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Review

A role for molecular genetics in biological conservation

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ABSTRACT **The recognition of recent accelerated depletion of species as a consequence of human industrial development has spawned a wide interest in identifying threats to endangered species. In addition to ecological and demographic perils, it has become clear that small populations that narrowly survive demographic contraction may undergo close inbreeding, genetic drift, and loss of overall genomic variation due to allelic loss or reduction to homozygosity. I review here the consequences of such genetic depletion revealed by applying molecular population genetic analysis to four endangered mammals: African cheetah, lion, Florida panther, and humpback whale. The accumulated genetic results, combined with physiological, ecological, and ethological data, provide a multifaceted perspective of the process of species diminution. An emerging role of population genetics, phylogenetics, and phylogeography as indicators of a population's natural history and its future prognosis provides valuable data of use in the development of conservation management plans for endangered species.**

Biological conservation did not start out as a science or even a wide-spread public concern until we read authors like Osa Johnson [*I Married Adventure* (1)], Joy Adamson [*Born Free* (2)], and Karen Blixen [*Out of Africa* (3)], who chronicled the depletion of African wildlife, or heard Rachel Carson's prophetic warning in *The Silent Spring* (4). The eerie prospect that our descendants might be denied the benefits of wildlife diversity and be destined to a world with but a handful of domestic plants and animals drew attention to the gradual but deliberate consequences of unrestricted human growth. The sad truth emerged that we are in the midst of the most rapid extinction event since the demise of the dinosaurs, and the primary cause is human development. Today conservation is a broadly supported goal of all peoples and the initiative to reverse the erosion of biodiversity involves many disciplines: politics, science, diplomacy, and economics, to name a few (5, 6). There is a role for each of these in conservation and the newest area involves biomedical and genetic technologies. In full understanding of the complicity of technology in facilitating the "taming of the wild," it may offer some small comfort that applications of biomedical and genetic technologies could have a role in reversing extinction processes or at least in developing management plans to curtail the rate of species extinction.

In the past two decades, the methods of molecular biology, clinical medicine, and reproductive physiology have been used to describe in precise detail the prospective status of several endangered species (7–9). The results obtained have provided important insight that critically affected management decisions and produced tangible benefits to the studied species. In addition, conservation applications have transformed population genetics from an academic discipline concerned with how flies, rodents, and plants handle natural selection to the forefront of global management decisions of critically endangered species. After a half century of exquisite theoretical and empirical development, the paradigms of population genetics are providing critical guidance for interpreting the history, present status, and future prognosis for threatened species. Further, evolutionary strategies encoded in the genomic architecture of natural populations are being revealed in ways that have direct bearing on human biology and offer provocative solutions to medical disease questions as well.

To understand the influence that molecular genetic analyses have exerted on conservation, it is necessary to understand what genetic surveys involve and measure. Molecular descriptions of the quality and quantity of genetic diversity in populations really began when Lewontin and Hubby (10) estimated the average genomic heterozygosity in populations of *Drosophila pseudoobscura* by using 18 protein and allelic isozyme (allozyme) loci. Their study stimulated similar estimates in hundreds of species, each looking at up to 50 allozyme loci for genetically controlled variation (11, 12). Most natural populations displayed 15–50% of their allozyme loci as polymorphic and the average heterozygosity was between 2 and 15%. Much of the early discussion of the variation dealt with how much random mutational variation a population could tolerate (Muller's genetic load concept), and later on, whether the patterns of variation supported an adaptive or a selectively neutral explanation (12–14).

The conservation community took notice of such studies when Bonnell and Selander (15) discovered that the endangered northern elephant seal displayed no variation in a survey of 24 allozyme loci. These authors interpreted their results as a consequence of a previously documented 18th century population bottleneck (due to hunting exploitation) followed by inbreeding (15–17). Their conclusions have been confirmed more recently with an allozyme study plus a mitochondrial DNA (mtDNA) sequence analysis that revealed that northern elephant seals had <5% of the genomic variation that occurs in the southern elephant seals (17). The significance of genetic uniformity to the future potential of elephant seals, however, was not obvious because the species had recovered to some 120,000 seals since it was afforded protection in 1922 by the governments of the United States and Mexico (17).

Ralls *et al.* (18, 19) demonstrated the severe cost of close inbreeding to wildlife biologists. Their analysis of breeding records of 24 captive wildlife species revealed that in every case but 1, infant mortality was greater when the parents were related. Their findings, which were reminiscent of the well-known examples of inbreeding depression in livestock and plants, alerted the wildlife conservation community to the dangers of close inbreeding in all species.

Since these pioneering reports, molecular genetics has been combined with ecological, clinical, reproductive, behavioral, and microbial data to describe the natural history of other threatened species. Equipped with multi-disciplinary approaches, species conservation plans have been constructed with better knowledge of actual threats to survival. To illustrate the power of integrated biomedical and genetic technology, I will briefly summarize case studies of four "charismatic" endangered species in which our group has been involved. They were chosen because their aesthetic popularity

Abbreviations: MHC, major histocompatibility complex; mtDNA, mitochondrial DNA; RFLP, restriction fragment length polymorphism.

prompted extensive life history studies and thereby the potential to assimilate information across bioconservation disciplines. As every biological species has a different adaptive and evolutionary history, each example provides a different perspective with important lessons for conservation, for biotechnology, and for genomic adaptation. Finally, the role of molecular genetic data in taxonomic issues related to endangered species protection is discussed briefly.

