al. 1993*a*, 1994*c*). Lack of evidence for extra-pair fertilizations (suspected from helpers) led to reassessment of effective population size estimates and progress towards achieving recovery goals based on N_e. Thus, helpers could not be counted in estimates of breeding birds (as had been done previously). These results were a significant contribution to a subsequent population viability analysis that evaluated various options for augmenting population growth in a small woodpecker population in South Carolina (Haig et al. 1993*b*). They are also being used in re-evaluation of the species recovery objective.

Population analyses using microsatellites are in their infancy, but the technique holds great potential for more accurate assessment of population structure. One excellent example of its potential use in conservation genetics is Taylor et al.'s (1994) work on the decline of the northern hairy-nosed wombat (*Lasiorhinus krefftii*), a species on the brink of extinction in Australia. Of three known colonies for this species, two went extinct about 1910 as a result of human activities. The third colony, discovered in 1939, may have numbered several thousand individuals in pre-European times, but only 20–30 individuals were found. Initially, both mtDNA and minisatellite DNA techniques were used to evaluate population structure in the remaining colony, in some tissue recovered from the extinct colonies, and in the closely related southern hairy-nosed wombat (*L. latifrons*). Neither technique yielded much variation among colonies. However, microsatellite variability indicated the extant colony retained 41% heterozygosity and 36% allelic diversity relative to the southern hairy-nosed wombat. These values correspond with an effective population size of <10 individuals over the past 120 yr. While these values are startlingly low, the population persists, giving hope for improved viability in the future.

Perhaps the newest genetic system to be evaluated for its relevance to population structure and conservation genetics is the major histocompatibility complex (MHC; Hedrick 1994). MHC gene products, used to identify potentially harmful foreign antigens, are extremely polymorphic. Studies to elicit population structure using MHC are just beginning (Schreiber and Tichy 1992). They have been used to describe genetic variability in populations of Swedish moose (*Alces alces*; Ellegren et al. 1996) and beluga whales (*Delphinapterus leucas*; Murray et al. 1995). As this technique is further developed, there are suggestions that captive or small populations should be designed to focus on allelic diversity in MHC genes (Hughes 1991). This controversial idea has been hotly debated as the function of specific genes is not known, hence managing for these genes would have unknown consequences (e.g., Gilpin and Wills 1991, Miller and Hedrick 1991, Vrijenhoek and Leberg 1991, Hedrick and Miller 1996). Most likely we will need some concrete examples from known populations prior to rendering judgment on this hypothesis. In the meantime, MHC polymorphisms may prove to be useful in evaluating specific population problems such as their susceptibility to disease.

Identification of unknown individuals

While characterization of general population structure is an important step in evaluating population viability, often the relationship among some individuals in the population is not known. In many cases, this may not be a problem, but in small and/or captive populations where each individual's contribution to past and future genetic diversity is critical, knowledge of relatedness is essential. For example, identification of relatedness among founders to a wild, translocated, reintroduced or captive population will help guide future population management. In some cases, identification of parentage alone will provide greater understanding of population structure. For example, pedigree relationships were derived for captive Waldrapp Ibises (*Geronticus eremita*) by comparing DNA fingerprints from each captive individual with each potential

mother–father pair (Signer et al. 1994). Using this approach, parentage was deduced for 29 of 33 birds. At that point, bird pairings could then be chosen so that their offspring would represent as diverse a population as possible.

Deriving more complex relatedness information has stymied scientists for years. In most cases, allozyme and mitochondrial data do not provide the detail necessary for such fine-tuned analyses. Minisatellites have been a great step forward, but analyses can still be problematic due to overlapping DNA similarity values for some degrees of relatedness and difficulty in scoring across autoradiograms. Microsatellites provide the best resolution to date, but have not been tested extensively (but see Blouin et al. 1996). Discussed below are several novel approaches that have proved helpful in the specific situations discussed. In all cases, note that knowledge of at least a partial pedigree has been the backbone of the analyses.

