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GENETIC AND PHYLOGENETIC ANALYSES OF ENDANGERED SPECIES[1]

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INTRODUCTION

To reverse the accelerated loss of biological species caused by human industrial development requires knowledge of the causes for extinction in small populations. As conservationists gather ecological and life-history data about threatened taxa, it is becoming clear that a variety of biological disciplines must be considered: principally ecology, behavior, demography, disease, and natural history (39, 150, 164). In the past two decades, a new field of conservation genetics has emerged with two general goals: (*a*) the precise description of the genetic changes affecting population survival that occur during range and

population contraction; and (*b*) application of genetic insight to successful management of threatened populations (5, 68, 101, 102, 138).

Conservation genetics draws upon the established theory of evolutionary genetics pioneered by Wright, Fisher, Haldane, Crow, Kimura, Dobzhansky, and their intellectual descendants, on the molecular genetic technologies that have revolutionized every field of biology, and on ecological phenomena that affect species survival. Integrated syntheses of genetic, biomedical, and ecological observations have uncovered cryptic threats and provided valuable perspective that benefited management imperatives for several endangered species. Genetic surveys of small populations have revealed phylogenetic differentiation that could be related to time intervals of geographic isolation, selective episodes, and other regulatory events in the history of the populations. Several examples of genomic homogenization, likely the result of historic demographic contraction followed by inbreeding and genetic drift, are discussed in this review. In some, but not all, cases physiological evidence of inbreeding depression in endangered taxa was displayed through increased incidence of developmental, congenital, and reproductive abnormalities.

Immunological, microbial, and genome-based screens for infectious disease are now being used to assess infectious disease load in several endangered species (16, 55, 82, 121, 139). Darwin recognized that infectious diseases would regulate growth or survival of populations, but failed to connect diseases with the process of natural selection (33). Haldane is generally credited as the first to emphasize the key role that disease outbreaks have on selecting genetically resistant survivors (53). He concluded, and many now agree, that infectious disease probably has as much influence as any ecological factor in determining the selective pressure on the genomes of surviving species (2, 3, 102). Because of these developments, molecular population genetics, clinical veterinary medicine, infectious disease, and reproductive physiology are now included as agenda items in developing conservation management plans for protecting endangered species in their native habitat.

Today's population genetic analysis has a demonstrated power in revealing a population's past history, present status, and future prognosis for survival. However, the interpretations are limited because the tested genetic markers are indices of genomic variation and not the object genes for natural selection and adaptation. Yet, advances in genetic analysis may soon change this. Gene-mapping projects of human, mammalian, and model species' genomes are beginning to identify genes that would be candidates for selective adaptation (30, 99, 114). The mammalian genome contains at least 50,000 coding genes and the aim of the Human Genome Project is to identify most of these in the next decade (29, 32). Already over 3000 human genes with a known function have been included. Among these are hundreds of loci that relate to reproduction (and thus to reproductive isolation), to neurophysiology (and thus to behavior), to immune defense (and thus to combating infectious disease), and to other physiological functions that themselves are the products of evolutionary struggles for existence. Comparative gene-mapping approaches in mouse, cat, livestock, and other species have revealed that mammalian genomes are highly conservative in linkage organization of homologous genes (61, 99, 114, 117, 120). This raises the prospect of using the human gene map as a index for reading and employing other mammal gene maps (mice, cats, whales, elephants, or kangaroos) to identify the stuff of evolution, of extinction, and of survival. Recent studies of sex-determining genes, of homeobox gene families, and of the major histocompatibility complex (MHC) interaction with infectious agents offer a preview of the emphasis in the next generation of evolutionary and conservation genetic research (49, 63, 64, 66, 144, 149).

Several reviews have chronicled the application of genetic principles to conservation management and summarized the state of genetic data on the few studied species (4, 5, 68, 101, 102, 135, 138). My goal here is to review some lessons learned by applying empirical population genetic approaches to define the factors that imperil fragile populations. I summarize both the limitations of our inference and the conclusions that we reached as a community of conservation scientists. Several examples will illustrate the synthesis of genetic interpretation with demographic, ecological, and life-history data to draw a cohesive picture of the threatened taxon. Most of my examples are endangered large charismatic carnivore species selected for two reasons. First, large carnivore species occupy the top position of a trophic chain for their ecosystem. They are often highly specialized and provide a sensitive barometer of an ecosystem's condition. Second, charismatic species attract long-term field studies that lay the groundwork for formulating falsifiable ecological and life-history hypotheses.

