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Intersection of Population Genetics and Species Conservation

The Cheetah's Dilemma

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INTRODUCTION

In 1966, Bruce Wallace, a young but distinguished member of the Cornell University Faculty, accepted me as a graduate student in genetics. Under the direction of Bruce and his colleagues, particularly Ross MacIntyre, I studied the principles, theory, and applications of population genetics, the behavior of genes, and genomic diversity. But I also learned important lessons from Bruce about the value of science and scientific investigation.

In the genomic era of today, population genetic inference has spawned the emergence of at least three critically important applied fields: (1) conservation genetics—assessing status and taxonomic recognition of endangered species; (2) human gene mapping, particularly using population association and linkage disequilibrium analysis as signals for genetic linkage; and (3) forensic genotyping for genetic individualization. Each of these fields, combined with the dazzling technological advances characterizing

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the Human Genome Project, has important social, legal, and ethical applications that stand on the framework of population genetic theory. To illustrate the integration of these concepts, I address here some of the significance and interpretations that have grown from our studies of the genetic and physiological analysis of natural populations of African cheetahs (*Acinonyx jubatus*).

CHEETAHS NEVER WIN

Over a decade ago, my collaborators and I reported the first results indicating that African cheetahs retain significantly less overall genomic variation than did other felid or mammal species, based on a survey of allozymes and fibroblast proteins resolved by two-dimensional gel electrophoresis (O'Brien *et al.*, 1983). Subsequent molecular studies have extended the original observation of genetic uniformity of the species (summarized in Table I) and led us to conclude that the cheetah's ancestors experienced a severe demographic reduction several thousand years ago (likely at the end of the Pleistocene, the time of the most recent Northern Hemisphere glaciation) (O'Brien *et al.*, 1985, 1986, 1987; Yuhki and O'Brien, 1990; Menotti-Raymond and O'Brien, 1993). We proposed that this population bottleneck resulted in inbreeding among close relatives, which led to reductions in overall genetic variability among all sub-Saharan cheetahs sampled to date. Correlated with the genetic uniformity are a number of physiological impairments that influence reproduction and contribute to difficulty in establishing a self-sustaining captive population (Table I). Further, the high levels of mortality in cheetahs from outbreaks of a frequently benign cat virus (feline infectious peritonitis virus) were interpreted as a consequence of the homogeneous state of genes that mediate immune defenses (O'Brien *et al.*, 1985, 1986; Heeney *et al.*, 1990; O'Brien and Evermann, 1988). Several of these immunologic loci are highly variable in other feline and mammalian species, particularly the major histocompatibility complex. High genetic variation at the genes involved in presenting microbial antigens to the immune system offers a selective advantage to a species at a population level by providing a "moving target" for microbial pathogens that are themselves rapidly evolving genetic strategies to overcome the immune defenses of the individuals within the population.

Partly because the cheetah is a charismatic endangered species, and partly because of the conservation implications of the genetic and physiological observations (O'Brien, 1994*a–c*; O'Brien *et al.*, 1985, 1996), the

TABLE I. Evidence for Genetic Uniformity in Cheetahs and Observed Physiological Correlates

Evidence and correlates	Reference
Indices of genetic variation reduced in cheetahs	
1. Allozyme, 52 loci.	O'Brien *et al.*, 1983, 1987
2. Two-dimensional PAGE, 155 loci.	O'Brien *et al.*, 1983
3. Allogeneic skin graft accepted.	O'Brien *et al.*, 1985
4. MHC RFLP, six restriction enzymes.	Yuhki and O'Brien, 1990
5. Mitochondrial DNA RFLP.	Menotti-Raymond and O'Brien, 1993
6. Microsatellite loci.	Menotti-Raymond and O'Brien, 1995
7. Increased fluctuating asymmetry of skeletal measurement.	Wayne *et al.*, 1986
Physiological correlates limited in cheetahs	
1. Diminished sperm count.	O'Brien *et al.*, 1983; Wildt *et al.*, 1983, 1987*b*
2. Elevated frequency of morphological abnormalities in sperm development (~70%).	O'Brien *et al.*, 1983; Wildt *et al.*, 1983, 1987*b*
3. Low fecundity in captive breeding attempts throughout history.	Marker and O'Brien, 1989; Marker-Kraus and Grisham, 1993
4. Captive population is not self-sustaining.	Marker and O'Brien, 1989; Marker-Kraus and Grisham, 1993
5. Relatively high incidence (~30%) of juvenile mortality, even among unrelated parents.	O'Brien *et al.*, 1985; Marker and O'Brien, 1989
6. Increased population vulnerability to infectious disease outbreaks, notably feline infectious peritonitis.	O'Brien *et al.*, 1985; Heeney *et al.*, 1990