The Cost of Inbreeding: African Cheetahs

Cheetahs are well known as the world's fastest land animal, exquisitely adapted for high-speed chase on the African plains with elongated legs, semi-retractile claws, an aerodynamic skull, and enlarged heart muscles and adrenals. The species has fascinated mankind for thousands of years with numerous regal potentates (Egyptian pharaohs, the Moghul emperor Akbar, and Kubla Khan) training the species for sport hunting. But as popular as cheetahs became, they posed a special problem to their keepers. They would seldom procreate in captivity.

In 30 years of breeding attempts in North American and European zoos, only ≈15% of the matings were successful (20, 21). Further, the extent of infant mortality in cheetahs was rather high (>30%) compared to other species where captive zoo breeding was attempted (18–22). Despite modest improvements in cheetah husbandry and behavioral management, low fecundity and high juvenile mortality (combined with a ban on cheetah imports by the U.S. Endangered Species Act of 1972) resulted in a captive population that was not self-sustaining as mortality outpaced birth increases among captive animals (20–23).

In the early 1980s, the difficulties in captive breeding of cheetahs prompted a biomedical approach to discover the reasons. Both captive and free-ranging African cheetahs were found to have relatively low sperm counts and an elevated level of sperm developmental abnormalities in their ejaculates (≈70% compared to ≈30% in lions or domestic cats) (24, 25). The spermatazoal defects provided our first hint that there was a physiological explanation for reproductive difficulties.

In addition, several measures of genomic diversity (listed in Table 1) indicated that the two major subspecies of cheetah (*Acinonyx jubatus jubatus* from southern Africa and *Acinonyx jubatus raineyi* from eastern Africa) displayed markedly reduced levels of genetic variation relative to other feline and mammal species (22, 26–30). The results of each of these approaches showed that the cheetah had levels of variation comparable to that of deliberately inbred strains of laboratory mice or livestock. These studies lent support to the hypothesis that the cheetah's ancestors had survived a historic period of extensive inbreeding (a population bottleneck), the modern consequences of which are 90–99% reduction in measurable allelic variation and a remarkable collection of correlative physiologic impairments (Table 2).

Table 1. Indexes of genomic variation that were reduced in cheetahs

Index	Ref.
Allozyme (52 loci)	26
Two-dimensional PAGE (155 loci)	26
Allogeneic skin graft accepted	22
MHC RFLP (six restriction enzymes)	27
mtDNA RFLP	28
Microsatellite loci	Unpublished
Increased fluctuating asymmetry of skeletal measurement	29

The cheetah's difficulties did not stop there. We knew from experience with inbred mice and livestock that inbreeding can contribute to an increased susceptibility of a population to infectious viruses, bacteria, and other pathogens. The interpretation for this phenomenon involves an understanding of the evolution of immune response. Several of the loci that mediate immune defenses seem to depend on extensive allelic variation within outbred populations as a "moving target" for rapidly evolving pathogens. The idea is that when a virus genetically changes (e.g., as do influenza or human immunodeficiency virus 1) to overcome the immune defenses of a single individual, the adaptations will not necessarily be as effective in another genetically distinct individual (32–36). This explanation seems to be the driving force for enormous genetic diversity at the mammalian major histocompatibility complex (MHC), whose role is to recognize and present foreign virus peptides on infected cellular surfaces to helper T lymphocytes as a prelude to cell-mediated immune destruction of infected cells (36, 37). The cheetah provided a vivid natural example of this scenario because the species was genetically monomorphic at the MHC (22). When a devastating outbreak of feline infectious peritonitis occurred at an Oregon cheetah breeding facility in the mid-1980s, it resulted in 100% morbidity (symptoms) and 60% mortality (22, 31), the worst recorded for this incurable disease in any feline species. In domestic cats, the mortality incidence of this virus is seldom >5%. The possibility that the cheetah population's nearly homogeneous response to the lethal peritonitis virus was related to its genetic homogeneity, particularly at the MHC (Table 1), was compelling.