THE MATERIALS AND RESOURCES FOR CONSERVATION GENETICS

When John Muir and Teddy Roosevelt camped in the shadow of El Capitan in Yosemite Valley nearly a century ago, they realized that part of their society's responsibility was the preservation of the earth's geological and biological heritage. But natural parks and protected areas were demarcated by majesty and convenience with little informed attention to the potential for continued extinction of inhabitants due to unforeseen ecological pressures spurred by human development. Popular books that chronicled the loss of biodiversity and conservation became a clarion call to reverse what is now recognized as the most dramatic extinction of species since the demise of the dinosaurs (1, 14, 25, 37, 62, 93). Darwin's prophetic vision of a "struggle for existence" could be pronounced as settled. Mankind and his technology, par-

ticularly agrarian development, were the clear winners; the losers, approximately 10 million biological species subjugated by our success.

The concept of endangered species became formalized as international agreements like the Convention of International Trade in Endangered Species (CITES) were established to end the hunting slaughter and to penalize illegal trade in skins and body parts. National legislation such as the 1973 *U.S. Endangered Species Act* were enacted to protect wildlife in their native countries (58, 78, 150). Ecological field studies began for several keystone species where radio telemetry permitted long-term observation of animal behavior and survival strategies (136, 137). Zoo managers changed their emphasis from capture and exhibition to captive propagation and conservation. Associations of zoo directors and curators formed "Species Survival Plans" to develop efficacious strategies for establishing self-sustaining captive populations of endangered species as a backup to wild populations (40, 70). Captive holdings of all zoo-bred species were incorporated into a computer database, the International Species Information System (ISIS), established in 1974 to provide the baseline for cooperative management (22). International studbook keepers were nominated by the International Union for Conservation of Nature (IUCN) and by Species Survival Plans to document births, deaths, and pedigree information for scores of endangered species held in captivity (159). This information was absolutely critical for determining the status of the captive populations and provided a framework for focused study on the reproduction and survivorship of endangered species (80, 143). By applying knowledge accumulated from laboratory animal models, zoo populations gradually became available for assessment of reproduction, immune response, and other components of fitness.

Records of breeding success and infant mortality provided the first evidence that endangered species, particularly in captivity, were subject to inbreeding depression. Ralls, Ballou, and colleagues examined breeding records of 32 captive wildlife species and revealed that infant mortality was greater when the parents were related, not an uncommon zoo situation because sib pairs of species were frequently mated (129–131). Their results reinforced what domestic plant and animal breeders have known by experience and what Darwin observed 150 years ago; most species lose fitness by close inbreeding due to expression of rare deleterious recessive genes (33, 38, 51). Muller termed the intrinsic population's threat "our load of mutations" (92) and Wallace (152, 153) showed empirically that the genetic load in *Drosophila* is so appreciable that X-irradiation, under certain conditions, can actually increase the fitness of a homozygous wild *Drosophila* chromosome. The concept of maximizing outbreeding became the standard of captive species survival plans, although not without some contention (see below).

The integration of ecological, genetic, and demographic information was

accomplished for scores of species through the effort of US Seal, who became head of IUCN Captive Breeding Specialist Group, a mix of zoo directors, curators, wildlife managers, and veterinary and medical scientists who shared a conservation resolve. The Population and Habitat Viability Analysis (PHVA) was established to assemble field workers, scientists, zoo-breeders, and government managers over a single species. The purpose was to assimilate available life-history information, to define recognized threats quantitatively as well as qualitatively, and to recommend needed action (27, 140). Critical to this exercise is the employment of demographic computer models that predict the probability of extinction in free-ranging populations (56, 69, 70) based upon estimates of species' life history and ecological parameters (age-specific mortality, reproductive success, litter size, population size, habitat-holding capacity). Also considered are genetic depletion characteristic of small populations and the predicted consequences. The projections of these demographic models have provided important barometers for survivorship of over 50 endangered species to date. Further, the PHVA reports assemble the estimates and opinions of available experts for each endangered species. These workshop reports summarize background information available about identified endangered species. The availability of this baseline information forms the framework for most genetic and evolutionary hypotheses we have encountered in our studies of threatened species.

Finally, the development of several pharmacological compounds for anesthesia has made the temporary capture of large free-ranging animals plus the examination of captive species both efficacious and safe (for both the clinician and the animal) (19, 20, 54, 57, 162, 163). The capture-anesthesia methods allow collection of blood, semen, skin biopsies, and other samples in a brief period. Skin biopsies, which can now be collected by a nonanesthetic dart biopsy (used, for example, in orangutans, elephants, and whales) can be viably frozen in liquid nitrogen in the field and reconstituted later as an immortal fibroblast cell culture (65, 71, 89). All of these parallel developments were critical to the collection of genetic and physiological data that form the basis of this review.