story of the cheetah's legacy has been debated, reinterpreted, and retold in both the scientific and popular media. Many of the derivative articles are largely accurate and serve an important role in illustrating how biomedical techniques can improve our understanding of the history of threatened species and optimize their chances for survival (May, 1995; Lewin, 1996). But some authors have confused the data and posed interpretations that I believe are inconsistent with the accumulated information (Merola, 1994; Caughley, 1994; Caro and Laurenson, 1994; Laurenson *et al.*, 1995*a*,*b*; Angier, 1992; Pennisi, 1993). I address here five specific misinterpretations that I have encountered commonly, occasionally from our scientific colleagues but also frequently from journalists and conservationists who are not trained to interpret the results and must depend on opinions of others. Each of these imprecise views has implications that could negatively influence conservation priorities for cheetahs and other threatened species. In an attempt to clarify my own impressions, I revisit the accumulated data

around the cheetah's fascinating genetic history by offering comment on five specific misleading inferences.

1. The cheetah has so many genetic and physiological problems, it is clearly doomed, so there is little justification for devoting resources to its conservation.

This view comes from some observers being overly convinced by the results summarized in Table I and reaching the erroneous conclusion that the prognosis for cheetah survival is hopeless. However, the prospects for cheetah conservation are actually much more encouraging. All available data indicate that the cheetah's bottleneck occurred an estimated 10,000 years before the present (Menotti-Raymond and O'Brien, 1993; O'Brien *et al.*, 1987). Since that time, the survivors of the bottleneck have undergone some 1,600 generations and risen to over 100,000 individuals a century ago (Myers, 1986). This is good news because we know that the majority of demographic and genetic damage occurs immediately after a bottleneck (Roelke *et al.*, 1993; Lande, 1988; Seal *et al.*, 1989). That the cheetah has survived for so long indicates that the physiological impairments (Table I) have not been overly limiting in natural populations. For example, a recent field study of cheetah cub survival in the Serengeti ecosystem suggested that the primary regulator of population growth was predation by lions and hyenas (Laurenson, 1994; Laurenson and Caro, 1994; Caro and Laurenson, 1994). A similar population regulation by predators was observed previously to explain the rapid rise of cheetahs on large private farmlands in Namibia after lions and hyenas had been eliminated by human-assisted depredation (Morsbach, 1987; Marker-Kraus and Kraus, 1993). It is rather clear that the major reason for 20th century reduction of cheetah numbers (to about 11,000 cheetahs) is habitat depletion caused by human development and not intrinsic genetic problems (Myers, 1986; Morsbach, 1987; Marker-Kraus and Kraus, 1993).

Captive breeding has also improved in the last several years, apparently through improvements in husbandry and behavioral insight (Marker-Kraus and Grisham, 1993; Wildt *et al.*, 1993). Further, there is at least one precedent, Northern elephant seals, *Mirounga angustirostrus*, for a species that suffered a severe bottleneck (down to fewer than eight individuals in 1892) and lost genetic diversity but recovered to over 100,000 individuals after being afforded legislative protection in 1992 (Bonnell and Selander, 1974; Hoelzel *et al.*, 1993; Le Boeuf and Bonnell, 1980). It is important to emphasize that there is a range of perils that afflict natural populations and that the significance of each varies over time and ecology. In addition, genomic evolution generates physiological redundancy that allows for alternative pathways to survivorship. This is not to say that the physiological