Although the evidence for a severe population bottleneck (or series of bottlenecks) in the cheetah's recent history was strong, the estimation of the time of the event was difficult. Population theory predicts that reconstitution of genetic diversity is slow, on the order of the reciprocal of mutation rate (10^{-6}–10^{-7} mutations per locus per gamete for allozymes, fibroblast proteins, or MHC loci) (38, 39); thus the near extinction could have occurred anytime from a few decades to a million years ago. To address this question, we took advantage of two DNA classes that evolve more rapidly, mtDNA and DNA fingerprints (28). These variants accumulate mutations at a rate of three to four orders of magnitude more rapidly than nuclear coding genes. Modern cheetahs display a modest amount of variation in both of these genomic families and the monophyletic similarity of modern DNA diversity led us to conclude that most of it was reconstituted by point mutations after the proposed bottleneck. By back-calculation from the quantity of diversity in these gene families, we estimated that the most recent demographic reduction was on the order of 10,000 years ago (lower Pleistocene), around the time of the last Northern Hemisphere glaciation. This date coincides with the most dramatic extinction events of large mammals in the fossil record (40–42). Before this event the cheetah's ancestors had a range that covered North America, Europe, Asia, and Africa and included several different species (43, 44). But when the large Pleistocene mammals

Table 2. Physiological measurements where cheetahs are limited compared to other felids

Measurement	Ref(s).
Diminished sperm count	24, 25
Elevated frequency of morphological abnormalities in sperm development (≈70%)	25
Low fecundity in captive breeding attempts throughout history	20, 22
Captive population is not self-sustaining	20, 21
Relatively high incidence of juvenile mortality even among unrelated parents	22
Increased population vulnerability to infectious disease outbreaks (notably, feline infectious peritonitis)	22, 31

such as the giant ground sloth, mastodon, sabertoothed cats, and American lion, became extinct, the cheetah's range changed abruptly. It is likely the cheetah barely escaped extinction, but not without a genomic vestige of its ancestry.

The messages from the studies of cheetah genetics were clear. (*i*) There were certainly undiscovered perils that can threaten populations that were not so apparent as traditional ecological parameters (Tables 1 and 2). (*ii*) When populations drop to very low numbers, as most endangered species do, if they do not become extinct, they still could suffer genetic depletion when inbreeding is close and persistent. (*iii*) Although every population bottleneck is different, they all carry the risk of inbreeding depression and the expression of congenital abnormalities resulting from homozygosity of deleterious genes. (*iv*) In addition to these heritable defects, inbreeding homogenizes variation at abundantly polymorphic genes that mediate immune response, increasing the population's risk of debilitating disease from pathogens that overcome the immune defenses of a single individual.

The Controlled Case of African and Asiatic Lions

The results from the cheetah caught the attention of the conservation community that did not have to look very far to discover genetic secrets in other species. Craig Packer and Ann Pusey (53) have studied behavioral ecology in a group of lion prides in the Serengeti ecosystem in Tanzania since 1980, and the population had been under continuous field observation since Schaller's seminal study in the late 1960s (45). When Packer and Pusey invited us to compare the genetic structure of a lion population in the Serengeti to the adjacent population isolated in the Ngorongoro Crater, we wondered if there might be genetic differences that reflected their unique natural histories. The Serengeti Plains are the home of a large outbred population of 3000 lions in a territory the size of Connecticut. Adjacent to the Serengeti is an extinct volcanic caldera, the Ngorongoro Crater with mountainous barriers surrounding a rich assemblage of East African wildlife (46–49). The ≈100 lions in the Crater are descended from a population bottleneck that occurred in 1962 due to an epizootic of biting fly (*Stomoxys calcitrans*) (46, 50). Only 10 lions survived (1 male and 9 females) and these lions plus 7 immigrant males from the Serengeti led to the founding of the present population (46). The Ngorongoro lions are an effective "island population" isolated from immigration (but not from emigration) since 1968 by steep walls and behavioral reinforcement. We also obtained samples from a group of lions originating from the Gir Forest Sanctuary located in the Gujarat Province of western India. These Asian lions, which show several morphological distinctions from their African counterparts, are a relict group of 250 individuals that also experienced a severe population contraction (to <20 animals) in the first quarter of this century due to hunting exploitation (47).

Genetic analysis of the three lion populations provided the natural equivalent of a case-controlled study for the effect of genetic depletion on reproductive parameters (27, 47–49). The Serengeti lions looked outbred with abundant molecular genetic variation estimated with allozymes, MHC restriction fragment length polymorphisms (RFLPs), and DNA fingerprinting (Table 3). The Gir lions were as bad as or worse than cheetahs with <5% of the variation found in Serengeti lions using all three genetic methods. The lions in the Ngorongoro Crater fell in between the two other populations with ≈30% of the variation seen in the outbred Serengeti population. Sperm abnormalities were remarkably correlated with genomic variation as the Asiatic lions had a high frequency of pleiomorphic sperm while the Serengeti lions did not (Table 3). Further, relative to Serengeti males there was a 20-fold reduction in circulating testosterone in Gir lion males and a 3- to 4-fold depletion in Ngorongoro males (51). Testosterone is known to play a key role in normal spermatogenesis in mammals and is a likely physiological explanation for the sperm development abnormality (as well as a reduced lion mane) seen in the Asiatic lions (47). The dramatic correlation between overall genetic variation, documented demographic history, and indices of reproductive function in free-ranging animals provided rather strong evidence for the cost of inbreeding in lion populations.