SOME EXAMPLES

Over a decade ago, we reported the first results indicating that African cheetahs (*Acinonyx jubatus*) retain significantly less overall genomic variation than other felid or mammal species based on a survey of allozymes and cell culture proteins resolved by two-dimensional polyacrylamide gel electrophoresis (PAGE) (119). In subsequent studies, additional molecular genetic data have confirmed the genetic uniformity of the species (summarized in Table 1) and led us to conclude that several thousand years ago (likely at the end of the

Pleistocene period, the time of the most recent north hemisphere glaciation), the cheetah's ancestors experienced a severe demographic reduction (85, 115, 118, 119, 157, 165). The proposed population bottleneck led to a significant range contraction and resulted in inbreeding among close relatives. Overall genetic variability of modern cheetahs is reduced 10–100-fold compared to other large cat species. Correlated with the genetic uniformity are a number of physiological impairments that influence reproduction and that contribute to difficulty in establishing a self-sustaining captive population. The physiological problems include reduced sperm count, elevated sperm development abnormalities, low fecundity, and high infant mortality (80, 81, 115, 162, 163). The sperm abnormalities are observed in both free-ranging and captive cheetahs and likely play a key role in the difficulty in achieving a self-sustaining captive population. Further, an extreme morbidity and mortality of cheetahs from outbreaks of a nearly benign cat virus (feline infectious peritonitis virus) was interpreted as a consequence of the homogeneous state of genes that mediate immune defenses (55, 115). Several of these immunological loci are highly variable in other feline and mammalian species, particularly the MHC. The evolutionary explanation for high variation among immune response loci would be that they offer a moving target for microbial pathogens, which themselves rapidly evolve genetic adaptations that override the immune defenses of individuals.

The cheetah example has served as a paradigm of the potential for hidden perils that threaten small populations from within. The interpretations have been reinforced by the variety of different genetic observations that support the bottleneck hypothesis. Evidence of the cheetah's genetic uniformity was obtained with five distinct measures of genomic variation (Table 1). Three genomic categories that do show modest variation in cheetahs (mitochondrial DNA-restriction fragment length polymorphism [mtDNA-RFLP], DNA fingerprint, and microsatellite) are rapidly evolving noncoding DNA families that likely accumulated variation by mutation since the hypothesized bottleneck (85, 86). The concordant genetic diminution of multiple estimators of genomic variation lent strong support to the occurrence of close inbreeding as a result of demographic collapse in the cheetah's history.

The developing legacy of the cheetah led our group to investigate the genetic structure of several other endangered species as well. In Table 1 is presented a list of six endangered carnivore species or subspecies, each of which also showed diminished overall genetic variation likely as a consequence of an historic population bottleneck. A population contraction was historically documented in the case of four of the taxa (lions in Ngorongoro Crater, Asiatic Gir lions, Florida puma, and northern elephant seals). Because the amount of measured genomic variation in a species is a relative measure, the populations of Florida puma and lions provided the equivalent of a case-controlled study

Table 1 Examples of species and populations that display multiple indices of relatively low genomic variation due to historic inbreeding in situ

	Nuclear coding genes		Rapidly evolving DNA families	
Species/Population	Allozyme	MHC-RFLP	mtDNA RFLP	DNA fingerprint
Cheetahs[1] *(Acinonyx jubatus)*	Lo (115, 119, 118)	Lo (165)	Hi (85)	Hi (85)
Lions—Ngorongoro Crater *(Panthera leo leo)*	Lo (109)	Lo (165)	Lo (60)	Hi (47)
Lions—Gir Forest[2] *(Panthera leo persica)*	Lo (109)	Lo (165)	Lo (60)	Lo (47)
Puma—Florida[3] *(Felis concolor coryi)*	Lo (133, 116)	N.D.	Lo (116)	Lo (133)
N. elephant seal *(Mirounga augustirostris)*	Lo (15, 56)	N.D.	Lo (56)	N.D.
Giant panda *(Ailuropoda melanoleuca)*	Lo (103, 146)	N.D.	Hi (106)	Hi (106)

[1] Cheetahs also have reduced variation in two-dimensional PAGE proteins and nuclear microsatellites. Reciprocal skin grafts between unrelated cheetahs are immunologically accepted and cheetah cranial characters show increased fluctuating asymmetry, an index of inbreeding in other species (86, 115, 119, 124, 157)

[2] Gir lions have three unusual morphological characters likely due to inbreeding: bifurcated infraorbital foramen, belly fold and reduced mane in males (104). The mane reduction may be a consequence of 10-fold depression in testosterone (161).