impediments in Table I are not significant in cheetah survival; rather, the species has grown in spite of these handicaps. One should not exclude, however, that genetically induced physiological impairments would become regulatory of population growth in certain situations (e.g., possibly in historic extinction of cheetahs in Asia, Europe, and North America); infectious disease outbreaks would pose a particularly serious threat to species as genetically uniform as cheetahs (Heeney *et al.*, 1990; O'Brien and Evermann, 1988; Brown *et al.*, 1993). The direct effects of genetic homogenization are dramatically apparent in at least two felid subspecies, Florida panther (*Felis concolor coryi*) and Asiatic lion (*Panthera leo persica*), which show marked reproductive, disease, and physiological impairments following severe genetic homogenization in nature (Roelke *et al.*, 1993; Wildt *et al.*, 1987*a*).

2. The cheetah example plus the documented cost of inbreeding depression among inbred species (Falconer, 1981; Wallace, 1970; Green, 1968; Wright, 1978; Black, 1992) makes genetic considerations the most important and perhaps the only major peril of small populations.

This interpretation also overextends the implication of our data. When a population drops to numbers sufficiently low to shed genetic diversity and suffer inbreeding depression, the acute threats are more demographic than genetic (Roelke *et al.*, 1993; Lande, 1988). As populations decline to very low numbers, individuals and indeed the whole population become vulnerable to a variety of stochastic processes. These can be age structure shifts, prey depletion, infectious disease, climatic extremes, or any calamity. Demographic factors can lead to extinction as surely as any other factor, including reduced genetic representation. The severe effects of demographic collapse have been observed directly in several species: Northern spotted owl (*Strix caurina occidentalis*), California condor (*Gymnogyps californianus*), Florida panther (*Felis concolor coryi*), black-footed ferret (*Mustela nigripes*), and giant panda (*Ailuropoda melanoleuca*) are familiar examples (Roelke *et al.*, 1993; Lande, 1988; Seal *et al.*, 1989; O'Brien and Knight, 1987; Geyer *et al.*, 1993). If a species is fortunate enough to increase its population size above the threshold of demographic danger (as the cheetah apparently did), then physiological and genetic costs begin to affect the population increase. Finally, the ecological status of the habitat should not be underestimated because population growth is affected by predators, prey availability, disease outbreaks, nutrition, and other factors under environmental influence in addition to a species' genetic potential.

3. Cheetahs are regulated in nature by ecological factors alone, and the genetic status and natural history are irrelevant to their survival.

Caro and Laurenson's recent field observations of Serengeti cheetahs revealed very high cub mortality in their lairs (71% cub mortality by 2 months old and 95% by one year of age) that was principally from lion or hyena predation (Laurenson, 1994; Laurenson and Caro, 1994; Caro and Laurenson, 1994), leading them to conclude that genetic factors were not regulating this population. Yet there are at least three reasons why their mortality estimates might be inflated. First, cub deaths from predation are not independent estimates because, once a cheetah den is discovered, all the cubs will be taken regardless of litter size. Second, it is difficult to exclude observer influence because a researcher in a vehicle watching a cheetah den would sometimes alert lions and hyenas to their subject. Third, confirmed mortality caused by predation was observed in only two of 36 studied lairs and merely inferred or deduced for seven other lost lairs (Laurenson, 1994; Laurenson and Caro, 1994). Of the 89 deaths at ≤2 months, they attribute 35.5 of the deaths (40%) to predation, but it is not clear how they estimated 73% death by predation in two subsequent correspondences (Caro and Laurenson, 1994; Laurenson *et al.*, 1995*a*,*b*). In their original report (Laurenson, 1994), 13 litters (48.5 cubs or 50% of the deaths) died of unknown causes. These caveats raise some question about the interpretations of the Serengeti study that dismissed the role of the cheetah's genetic status in their survival.