It is now becoming fairly well accepted that a significant relationship exists between minisatellite DNA similarity and relatedness values calculated from most pedigrees of wild or captive individuals (e.g., Gilbert et al. 1991, Haig et al. 1993, 1994*b*, *c*, 1995, Rave et al. 1994). The exception may be in pedigrees with highly inbred animals (Butler et al. 1994). Establishment of this relationship allows further hypotheses to be tested regarding individual relationships. For example, in Guam Rails and Micronesian Kingfishers (*Halcyon cinnamomina cinnamomina*; Haig et al. 1994*b*, 1995), relative relatedness among founders to their respective captive populations was determined by testing, via regression, various hypotheses regarding possible founder relationships. In both species, the most helpful hypothesis incorporated use of UPGMA (Unweighted Pair Group Method Analysis) cluster analyses of individual similarity values among all founders calibrated to known relatedness/similarity values from the remainder of the pedigree. Once the population structure was determined, captive pairings could be made that minimized mean kinship in captive and newly introduced populations.

Hypothesis testing was also used in deriving relatedness among several unknown captive Rothschild's Mynahs (*Leucopsar rothschildi*), an endangered species for which population management program was just beginning. Ashworth and Parkin (1992) used simple and multiple regression to examine the relationship (slope) between similarity and relatedness for various hypotheses potentially explaining the relationship among unknown birds. A more accurate, but complex approach was used by Geyer and Thompson (1995) to estimate founder relatedness among captive California Condors (*Gymnogyps californianus*). Their use of maximum likelihood estimates appears to make optimal use of all DNA similarity data, but is computationally complex and realistically impractical for pedigrees larger than 20 individuals. Finally, the potential for two previously unknown wild Speke's gazelles (*Gazella spekei*) to be considered as additional founders (i.e., unrelated to the remaining population) to the extremely inbred captive population was evaluated by estimating relatedness between the new animals and captive founders using a permutation test on all pairwise DNA similarities (Butler et al. 1994). The wild individuals were determined to be unrelated and they have now added new, desperately needed genes to the captive population.

Analysis of microsatellites is just beginning to make a contribution in individual identification. In long-finned pilot whales (*Globicephala melas*), identification of pod members via microsatellites revealed that pod structure is made up of single extended families often containing >100 individuals (Amos et al. 1993). That is, offspring of neither sex disperse, yet inbreeding is not a factor. Matings occur between members of different pods when pods happen upon each other. This remarkable social system allows males to help related females, increasing their inclusive fitness, without introducing inbreeding within the pod. The conservation implications for this and any other species with a similar social organization are daunting. If there is little or no movement between pods, as many pods as possible must be conserved to maintain existing genetic diversity.

Conclusions

In this paper, recent case studies illustrated how molecular tools can be used to address specific conservation issues. Studies that used several molecular techniques to address related questions provided a more definitive assessment of an issue than those that used only one technique. Molecular techniques also provide insight into the role of individuals in a population, relationships among populations, and differences among species. The greatest understanding of molecular information will come, however, when it is used in conjunction with ecological, demographic, behavioral, and physiological data collected in the field. In the future, the rapid evolution of new molecular techniques will enable workers to identify individuals and population structure with great resolution, and potentially to trace the effects of factors such as disease (via MHC) on population viability.

Acknowledgments

I thank P. Parker for inviting this paper, D. Simberloff for encouraging me to think more broadly about the relative importance of genetic issues in conservation, and J. Ballou for expanding my vision of conservation genetics to include pedigree analyses. P. Kareiva, T. Mullins, P. Parker, A. Snow, and S. Wagner provided helpful comments on the paper.

LITERATURE CITED

Allendorf, F., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the Cutthroat Trout. Conservation Biology **2**:170–184.

Allendorf, F. W., and R. S. Waples. 1995. Conservation and genetics of salmonid fishes. Pages 238–280 *in* J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case histories from nature. Columbia University Press, New York, New York, USA.

Amos, B., C. Schlotterer, and D. Tautz. 1993. Social structure of pilot whales revealed by analytical DNA profiling. Science **260**:670–672.

Ankney, C. D., D. G. Dennis, L. N. Wishard, and J. E. Seeb. 1986. Low genic variation between Black Ducks and Mallards. Auk **103**:701–709.

Ashworth, D., and D. T. Parkin. 1992. Captive breeding: can genetic fingerprinting help? Symposium of the Zoological Society of London **64**:135–149.