[3] Florida pumas have two unusual morphological characters associated with inbreeding: a dorsal anterior cowlick and a "kink" in tail vertebrae (12, 133).

N.D. Not determined

for the consequences of genetic depletion (104, 109, 116, 133, 161). The two lion populations had dramatically reduced allozyme, MHC, and DNA fingerprint minisatellite variation relative to a larger outbred lion population living in the Serengeti (47, 109, 122, 165). Both lion populations also had displayed elevated sperm abnormalities and large reduction in circulating testosterone concentration relative to the outbred Serengeti lion counterparts (104, 161). Further, reproduction of the most genetically deficient lion population, the Asiatic lion, was severely compromised with very limited male founder contribution in the captive population (104). When Asiatic lions were inadvertently bred to African lion subspecies in North America, the fecundity, reproductive success, and spermatozoa development improved dramatically (104). Combined with the cheetah result, it seemed clear that inbreeding, at least in the Felidae, had a direct effect on reproductive performance.

A compelling addition to this inference came from the dramatic story of the Florida panther (*Felis concolor coryi*), a native American subspecies of puma (111, 116, 133). Human depredation spurred principally by fear, legends of

ferociousness toward livestock and mankind, plus the imposition of bounties reduced the panther's range from the entire American southeast to hardwood swamps and adjoining Everglades National Park ecosystems in south Florida, the only habitat east of the Mississippi occupied by wild pumas today. The major threats to the Florida panther were demographic as road kills and illegal hunting accounted for 63% of documented mortalities since 1973. Genetic studies (Table 1) revealed that the Florida panther retained less genomic variation than any puma subspecies from North or South America, and several cases of incestuous (father/daughter) matings were documented in situ (133).

The cost of inbreeding in this population was dramatic. Florida panthers have the worst sperm we have seen anywhere; about 95% of the sperm in each ejaculate are malformed (11, 133). Further, the incidence of cryptorchidism, a rare heritable defect that causes one or two testicles to remain undescended, has risen from 0.0 to 80% in the males in the past 15 years. In addition, a new fatal congenital cardiac abnormality has recently appeared in three panther kittens. Finally, Florida panthers are riddled with pathological viruses, bacteria, and parasites, representing a time bomb waiting to explode as the animals develop debilitating disease. One of the viruses that is endemic in Florida panthers is a close relative of the feline version of the human AIDS virus, feline immunodeficiency virus (FIV) (121). FIV causes severe immunodeficiency in domestic cats, but we are not sure if it causes disease in panthers, at least not yet.

Two other species (Table 2), northern elephant seals and giant pandas, are also remarkably uniform in genetic variability compared to other closely related species (southern elephant seals and bears, respectively). Northern elephant seals have descended from a bottleneck of fewer than eight individuals due to rapacious hunting of animals on beaches off the west coast of North America (15, 56, 73). The cost of historic inbreeding in the species may not have been severe as the species has recovered to over 120,000 animals since legislative protection was afforded in 1922. The giant panda also has limited genetic variation based on two allozyme studies (103, 146); however, appreciable variation (106) was observed in more rapidly evolving DNA markers (mtDNA and DNA fingerprint minisatellite families). As had been observed in cheetahs (85), such a result implies an ancient bottleneck, the descendants of which have accumulated variation by mutation since the event. Giant pandas and northern elephant seals may be examples that offer some hope to conservationists since physiological correlates of inbreeding are not immediately apparent (73, 105, 113). The optimism should be cautious, however, as their immune system loci, if comparably homogenized, would still be vulnerable to pathogens that may evade immune surveillance in the future (105, 113).

The lessons we have learned from the studies of the six carnivore examples in Table 1 are several. First, undiscovered perils that were not so apparent from traditional ecological observations can threaten populations. Second, when populations drop to very low numbers, as most endangered species are wont to do, if they do not go extinct they still could suffer genetic depletion when inbreeding is close and persistent. Third, although every population bottleneck is different (see next section), they all carry the risk of inbreeding depression and the expression of congenital abnormalities resulting from homozygosity of rare deleterious genes. These genes can affect any aspect of development, survivorship, or reproduction in an unpredictable manner. Fourth, in addition to these heritable defects, inbreeding homogenizes variation of abundantly polymorphic genes that mediate immune response, increasing the population's risk of extinction from pathogens that abrogate the immune defenses of an individual.