A surprising twist emerged when we looked at the breeding success of the inbred Asian lions in captivity. An Asiatic lion pedigree from the Sakkarbaug Zoo in India showed reproductive difficulties particularly in male representation (very few successfully bred), while a North American pedigree of Asiatic lions (Species Survival Plan of the American Zoo Association) actually was breeding very prolifically even though it was derived from only five founder lions (47). The explanation came when it was discovered that two of the original North American founders were genetically from African lion stock. The relative reproductive success of the North American pedigree was likely due to between-subspecies hybridization (hybrid vigor) discovered retrospectively with genetic techniques three generations later (47).

Molecular genetic analysis of the Serengeti lions also provided an opportunity to test various hypotheses about the adaptive value of pride behavior (52). Lions are the only cat that has a cooperative social organization based on the temporally stable pride unit (45, 53). Lion prides consist of 1 to 18 adult females and subadults plus a coalition of 1 to 7 adult males that live in definable territories. Females raise young communally and resident male coalitions are replaced every few years by unattached male coalitions that "take over" an existing pride group by social intimidation. Packer and Pusey (53, 54) had noticed that the major determinant for pride "takeover" success was the size of the coalition (number of males). Since male coalitions differ with respect to relatedness (some are brothers and some are unrelated), the adaptive value of wandering males joining up with unrelated males who might be competitors for mating with pride females was puzzling (52).

By developing feline-specific minisatellite (DNA fingerprint) probes, Dennis Gilbert and his collaborators (49, 52) were able to identify parentage (both mother and father) of 78 cubs born in 11 prides over a 10-year period. The precise identification of familial relationship among 200 lions permitted us not only to assess mating success of males from different coalitions but also to draw a calibration curve between DNA fingerprint

Table 3. Correlation of genetic variation and reproductive parameters in three lion populations

Parameter	Serengeti, Tanzania	Ngorongoro Crater, Tanzania	Gir Forest, India
Heterozygosity, %			
Allozyme	3.1	1.5	0.0
MHC RFLP	21.8	8.0	0.0
DNA fingerprint	48.1	43.5	2.8
Reproductive measure			
Sperm count ($\times 10^{-6}$)	34.4 ± 12.8	25.8 ± 11.01	3.3 ± 2.8
% sperm abnormality	24.8 ± 4.0	50.5 ± 6.8	66.2 ± 3.6
No. motile sperm ($\times 10^{-6}$) per ejaculate	228.5 ± 65.5	236.0 ± 93.0	45.3 ± 9.9
Testosterone, ng/ml	1.3–1.7	0.5–0.6	0.1–0.3

Refs. 27, 47–49, and 51 are sources. When indicated, data are the mean ± SEM.

band-sharing and actual genetic relatedness. These results showed that large coalitions (4–9 males) were always composed of first-order relatives (brother, father, and son), whereas smaller coalitions (2 or 3 individuals) were unrelated ≈50% of the time. In nearly all cases, however, paternity was restricted to two males regardless of coalition size.

Thus, the explanation now made sense (52). Singleton or doubleton male groups would join with an unrelated partner to increase the chance of takeover but would not reduce their chance of reproduction appreciably since at least one of the brothers would likely father cubs. Larger brother coalitions would avoid addition of nonrelatives because there was no benefit in takeover success and a large fraction of a nonbreeder's genes were transmitted since their siblings would father the cubs. The conclusions derived from the DNA fingerprint study in lions (49, 52) had important implications for the evolution of cooperative behavior and also demonstrated the power of high-resolution DNA polymorphism in approaching ecological questions in natural populations. Our success in combining ethological and DNA-fingerprint technology in the Serengeti lion study lent confidence to this application for assessing paternity and kinship in natural settings despite persuasive theoretical arguments questioning the feasibility of such an approach (55, 56).

The Florida Panther: An Extreme Case

Although species are disappearing at an alarming rate, field observation of the actual process of species collapse has been rare. The case of the Florida panther, documented in exquisite detail by Melody Roelke and her collaborators, is a chilling scenario of a tiny population on the very edge of extinction (57). The Florida panther is a subspecies of puma that a century ago occupied a range roughly equivalent to the Confederacy (Fig. 1). Human depredation, spurred principally by fear, legends of ferociousness toward livestock and humankind, and the payment of bounties reduced the panther's range to the hardwood swamps and cypress prairies of south and central Florida by 1920. Today, <30 adult Florida panthers survive in the Big Cypress Swamp and adjoining Everglades National Park ecosystems in south Florida, the only wild pumas found east of the Mississippi.

At first, the major threat to the Florida panther seemed obvious: road kills and illegal hunting accounted for 63% of documented mortalities since 1973 (57). Genetic studies (allozymes, mtDNA, and DNA fingerprinting) revealed that the Florida panther has less molecular genetic variability than any puma subspecies from North or South American and several cases of incestuous matings were documented since 1984 (57, 58).