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos **63**:62–76.

———. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conservation Biology **9**:686–690.

Avise, J. C., C. D. Ankney, and W. S. Nelson. 1990. Mitochondrial gene trees and the evolutionary relationship of Mallard and Black Ducks. Evolution **44**:1109–1119.

Avise, J. C., S. M. Haig, O. A. Ryder, M. Lynch, and C. J. Geyer. 1995. Descriptive genetic studies: applications in population management and conservation biology. Pages 183–244 *in* J. D. Ballou, M. Gilpin, and T. J. Foose, editors. Population management for survival and recovery. Columbia University Press, New York, New York, USA.

Avise, J. C., and J. L. Hamrick, editors. 1995. Conservation genetics: case histories from nature. Columbia University Press, New York, New York, USA.

Avise, J. C., and W. S. Nelson. 1989. Molecular genetic relationships of the extinct Dusky Seaside Sparrow. Science **243**:646–648.

Ball, R. M., Jr., and J. C. Avise. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. Auk **109**: 626–636.

Baker, C. S., F. Cipriano, and S. R. Palumbi. 1996. Molecular genetic identification of whale and dolphin products from commercial markets in Korea and Japan. Molecular Ecology **5**:671–685.

Baker, C. S., S. R. Palumbi, R. H. Lambertsen, M. T. Weinrich, J. Calambokidis, and S. J. O'Brien. 1990. Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. Nature **344**:238–240.

Baker, C. S., and S. R. Palumbi. 1995. Population structure, molecular systematics, and forensic identification of whales and dolphins. Pages 10–49 *in* J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case histories from nature. Columbia University Press, New York, New York, USA.

Baker, C. S., R. W. Slade, J. L. Bannister, R. B. Abernathy, M. T. Weinrich, J. Lien, J. Urban, P. Corkeron, J. Calmabokidis, O. Vasquez, and S. R. Palumbi. 1994. Hierarchical structure of mitochondrial DNA gene flow among Humpback Whales *Megaptera novaeangliae*, worldwide. Molecular Ecology **3**:313–327.

Barone, M. A., M. E. Roelke, J. G. Howard, J. L. Brown, A. E. Anderson, and D. E. Wildt. 1994. Reproductive characteristics of male Florida Panthers: comparative studies from Florida, Texas, Colorado, Latin America, and North American zoos. Journal of Mammalogy **75**:150–162.

Barrowclough, G. F. 1983. Biochemical studies of microevolutionary processes. Pages 223–261 *in* A. H. Brush and G. A. Clark, editors. Perspectives in ornithology. Cambridge University Press, New York, New York, USA.

Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinction. Conservation Biology **8**:60–71.

Blouin, M. S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. Molecular Ecology **5**:393–402.

Bonnell, M. L., and R. K. Selander. 1974. Elephant seals: genetic variation and near extinction. Science **184**:908–909.

Bowen, B. W., and J. C. Avise. 1995. Conservation genetics of marine turtles. Pages 190–237 *in* J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case histories from nature. Columbia University Press, New York, New York, USA.

Bowen, B. W., A. B. Meylen, and J. C. Avise. 1991. Evolutionary distinctiveness of the endangered Kemp's Ridley sea turtle. Nature **352**:709–711.

Browne, R. A., C. R. Griffin, P. R. Chang, M. Hubley, and A. E. Martin. 1993. Genetic divergence among populations of the Hawaiian Duck, Laysan Duck, and Mallard. Auk **110**:49–56.

Butler, M. A., A. R. Templeton, and B. Read. 1994. DNA fingerprinting in Speke's Gazelle: a test for genetic distinctness, and the correlation between relatedness and similarity. Molecular Ecology **3**:355–361.

Cheverud, J., E. Routman, C. Jaquish, S. Tardiff, G. Peterson, N. Belfiore, and L. Forman. 1994. Quantitative and molecular genetic variation in captive Cotton-top Tamarins (*Saguinus oedipus*). Conservation Biology **8**:95–105.

Crawford, D. J., T. F. Steussy, M. B. Cosner, D. W. Haines, D. Wiens, and P. Penaillo. 1994. *Lactoris fernandeziana* (Lactoridaceae) on the Juan Fernandez Islands: allozyme uniformity and field observations. Conservation Biology **8**: 277–280.