EACH POPULATION BOTTLENECK IS DIFFERENT AND QUALITATIVELY UNPREDICTABLE

The consequences of a population bottleneck in a small population are difficult to predict because of a limitless variety of genomic variation generated by ancestral mutation and recombination over time. Mammal genomes contain between 50,000 and 100,000 structural genes and in most species 15–40% of these loci are highly polymorphic, and an even higher percentage have rare allelic variation (76, 97). When a species is reduced to a handful of individuals sufficient to deplete overall variation, the combination of surviving alleles will always be unique due to the stochastic nature of a founder event. For the same reason, every successful species is a unique product of numerous combinations of allelic interaction, selection, and genetic drift.

Table 2 presents a list of animal species that are presumed or known to have experienced a demographic contraction in their recent past. The reduction was actually witnessed in most taxa and in several cases subsequent molecular genetic estimates show a reduction in genomic variability. The differences in outcome have been described in detail, but it is striking how heterogeneous were the end results. The Florida panther represents the most extreme case, although other species also show problems that could have been exaggerated by historic inbreeding. For example, several taxa (black-footed ferret, bighorn sheep, cheetah, Pere David deer) were afflicted with post-bottleneck epizootics of infectious disease that may have been accelerated by the genetic structure of the population. By contrast, other nonendangered taxa (Swiss mice, Channel Island foxes, gypsy moths, northern elephant seals) have survived historic bottlenecks quite well, suggesting that they likely acquired a favorable combination of alleles. These examples suggest that the consequences of a population bottleneck are determined by both the quantity of variation lost as well as the stochastically determined quality of allelic representation that remains.

Table 2 Taxonomic units that have suffered demographic crashes: genetic and epidemiologic information

Species	Population bottleneck: Locale	Apparent cause	Lowest numbers of individuals diversity	Date	Measured loss of molecular genetic variation	Post-bottleneck parasite	Ref.
I. Endangered species:							
Northern elephant seal *(Mirounga angustirostris)*	West U.S. Coast	Overhunting	8	1892	Yes	Unknown	15, 56, 73
Cheetah *(Acinonyx jubatus)*	East and South Africa	Pleistocene extinction of large mammals	Unknown	10,000 ybp[a]	Yes	Feline infectious peritonitis	55, 115, 118
Black-footed ferret *(Mustela nigripes)*	Central U.S.	Poisoning of prey species	≤17	1900–1980	Yes	Canine distemper	108, 141
Big horn sheep *(Ovis canadensis)*	Western U.S.	Habitat loss	Unknown	1900–1980	Yes	Lungworm, *Pasteurella*, respiratory syncytial virus	36
California condor *(Gymnogyps californianus)*	Western U.S.	Habitat loss	30	1985	Yes		45
Giant panda *(Ailuropoda melanoleuca)*	China	Habitat loss	Unknown	1800–1980	Yes	Unknown	103, 146
Golden lion tamarin *(Leontopithecus chrysomelas)*	Brazil	Habitat loss	Unknown	1950–1980	Yes	Unknown	41
Humpback whale *(Megaptera novaengliae)*	Atlantic and Pacific Oceans	Overhunting	Unknown	1800–1970	No	Unknown	9, 10
Pere David deer *(Elaphurus davidianus)*	China	Hunting	3		Yes	Gamma Herpesvirus[b]	
II. Endangered subspecies:							
African lion *(Panthera leo leo)*	Ngorongoro	*Stomoxys*	6–16	1962	Yes	Unknown	47, 109, 123
Asiatic lion *(Panthera leo persica)*	Gir Forest	Overhunting	≤20	1880–1920	Yes	Papilloma, FIP	90, 104, 109, 161
Florida panther *(Felis concolor coryi)*	Florida	Habitat loss	30–50	1900–present	Yes	FIV	116, 121, 133
III. Nonendangered populations:							
Channel Island foxes *(Urocyon littoralis)*	Channel Islands	Founder effect	Unknown	2200 ybp[a]	Yes	Unknown	46
Domestic dog *(Canis familiaris)*	Worldwide	Domestication	Unknown	20,000 ybp[a] to present	Yes	Canine parvovirus	125, 154
Swiss mouse *(Mus musculus)*	U.S.	Founder effect	9	1926	No	Unknown	132
Lake Casitas mouse *Mus musculus domesticus)*	California	Founder effect	Unknown	19th Century	No	B-tropic MuLV	43
Gypsy moth *(Lymantira dispar)*	Northeast U.S.	Founder effect	Unknown	1960	Yes	*Entomophaga mimaiga*	52
Wildebeest *(Connochaetes gnou)*	East Africa	Rinderpest	Unknown	1889	Yes	Rinderpest	31, 144