The cost of inbreeding in this population is dramatic (57–59). Florida panthers have the worst sperm seen in any species; ≈95% of the sperm in each ejaculate are malformed (59). Further, the incidence of cryptorchidism, a rare heritable defect that causes one or two testicles to remain undescended, has risen from 0 to 80% in the males in the last 15 years. In addition, a new congenital and fatal cardiac abnormality has recently appeared in three kittens. Finally, the microbial parasite disease load in Florida panthers is enormous. A score of microbial pathogens have contributed to debilitating disease or mortality in at least eight panthers to date. One of the viruses discovered in Florida panthers is a close relative to the feline version of the human AIDS virus, feline immunodeficiency virus (FIV) (60). FIV causes severe immunodeficiency in domestic cats, but there is no evidence yet that it causes disease in panthers.

The Florida panther's immediate problems are demographic; unless its numbers are increased the probability of extinction is almost certain (57, 61, 62). Yet if left alone to breed, even if numbers increase to several hundred, the genetic hangover caused by inbreeding is formidable and may impede population growth or survival.

Faced with the genetic, reproductive, epidemiological, and demographic data on the Florida panther, an October 1992 Florida panther management workshop (63) made two recommendations: (*i*) A captive breeding program was initiated to protect against the high probability of extinction in the wild. (*ii*) A plan was developed to introduce Texas-born pumas (*F. concolor stanleyana*) into Florida to assuage the genetic impoverishment. The logic was that the panther's critical status demanded genetic addition, particularly since the Texas puma and Florida panthers had a range overlap and probable genetic exchange during the 19th century. The reintroduction proposal was controversial because of the question of subspecies hybridization (refs. 64 and 65 and see below) but the potential to avert both demographic and genetic calamity in this population was overwhelming in influencing the recommendations.

Tales of the Humpback Whale

Behavioral research that tracked individuals by tail fluke patterns revealed that humpback whales (*Megaptera novaeanglia*) migrate >10,000 km each year from summer feeding grounds to winter breeding grounds (Fig. 2). In a fascinating study that examined mtDNA RFLP haplotypes in Pacific populations, Scott Baker and his associates (62) discovered a phylogeographic mtDNA genotype separation between feeding ground populations (from Alaska and California coasts) that intermixed in the Hawaii waters breeding grounds. As there were no obvious geographic barriers and considering the maternal inheritance of mtDNA genotypes, the results likely reflected a migration of humpback whale pods to specific feeding ground locales as a consequence of maternal-directed home-range fidelity.

The number of living humpback whales dropped dramatically from a high of 125,000 to <5000 due to hunting exploitation before international protection was afforded in 1966 (66). The overall amount of genomic variation found in humpback populations and in the entire species was moderate as determined by DNA fingerprints, mtDNA RFLP, and D-loop (control region) sequences (66–68). The variation was partitioned geographically among three oceanic populations: North Pacific, North Atlantic, and the southern oceans (Fig. 3). These results indicated that the rapacious slaughter over two centuries of commercial whaling was not sufficient to cause a significant genetic loss in the species, a likely consequence of the 11th-hour ban on exploitation afforded by international protection.

A phylogenetic analysis of mtDNA control-region DNA sequences of 90 whales from separate populations of the three ocean basins illustrates the dramatic power of this approach for reconstructing natural histories (Fig. 3). A total of 37 distinct control-region sequence genotypes assorted in three major phylogenetic clusters (or monophyletic clades), each predominant in a different ocean. Within each cluster were "microclades" or monophyletic groups collected from an ocean that was different from the origin of other genotypes in the major clade. The simplest interpretation of the control-region phylogeny would be an ancient divergence of whales in the three oceans (separated by continents and by seasonal opposition of hemispheres; see Fig. 2) punctuated by a few (four to be exact) migration events between oceans that were followed by monophyletic divergence of descendent microclades (67).

When the extent of control-region divergence between humpback whale genotypes was estimated, the value (3% between the 37 genotypes) was very large. Using the extent of divergence in the homologous mtDNA region across three families of whales as a molecular clock, the humpback mtDNA lineages were estimated as dating back 3–5 million years ago (67). Relative to the comparable estimate for humans (166,000–249,000 years) (69, 70), the humpback lineage