Ellegren, H., S. Mikko, K. Wallin, and L. Andersson. 1996. Limited polymorphism at major histocompatibility complex (MHC) loci in the Swedish moose *A. alces*. Molecular Ecology **5**:3–9.

Emerson, B. C., and G. P. Wallis. 1994. Species status and population genetic structure of the flightless chafer beetles *Protodontria modesta* and *P. bicolorata* (Coleoptera; Scarabaeidae) from South Island, New Zealand. Molecular Ecology **3**:339–345.

FitzSimmons, N. N., S. W. Buskirk, and M. H. Smith. 1995. Population history, genetic variability, and horn growth in bighorn sheep. Conservation Biology **9**:314–323.

Frankel, O. H., and M. E. Soulé. 1981. Conservation and evolution. Cambridge University Press, Cambridge, UK.

Garcia-Moreno, J., M. D. Matocq, M. S. Roy, E. Geffen and R. K. Wayne. 1996. Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. Conservation Biology **10**:376–389.

Gebczynski, M., and Tomaszewska-Guszkiewicz. 1987. Genetic variability in the European bison. Biochemical Systematics and Ecology **15**:285–288.

Geyer, C. J., and E. A. Thompson. 1995. A new approach to the joint estimation of relationships from DNA fingerprint data. Pages 245–260 *in* J. D. Ballou, M. Gilpin, and T. J. Foose, editors. Population management for survival and recovery. Columbia University Press, New York, New York.