[a]ybp, years before present
[b]P. Fennessey, personal communication

Another important consideration that also affects the outcome of bottlenecks is the amount of "genetic load", or quantitatively, the number of lethal equivalents, present in the original pre-bottleneck population (24, 153). If a species persists for a long period without contraction, deleterious mutations accumulate to a level that is proportional to the time elapsed since the last bottleneck (bottlenecks purge or "flush" the population of deleterious recessive and lethal equivalent genes by genetic homogenization, selection, and genetic drift). Ralls et al (129, 130) have used infant mortality as an index of fitness in studbook pedigrees of 40 zoo-bred species to estimate the fraction of lethal equivalents present in the species. This approach provides not only a predictor of the relative time since the last bottleneck or population flush, but also an indication of the relative sensitivity of different species to future inbreeding events.

It is possible in certain cases to estimate the time since historic bottlenecks or founder effects occurred by examining the pattern of genetic variation at rapidly evolving DNA families such as mtDNA or minisatellites (21, 85, 151). These molecules evolve at rates of 2–3 logarithms more rapidly than structural genes and have been used as an index of time elapsed since the last bottleneck in a species. For example, the amount of mtDNA sequence variation, due to lack of mtDNA recombination and maternal inheritance transmission, is a sensitive indicator of historic population reduction. Based upon the clocklike and monophyletic accumulation of haplotype variation since their respective most recent bottlenecks, estimated dates (21, 85, 133, 151) have been inferred for Florida panther (100 years ago), cheetah (10,000 years ago), and even humans (170,000 years ago). The time of the cheetah's bottleneck was determined by back-calculating mtDNA and minisatellite variation under the presumption that these loci were rendered monomorphic by the event and accumulated variation in the meantime (85). In other species (humpback whales, orangutans, and leopards) abundant mtDNA variations, sometimes with deep phylogenetic node divergences (see below), have been interpreted as evidence for the absence of recent genetically significant contractions (10, 59, 88). It seems clear that the combination of molecular genetics, studbook pedigree analysis, and evaluation of fitness components are providing penetrating new insights into the intrinsic threats to endangered species.

SOME CRITIQUES OF THE BOTTLENECK HYPOTHESIS

The genetic unpredictability of population bottlenecks has led to controversy over the implications of such an event and the prognosis for survivorship and fitness (18, 23, 50, 75, 147, 148). The debates have involved not only the theoretical consequences of inbreeding but also the implications of inbreeding for conservation. Certain misinterpretations, particularly that of the cheetah's genetic status, have been discussed elsewhere (100, 101) and are not repeated here. However, the general prediction that a population bottleneck should be avoided due to expression of deleterious recessive genes and homogenization of the immune systems has received some challenges that merit comment.

Two papers (18, 50) appeared in 1987 that pointed to an increase in variation following inbreeding, particularly at multiple morphological loci regulated by epistatic interaction. Bryant et al (18) measured quantitative variation at eight metric morphological measurements in houseflies after forcing strains through bottlenecks of 1, 4, or 16 pairs of individuals. They observed an increase of variation for six characters in survivors of moderate bottlenecks (4 and 16 pairs) and interpreted their observations as an empirical contradiction to the prevailing expectation that inbreeding would decrease variation (72, 96). A derivative news article suggested that their results challenged the dogma that "newly founded populations are at risk", and highlighted the cheetah example on the same pages (75).

Did the housefly data challenge the dogma? Probably not. First, the increase in variance of morphological traits upon inbreeding is well known; in fact a similar increase in variance of symmetrical morphormetric cranial measurements was reported in cheetahs (124, 157). Increases in asymmetry are common among inbred livestock apparently due to homogenization of several quantitative loci that are developmentally buffered in outbred individuals, a phenomenon termed genetic homeostasis (74, 124, 157). Second, the housefly data were based on characters with so little genetic definition that the authors offered several alternative and somewhat speculative models to explain them. Third, there were no measurements of fitness in the housefly data, so inferences about "risk" or fitness were not assessed. The observation was provocative, but hardly a serious exception to the paradigm; it was rather a new example of inbreeding affecting developmental homeostasis.

An interesting strategy for conserving endangered species was attempted at the St. Louis Zoo in a direct attempt to rescue the Spekes gazelle, *Gazella spekei*, from a founder effect (147, 148). The entire captive population was derived from one male and three female founders imported into the U.S. from their African range near the Ethiopia-Somalia border. It was impossible to avoid inbreeding with this population and the consequences of inbreeding depression were evident in regression analysis of 30-day and 1-year mortality with the coefficient of inbreeding. After several generations of close inbreeding with high mortality, maximal outbreeding was initiated with demonstrable improvement in survivorship and growth of the population. Templeton & Read (147, 148) correctly point out that bottlenecks are all different and that the early inbreeding may have eliminated a portion of the "lethal equivalents" by natural selection.