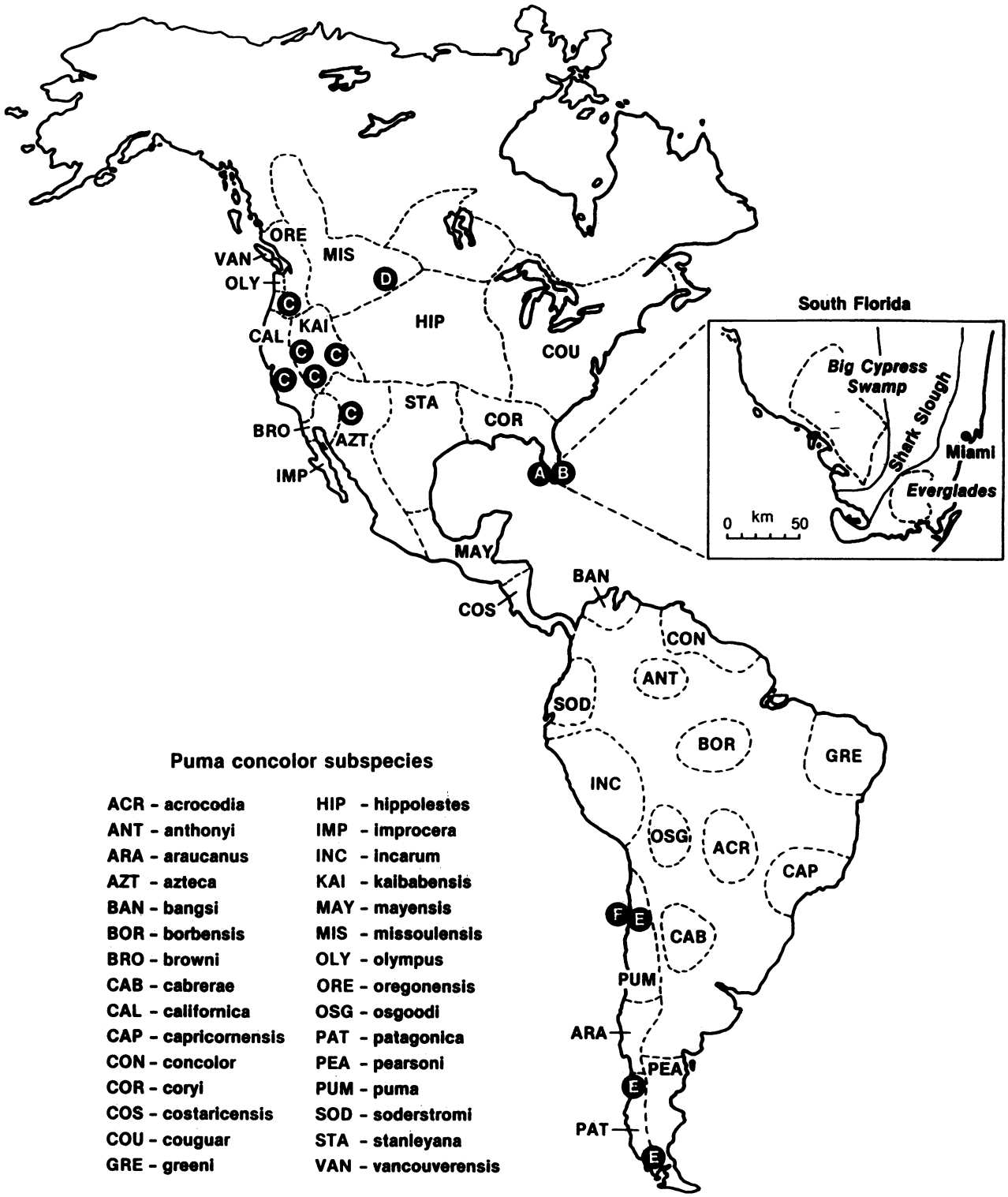

FIG. 1. Distribution of *Felis concolor* subspecies, including the historic and present (*Inset*) ranges of *Felis concolor coryi* (43, 57). Solid circles indicate the locales of specimens collected for genetic analysis; letters inside the circles represent the mtDNA RFLP haplotypes defined by restriction enzyme typing of 109 restriction sites (58). Haplotypes A, C, and D differ from each other by one or two restriction sites.

seems very ancient. Thus the humpback whale data offer some encouragement to the conservation managers of this species. If protection continues, recovery should occur, although the slow rate of increase for this species would require protection for hundreds of years. Further, the extremely ancient origin of mtDNA diversity is a signal that the species has not undergone a population bottleneck (with founder effect, inbreeding, and genetic depletion) for several million years. If we presume that accumulation of lethal equivalents in the mammalian genome is a time-dependent process (71, 72), then the humpbacks probably have a large dose of such a genetic load simply as a consequence of so long a period since the last genetic homogenization.

Taxonomy and Endangered Species

Taxonomy, the systematic classification of plants and animals, had little relevance outside of academic institutions in the mid-1970s. Before then species and subspecies