Gibbs, H. L., K. A. Prior, and P. J. Weatherhead. 1994. Ge-

netic analysis of populations of threatened snake species using RAPD markers. Molecular Ecology **3**:329–337.
Gilbert, D. A., C. Packer, A. E. Pusey, J. C. Stephens, and S. J. O'Brien. 1991. Analytical DNA fingerprinting in lions: parentage, genetic diversity, and kinship. Journal of Heredity **82**:378–386.
Gillespie, G. D. 1985. Hybridization, introgression, and morphometric differentiation between Mallard (*Anas platyrhynchos*) and Grey Ducks (*Anas superciliosa*) in Otago, New Zealand. Auk **102**:459–469.
Gilpin, M., and C. Wills. 1991. MHC and captive breeding: a rebuttal. Conservation Biology **5**:554–555.
Godt, M. W., J. L. Hamrick, and S. Bratton. 1995. Genetic diversity in a threatened wetland species, *Helonias bullata* (Liliaceae). Conservation Biology **9**:596–604.
Godt, M. W., B. R. Johnson, and J. L. Hamrick. 1996. Genetic diversity and population size in four rare southern Appalachian plant species. Conservation Biology **10**:796–805.
Gotelli, D., C. Sillero-Zubiri, G. D. Applebaum, M. S. Roy, D. J. Girman, J. Garcia-Moreno, E. A. Ostrander, and R. K. Wayne. 1994. Molecular genetics of the most endangered canid: the Ethiopian wolf (*Canis simensis*). Molecular Ecology **3**:301–312.
Grant, P. R., and B. R. Grant. 1992. Hybridization of bird species. Science **256**:193–197.
Haig, S. M., and J. D. Ballou. 1995. Genetic diversity in two avian species formerly endemic to Guam. Auk **112**: 445–455.
Haig, S. M., J. D. Ballou, and N. J. Casna. 1994*b*. Identification of kin structure among Guam Rail founders: a comparison of pedigrees and DNA profiles. Molecular Ecology **5**:109–119.
Haig, S. M., J. D. Ballou, and N. J. Casna. 1995. Genetic identification of kin in Micronesian Kingfishers. Journal of Heredity **86**:423–431.
Haig, S. M., J. R. Belthoff, and D. H. Allen. 1993*a*. Examination of population structure in Red-cockaded Woodpeckers using DNA profiles. Evolution **47**:185–194.
Haig, S. M., J. R. Belthoff, and D. H. Allen. 1993*b*. Population viability analysis for a small population of Red-cockaded Woodpeckers and an evaluation of population enhancement strategies. Conservation Biology **7**:289–301.
Haig, S. M., R. Bowman, and T. D. Mullins. 1996. Population structure of Red-cockaded Woodpeckers in south Florida: RAPDs revisited. Molecular Ecology **5**:725–734.
Haig, S. M., C. L. Gratto-Trevor, T. D. Mullins, and M. A. Colwell. 1997. Population identification of western hemisphere shorebirds throughout the annual cycle. Molecular Ecology **6**:413–427.
Haig, S. M., J. M. Rhymer, and D. G. Heckel. 1994*a*. Population differentiation in randomly amplified polymorphic DNA of Red-cockaded Woodpeckers. Molecular Ecology **3**:581–595.
Haig, S. M., J. R. Walters, and J. H. Plissner. 1994*c*. Genetic evidence for monogamy in the Red-cockaded Woodpecker, a cooperative breeder. Behavioral Ecology and Sociobiology **23**:295–303.
Hall, P., S. Walker, and K. Bawa. 1996. Effects of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. Conservation Biology **10**:757–768.
Hamer, T. E., E. D. Forsman, A. D. Fuchs, and M. L. Walters. 1994. Hybridization between Barred and Spotted Owls. Auk **111**:487–492.
Hartl, G. B., and Z. Pucek. 1994. Genetic depletion in the European bison (*Bison bonasus*) and the significance of electrophoretic heterozygosity for conservation. Conservation Biology **8**:167–174.
Heath, D. D, N. J. Bernier, and T. A. Mousseau. 1995. A single-locus minisatellite discriminates Chinook Salmon (*Oncorhynchus tshawytscha*) populations. Molecular Ecology **4**:398–93.
Hedrick, P. W. 1994. Evolutionary genetics of the major histocompatibility complex. American Naturalist **143**:945–964.
Hedrick, P. W., and P. S. Miller. 1996. Rare alleles, MHC and captive breeding. Pages 187–204 *in* V. Loeschcke, J. Tomiuk, and S. K. Jain, editors. Conservation genetics. Birkhauser, Basel, Switzerland.
Hoezel, A. R., J. Halley, S. J. O'Brien, C. Campagna, T. Arnbom, B. LeBoeuf, K. Ralls, and G. A. Dover. 1993. Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. Journal of Heredity **84**:443–449.
Houlden, B. A., P. R. England, A. C. Taylor, W. D. Greville, and W. B. Sherwin. 1996. Low genetic variability of the koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. Molecular Ecology **5**:269–281.
Hughes, A. L. 1991. MHC polymorphism and the design of captive breeding programs. Conservation Biology **5**:249–251.
Johnson, N. K., J. A. Marten, and C. J. Ralph. 1989. Genetic evidence for the origin and relationships of Hawaiian Honeycreepers (Aves Fringillidae). Condor **91**:379–396.
Kimberling, D. N., A. R. Ferreira, S. M. Shuster, and P. Keim. 1996. RAPD marker estimation of genetic structure among isolated northern leopard frog populations in the southwestern USA. Molecular Ecology **5**:521–529.
Krajewski, C. 1994. Phylogenetic measures of biodiversity: a comparison and critique. Biological Conservation **69**:33–39.
Lande, R. 1988. Genetics and demography in biological conservation. Science **241**:1455–1460.
Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution **46**:477–494.
Lehman, N., A. Eisenhawer, K. Hansen, L. D. Mech, R. O. Peterson, P. J. P. Gogan, and R. K. Wayne. 1991. Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. Evolution **45**:104–119.
Lesica, P., R. F. Leary, F. W. Allendorf, and D. E. Bilderback. 1988. Lack of genetic diversity within and among populations of an endangered plant *Howellia aquaticus*. Conservation Biology **2**:275–282.
Longmire, J. L., R. E. Ambrose, N. C. Brown, T. J. Cade, T. L. Maechtle, S. W. Seegar, F. P. Ward, and C. M. White. 1991. Use of sex-linked minisatellite fragments to investigate genetic differentiation and migration of North American populations of the Peregrine Falcon (*Falco peregrinus*). Pages 217–229 *in* T. Burke, G. Dolf, A. J. Jeffreys, and R. Wolff, editors. DNA fingerprinting: approaches and applications. Birkhauser Verlag, Basel, Switzerland.
Longmire, J. L., A. K. Lewis, N. C. Brown, J. M. Buckingham, L. M. Clark, M. D. Jones, L. J. Meincke, J. Meyne, R. L. Ratliff, F. A. Ray, R. P. Wagner, and R. K. Moyzis. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat region in the family Falconidae. Genomics **2**:14–24.
Mercure, A., K. Ralls, K. P. Koepfli, and R. K. Wayne. 1993. Genetic subdivisions among small canids: mitochondrial DNA differentiation of swift, kit, and Arctic foxes. Evolution **47**:1313–1328.