An erroneous interpretation of their experiments would be that early inbreeding was a "good idea" that might be applied to other endangered species

to facilitate the "flush" elimination of lethal equivalents in the survivors (87). The difficulties in following this logic are twofold. First, there was no alternative for the Speke's gazelle but to inbreed due to the availability of only four founders. There was never a conscious decision to inbreed this population as a survival strategy. Second, the fitness of the pre-bottleneck population (the outbred founder population) was unavailable for assessment so there is no evidence that the now maximally outbred captive group was as good as, much less greater in fitness, than the ancestral wild outbred stock. Although the Speke's example did provide empirical support for the shedding of accumulated lethal equivalents in the original founders, the resultant population nearly died out until outbreeding of the derivative lineages commenced. Since most inbreeding experiments lead to dead ends (or extinction), to intentionally inbreed a small threatened population should never be encouraged.

MOLECULAR PHYLOGENY AND ENDANGERED SPECIES

Advances in molecular phylogenetic inference have greatly increased our understanding of the hierarchical relationship between related species. In the past 30 years, the field of molecular evolution has matured in the provision of powerful analytical methods for building topologies and recapitulating evolutionary lineages (39, 77, 79, 155, 158). Molecular data, when combined with traditional "form and function" morphological descriptions, provide a keystone for biological classification or taxonomy. For endangered species, these advances are critically important because recognition of the categories of systematics (species, subspecies, genera, family, etc) provide the bases for legislative protection. Errors of "over-splitting" and "over-lumping" based on guesswork have led to several mistaken legal judgments that were retrospectively revealed by molecular approaches (8, 34, 44, 156).

Because it is unlikely that all endangered species will be afforded equal protection and resources, a proposed basis for ranking the priority of endangered species involves taxonomic distinctiveness or depth of phylogenetic divergence relative to other endangered species (83). For example, if an imperative came to choose between conservation of red pandas, snow leopards, and golden lion tamarins, the differences in their phylogeny would rank red panda first because the species has no close relatives and a 30-million-year old divergence from other procyonids (112, 126). Snow leopards would rank second as a member of a polyphyletic two-million-year-old genus that also includes lion, tiger, leopard, and jaguar (28). On taxonomic distinctiveness criteria, golden lion tamarin would rank third, as it is closely related in time (about 200,000 years) with two species, golden headed lion tamarin and black lion tamarins (41).

Molecular phylogenetic reconstruction can also be used to provide the temporal divergence hierarchy as a backdrop for interpreting functional adaptation (6). Molecular topologies are frequently based upon stochastic (selectively neutral) DNA segments, which are likely not involved in species adaptation. This adaptive "neutrality" is the reason why the DNA segments are useful for evolutionary tree building, because their mutational accumulation is cumulative, monotonic, and clocklike. The derived chronological topology can subsequently be applied to interpreting morphological adaptation in the context of the phylogenetic hierarchy. An example of this approach is illustrated by interpreting functional and chromosomal evolution in pandas and bears (94, 112).

In addition to resolving hierarchical species evolution, phylogenetic analysis has also revealed other surprising historic events in the background of endangered species. Two illustrations involve hybridization between taxa of two endangered species: Florida panther (*F. concolor coryi*) and red wolf (*Canis rufus*). In 1988, two family groups of pumas were discovered in the Everglades National Park that did not share the morphological characteristics of Florida panthers living adjacent to the Everglades in Big Cypress Reserve. An mtDNA–RFLP analysis revealed that the Everglades pumas had a haplotype that differed by 10 restriction sites from pumas in Big Cypress or from pumas in western USA (116). Phylogenetic analysis aligned the Everglades type with South/Central American pumas, which suggested a mysterious introgression into Florida of pumas originating from South America. The suspicion was confirmed when archives of the Everglades National Park revealed records of release of nonnative pumas to the Everglades between 1957–1967 (116).

A similar story involves the endangered red wolf, *Canis rufus*. This species was suspected to be a de novo hybrid between wolves and coyotes because red wolves have morphology intermediate between the two species and these species are known to interbreed in situ, particularly when the range and habitat of wolves are imperiled (84). A phylogenetic analysis of mtDNA haplotype and sequences revealed that living red wolves plus historic museum specimens had mtDNA genotypes that assorted phylogenetically with either wolf or coyote types but had no unique phylogenetic lineage (156). More recent analysis of nuclear microsatellite (dinucleotide repeat) loci supported this inference as no unique red wolf lineage was apparent (134). Both of these hybridization examples show the power of phylogenetic analysis to reveal historic isolation or introgression events that occur in nature.