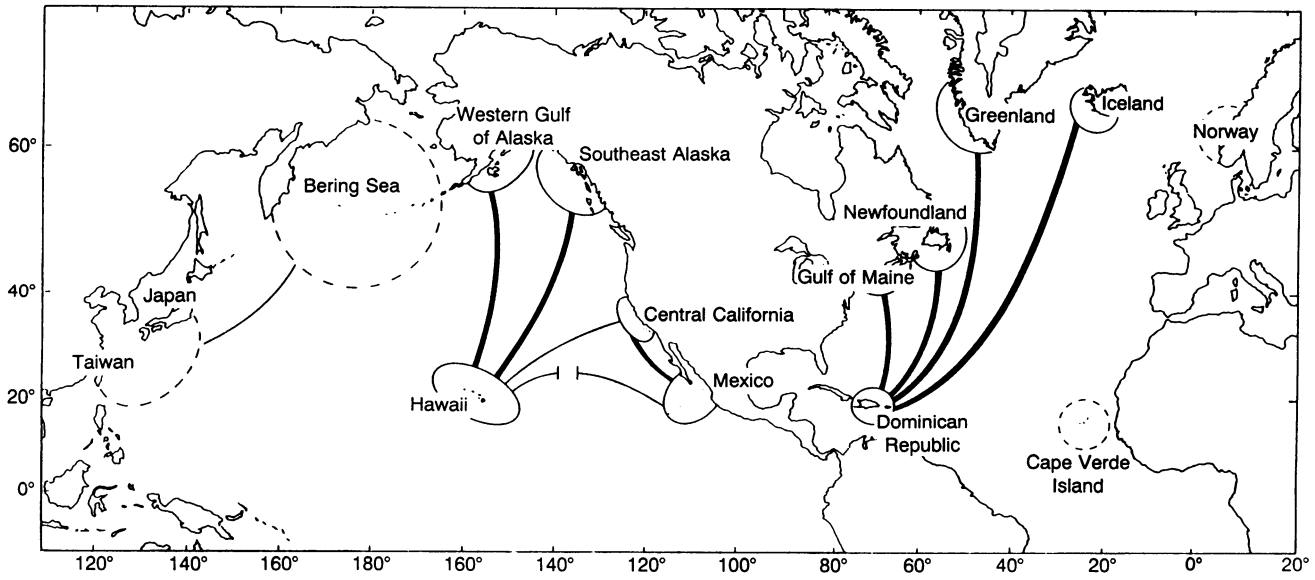

FIG. 2. Migratory destinations and population structure of humpback whales in the North Pacific and western North Atlantic oceans, based on observations of marked individuals (66–68). Regions encircled by a broken line are defined by historical patterns of distribution during periods of commercial whaling. Arrows connect seasonal habitats visited by individually identified whales but do not necessarily indicate migratory routes.

were identified, described, and named on the basis of a few specimens collected on expeditions that celebrated the tradition of Darwin's voyage on the *Beagle*. Species were grouped according to morphological type specimens into genera, genera into families, families into orders, and so on. Systematic uncertainties had little relevance to everyday life and the methods of taxonomic resolution were limited. When the taxonomic distinctions became the basis for legal protection afforded by the Endangered Species Act of 1973 (73), this innocence was lost forever. Disagreements over taxonomic status fueled legal assaults on the Act and misclassifications led to inappropriate conservation resulting in losses of some species (9, 74–78). Even today with vastly improved molecular methods for discriminating between taxonomic groups, there remains considerable confusion about units of conservation that the Endangered Species Act was designed to protect (75, 79, 80).

Formalized taxonomy is critical to conservation because it provides the basis for recognition (and therefore protection) of endangered species. The U.S. Endangered Species Act (73) extends legal protection to three categories of taxa: species, subspecies, and certain vertebrate populations. To date there are 681 U.S. plant and animal species and 528 foreign species listed as endangered; some 3500 candidates await classification. Unfortunately, many of the classification schemes are based on inadequate type descriptions of a few morphological specimens (skins or skeletons) collected by 19th century naturalists (e.g., Fig. 1 or subspecies in ref. 43). Although most observers agree that there exists a systematic hierarchy based on evolutionary time and adaptive radiation in taxon emergence, there is little agreement on the specifics.

Taxonomic imprecision has contributed to errors both over "splitting" of genetically indistinguishable groups (e.g., Dusky seaside sparrow, leopards, and pumas) and over "lumping" of significantly divergent taxa (e.g., the three species of tauratora) (9, 77, 78, 81, 82). Because phylogenetic distinction is often considered in ranking species recovery plans and in allocating resources, precise taxonomic hierarchies become critical (83).

Another area that has led to confusion and to legal assaults on protection involves the question of *in situ* hybridization of endangered species or subspecies. Historically, the U.S. Fish and Wildlife Service had interpreted that "hybrids" between taxa listed by the Endangered Species Act would not be eligible for protection, largely to concentrate responses on "pure" endangered species. So when molecular genetics revealed a natural hybridization involving the Florida panther (58), or geographically restricted hybrids between wolves and coyotes (84, 85), litigious challenges to their protection were based on the precedent of the so-called "hybrid policy" to preclude protection (64). Fortunately, the hybrid policy was suspended when Ernst Mayr and I argued that these sorts of hybrid events were natural outcomes of evolution and that the species should not be penalized due to bureaucratic precedent that did not consider the resolving power of molecular genetics (see ref. 64). The new molecular technologies offer considerable precision to identify and categorize species, subspecies, and population level differentiation. As they are applied to threatened populations, combining genetics, phylogeny, and geography, the data required to classify these groups based on their own genetic and evolutionary history will be collected.

Conclusions

The last decade has seen the beginning of a field that applies the principles and methods of population genetics to species conservation. As for other areas of molecular biotechnology, conservation genetics is an applied science with the important goal of describing explicitly the composite genomes of small endangered populations. By comparison to better studied examples such as those reviewed here, one can make realistic approximations of the recent natural history, present status, and future prognosis of endangered populations. When combined with data from other disciplines (e.g., reproduction, infectious disease, and field ecology), the synthesis offers some valuable insight that can be applied directly to species management plans.