Miller, P. S., and P. W. Hedrick. 1991. MHC polymorphism and the design of captive breeding programs: simple solutions are not the answer. Conservation Biology **5**:556–558.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology **3**:401–411.

Murray, B. W., S. Malik, and B. N. White. 1995. Sequence variation at the major histocompatibility complex locus *DQB* in beluga whales (*Delphinapterus leucas*). Molecular Biology and Evolution **12**:582–593.

Nielsen, J. L., D. Tupper, and W. K. Thomas. 1994. Mitochondrial DNA polymorphism in unique runs of chinook salmon (*Oncorhynchus tshawytscha*) from the Sacramento-San Joaquin River Basin. Conservation Biology **8**:882–884.

Norman, J. A., C. Moritz, and C. J. Limpus. 1994. Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. Molecular Ecology **3**:363–373.

O'Brien, S. J. 1994. A role for molecular genetics in biological conservation. Proceedings of the National Academy of Sciences (USA) **91**:5748–5755.

O'Brien, S. J., and E. Mayr. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. Science **251**:1187–1188.

O'Brien, S. J., M. E. Roelke, N. Yuhki, K. W. Richards, W. E. Johnson, W. L. Franklin, A. E. Anderson, O. L. Bass, R. C. Belden, and J. S. Martenson. 1990. Genetic introgression within the Florida Panther *Felis concolor coryi.* National Geographic Research **6**:485–494.

O'Brien, S. J., D. E. Wildt, D. Goldman, C. R. Merril, and M. Bush. 1983. The cheetah is depauperate in genetic variation. Science **221**:459–461.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology **4**:347–354.

Paetkau, D., and C. Strobek. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology **3**:489–495.

Parker, P. G., A. A. Snow, M. D. Schug, G. C. Booton, and P. A. Fuerst. 1998. Molecular markers for population biology. Ecology **79**:000–000.

Ralls, K., K. Brugger, and J. D. Ballou. 1979. Inbreeding and juvenile mortality in small populations of ungulates. Science **206**:1101–1103.

Ranker, T. A. 1994. Evolution of high genetic variability in the rare Hawaiian fern *Adenophorus periens* and implications for conservation management. Biological Conservation **70**:19–24.

Rassmann, K., W. Arnold, and D. Tautz. 1994. Low genetic variability in a natural alpine Marmot population (*Marmota marmota,* Sciuridae) revealed by DNA fingerprinting. Molecular Ecology **3**:347–353.

Rave, E. H., R. C. Fleischer, F. Duvall, and J. M. Black. 1994. Genetic analyses through DNA fingerprinting of captive populations of Hawaiian Geese. Conservation Biology **8**: 744–751.

Rhymer, J. M., M. J. Williams, and M. J. Braun. 1994. Mitochondrial analysis of gene flow between New Zealand Mallards (*Anas platyrhynchos*) and Grey Ducks (*A. superciliosa*). Auk **111**:970–978.

Rieseberg, L. H. 1991. Hybridization in rare plants: insights from case studies in *Cercocarpus* and *Helianthus.* Pages 171–181 *in* D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, Cambridge, UK.

Rieseberg, L. H., S. Zona, L. Aberbom, and T. D. Martin. 1989. Hybridization of the island endemic, Catalina Mahogony. Conservation Biology **3**:52–58.

Robinson, N. A. 1995. Implications from mitochondrial DNA for management to conserve the eastern barred bandicoot (*Perameles gunnii*). Conservation Biology **9**:114–125.

Roelke, M. E., J. S. Martenson, and S. J. O'Brien. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Current Biology **3**:340–350.

Rossetto, M., P. K. Weaver, and K. W. Dixon. 1995. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Molecular Ecology **4**:321–329.