A third application of phylogenetic analysis is the identification of subspecies, natural phylogeographic subdivisions below the species level. Subspecies genetic differentiation is thought to reflect geographic isolation for a time period sufficient for population genetic subdivision to be recognizable

with molecular methods, and by implication for adaptation to be possible. "Subspecies" is a much maligned concept, primarily because the definition is not universally agreed upon—not to mention the controversies over species definition. Further, many endangered species have been "split" excessively by nineteenth century naturalists who named nearly every population they encountered as distinct often based on a single type specimen. For example, pumas have been split into 30 different subspecies and leopards into 33 distinct trinomials (95, 142). Miththapala (88) has addressed the leopard subspecies question by a combined morphological and molecular assessment of leopard subspecies. Using concordant phylogenetic distinctiveness as a criterion (7), allozymes, mtDNA-RFLP, and DNA fingerprints identified eight differentiated clades of leopards (88). The molecular results were largely supported by a multivariate analysis of cranial measurements and provide a formal basis for assimilating leopard subspecies into eight revised subspecies. A parallel analysis of molecular differentiation in puma subspecies, using additional DNA families (microsatellites, DNA sequence, single-stranded conformation polymorphism) is under development in our laboratory.

Two additional applications of phylogenetic analysis relating to endangered species also deserve mention. The matrilineal inheritance of mtDNA haplotypes played a role in interpreting migration patterns of humpback whales by Baker and colleagues (9). Although humpbacks migrate from separate summer feeding grounds in the North Pacific to a common breeding ground near Hawaii each winter, their migratory destinations to natal feeding grounds are apparently maternally directed since mtDNA genotypes in each feeding ground are phylogenetically nonoverlapping and largely monophyletic. The phylogenetic analysis of a maternally inherited genome led directly to insight into the behavioral migratory strategies used by a large endangered marine mammal.

An area of increasing interest relative to natural populations in general and endangered species in particular is the phylogenetic analysis of pathogens. Viruses, particularly retroviruses, accumulate mutations in their genomes rapidly, on the order of one mutation per 8-kilobase genome replication (48, 107). The evolutionary relationship of virus genomic clades reflects evolution within a host species such that the occurrence of a virus sequence clade with deep phylogenetic roots of divergence generally indicates cross-species transmission. This type of inference has led to provocative reconstruction of virus transfer of human immunodeficiency virus (HIV-1 and HIV-2) from African primates to man (35, 42). More extensive between-species transfer followed by host monophyletic divergence has been described for human T-lymphotropic virus (HTLV-I), the etiologic agent for adult T-cell leukemia and tropical spastic paraparesis (67).

The cat family, Felidae, has its own version of the AIDS lentivirus, termed feline immunodeficiency virus (FIV) (127, 128). The virus causes immune cell collapse and death in domestic cats. Phylogenetic relatives of FIV have been discovered in several free-ranging endangered species including lions, cheetahs, leopards, pumas, and many others. Phylogenetic analysis of conserved viral polymerase genes reinforces species monophyly of the viruses, but with very deep evolutionary roots (17, 121). Because disease is not so apparent in wild cats, the viruses may have achieved a period of virulence amelioration or balance following an historic outbreak and genetic accommodation. As commensal or symbiotic hitchhikers, these lentiviruses offer a handle on population differentiation as well as a rare glimpse of coevolution of pathogen and host in situ.

CONCLUSIONS

Conservation genetics is just getting started, but its influence can already be seen in conservation planning. Most captive species are managed by maximizing outbreeding and founder representation. The cheetah legacy led to a concerted effort to improve genetics, reproduction, and husbandry, a program that has seen significant improvements in captive propagation (160). Natural hybridization of Florida panthers and red wolves in nature led to the US Fish and Wildlife Service dropping its ill-conceived "Hybrid Policy," which precluded protection of hybrids between species or subspecies (111). The genetic horrors of the Florida panther justified a backup captive breeding program and facilitated introduction of Texas cougars (*F. concolor stanlezi*) to Florida (133). Black-footed ferrets were captured, immunized against canine distemper, bred up to several hundred, and released (141). These are but a few examples of the power and direct implementation of genetic technology to species conservation. No one aspect of conservation biology offers a unique and general solution, but each of these, including genetics, can provide crucial guidance in the important goal of reversing extinction of endangered species.

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