On the surface genetic surveys of natural populations appear to offer a limited view: namely, (*i*) the quantity of overall genomic variation, (*ii*) evidence for genetic differentiation between taxa, and (*iii*) phylogenetic relationships between geographically isolated populations. However, I have attempted to illustrate that the interpretation of these results with other disciplinary data can advance our understanding appreciably and thereby limit the guesswork associated with conservation management decisions. Finally, the methods of genetic technology are being improved continu-

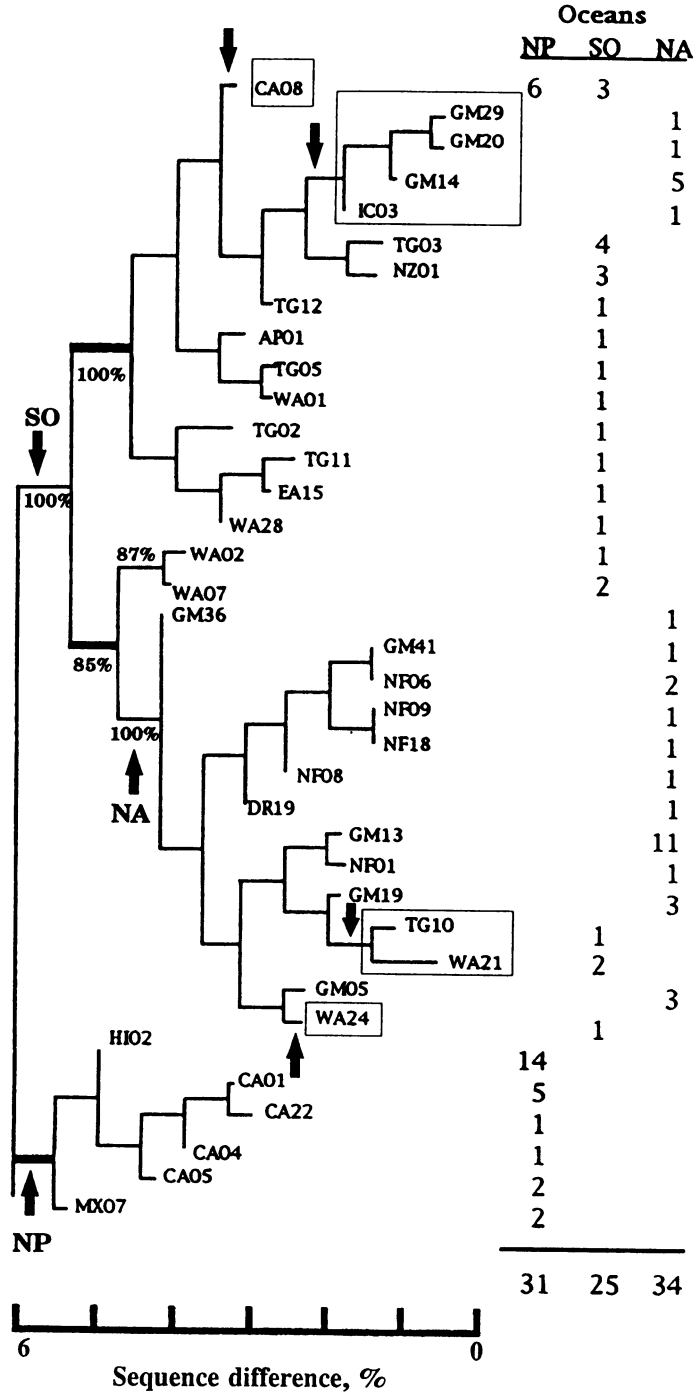

FIG. 3. Phylogenetic relationship between 37 unique mtDNA control-region sequences from 90 humpback whales from the North Pacific (NP), North Atlantic (NA), and southern oceans (SO) (modified from ref. 67). The tree was constructed by the neighbor-joining method (67) and rooted with the homologous sequence of the fin whale. To the right of the phylogenetic tree is the number of each humpback whale mtDNA genotype collected in the three oceans. Percentages represent the fraction of bootstrap iterations (out of 200) that support the inclusion of the descendent mtDNA genotypes commented by the node. Labeled arrows (NP, NA, and SO) indicate the common ancestors of the mtDNA genotypes found in the three oceans. Other arrows represent hypothesized points of migrations; boxed "microclades" represent monophyletic descendants of the ancestral migrant within the ocean region that received the migrating ancestor (67) (see text).

ally. DNA sequence analysis is now routine. DNA polymorphisms are detected as easily as allozymes were 20 years ago, and powerful computer algorithms for analysis of complex phylogenetic and population data sets make analyses more statistically robust. The potential to approach conservation questions rigorously can now be realized and applications of findings are now agenda items for species conservation plans world-wide. I personally hope (and believe) that it is not too late to put the experience of each of the disciplines, including molecular genetics, to task in many conservation initiatives.

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