Roy, M. S., E. Geffen, D. Smith, E. A. Ostrander, and R. K. Wayne. 1994. Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution **11**: 553–570.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution **1**:9–10.

Ryman, N., F. Utter, and K. Hindar. 1995. Introgression, supportive breeding, and genetic conservation. Pages 341–365 *in* J. D. Ballou, M. Gilpin, and T. J. Foose, editors. Population management for survival and recovery. Columbia University Press, New York, New York, USA.

Schemske, D. W., B. C. Husband, M. H. Ruckelshaus, C. Goodwillie, I. M. Parker, and J. G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology **75**:584–606.

Schreiber, A., and H. Tichy. 1992. MHC polymorphisms and the conservation of endangered species. Symposium of the Zoological Society of London **64**:103–121.

Sherwin, W. B., N. D. Murray, J. A. Marshall Graves, and P. H. Brown. 1991. Measurement of genetic variation in endangered populations: bandicoots (Marsupialia: Peramelidae) as an example. Conservation Biology **5**:103–108.

Sibley, C. G., and J. E. Ahlquist. 1982. Relationships of the Hawaiian Honeycreepers (*Drepaninini*) as indicated by DNA-DNA hybridization. Auk **99**:130–140.

Signer, E. N., C. R. Schmidt, and A. J. Jeffreys. 1994. DNA variability and parentage testing in captive Waldrapp Ibises. Molecular Ecology **3**:291–300.

Stangel, P. W., M. R. Lennartz, and M. H. Smith. 1992. Genetic variation and population structure of Red-cockaded Woodpeckers. Conservation Biology **6**:283–292.

Storfer, A. 1996. Quantitative genetics: a promising approach for the assessment of genetic variation in endangered species. Trends in Ecology and Evolution **11**:343–348.

Sun, M. 1996. Effects of population size, mating system, and evolutionary origin on genetic diversity in *Spiranthes sinensis* and *S. hongkongensis*. Conservation Biology **10**: 785–795.

Swensen, S. M., G. J. Allan, M. Howe, W. J. Elisens, S. A. Junaks, and L. H. Rieseberg. 1995. Genetic analysis of the endangered island endemic *Malacothamnus fasciculatus* (Nutt.) Greene *var. nesioticus* (Rob.) Kearn. (Malvaceae). Conservation Biology **9**:404–415.

Tarr, C. L., and R. C. Fleischer. 1993. Mitochondrial DNA variation and evolutionary relationships in the 'Amakihi' complex. Auk **110**:825–831.

Taylor, A. C., W. B. Sherwin, and R. K. Wayne. 1994. Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. Molecular Ecology **3**:277–290.

Vrijenhoek, R. C. 1995. Conservation genetics of North

American desert fishes. Pages 367–397 *in* J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case histories from nature. Columbia University Press, New York, New York, USA.

Vrijenhoek, R. C., and P. L. Leberg. 1991. Lets not throw the baby out with the bathwater: a comment on management for MHC diversity in captive populations. Conservation Biology **5**:252–254.

Wathen, W. G., G. F. McCracken, and M. R. Pelton. 1985. Genetic variation in black bears from the Great Smoky Mountains National Park. Journal of Mammalogy **66**:564–567.

Wayne, R. K. 1995. Conservation genetics in the canidae. Pages 75–118 *in* J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case histories from nature. Columbia University Press, New York, New York, USA.

Wayne, R. K., and S. M. Jenks. 1991. Mitochondial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus.* Nature **351**:565–568.

Wayne, R. K., N. Lehman, M. A. Allard, and R. L. Honeycutt. 1992. Mitochondrial DNA variability of the gray wolf: genetic consequences of population decline and habitat fragmentation. Conservation Biology **6**:559–569.

Young, A. G., and A. H. D. Brown. 1996. Comparative population genetic structure of the rare woodland shrub *Daviesia suaveolens* and its common congenor *D. mimosoides.* Conservation Biology **10**:1220–1228.

Zink, R. M., and H. W. Kale. 1995. Conservation genetics of the extinct Dusky Seaside Sparrow *Ammodramus maritimus nigrescens.* Biological Conservation **74**:69–71.