Even if one accepts that ecological factors are primary regulators of cheetahs in the Serengeti, to conclude that "genetics may only be relevant to free-living populations under limited conditions" (Caro and Laurenson, 1994) seems to be an unsupported extrapolation of their observations. The consequences of inbreeding among many originally outbred populations (congenital abnormalities, reproductive impairment, increase population susceptibility to infectious disease) are well documented and in several species including Asiatic lions, Florida panther, Spekes gazelle, black-footed ferrets, inbred mice, cheetahs, and several livestock species (O'Brien *et al.*, 1985; Roelke *et al.*, 1993; Wildt *et al.*, 1987*a*,*b*; Falconer, 1981).

Human populations are not immune. Black (1992) suggested that the massive infectious disease mortalities of native Americans following European contact were aggravated by a threefold reduction in genetic diversity at the human major histocompatibility complex, HLA. Further, many recent studies point to the critical role of genetic diversity in response to infectious disease in man (Sorensen *et al.*, 1988; Dean *et al.*, 1996). Although one might prefer that the cheetah's genetic legacy would not threaten populations in different habitats, ecosystems or in different times (as they clearly do under captive conditions), to disregard the implications of the genetic results would be a mistake.

4. Cheetahs breed quite well in captivity, and there is noting inherently wrong with them; their developmentally abnormal sperm have little effect on reproductive potential.

This conclusion, highlighted by an article published in the *New York Times* (Angier, 1992) and derivative popular pieces (Pennisi, 1993), is based on a study of 12 male cheetahs housed at the San Diego Zoo from 1982 to 1991 that were selected for sperm and breeding evaluation (Lindburg *et al.*, 1993). All the males had a high frequency of morphologically abnormal sperm (mean ± S.E.M. 66.8 ± 3.7%), but ten of 12 (83.3%) successfully produced pregnancies. The authors concluded that "Semen quality . . . is by itself a poor predictor of fertility in cheetahs." Lindburg commented" . . . recent studies with cheetahs show unequivocally that there is nothing inheretly wrong with them, only with the ways they are handled" (Lindburg, 1993).

Certain aspects of the San Diego Zoo study lead us to draw somewhat different conclusions. First, only one of the six physiological parameters listed in Table I, fecundity, was challenged; two others, elevated teratospermia and high infant mortality, were affirmed, and the others undisputed. Second, the San Diego sample is small (12 male subjects) compared to the complete studbook estimates (Marker-Kraus, 1992) of fecundity that our earlier studies used to infer breeding difficulties (O'Brien *et al.*, 1985; Marker and O'Brien, 1989) (which also included the San Diego breeding results). The studbook breeding data estimates were based on a sample of 438 cheetahs taken into captivity over a 20-year interval (Marker and O'Brien, 1989; Marker-Kraus and Grisham, 1993; Marker-Kraus, 1992) compared to 12 male animals in the San Diego study. Marker and O'Brien (1989) reported that 52 of 349 wild-caught animals (15%) successfully reproduced in 22 of 50 facilities that housed cheetahs up until 1986 (when that report was completed). Since then, breeding improved to 28% success (25/89 paired cheetahs bred in 1987–1991), reflecting both better management and perhaps outbreeding between two subspecies of cheetahs (*A. jubatus raineyi* from central eastern Africa and *A. jubatus jubatus* from southern Africa), as 39% of the offspring were derived from between-subspecies crosses (Marker-Kraus and Grisham, 1993). An additional study demonstrated that hybridizing between different cheetah subspecies resulted in a large (>50%) reduction in cub mortality at Whipsnade Park, U.K. (O'Brien *et al.*, 1985).

Lindburg *et al.* (1993) stated that "eleven [of the 12 male cheetahs studied] had no reproductive history at the time of entry into the test program, thereby precluding the selection of samples biased in terms of reproductive potential." A retrospective analysis of breeding successes for the San Diego Zoo (based on studbook records reported by that facility)

did not support this statement. During the study period, 1982 to 1991, nine of 25 adult male cheetahs with no reproductive history at San Diego fathered cubs. If all 25 available males were paired for breeding attempts (as was reported to the cheetah species survival plan), the success rate would be 9/25 = 36%, not 83% as claimed. Further, presuming that the single additional animal (the one with a reproductive history) was a known breeder, then the ten breeders in the study included one proven breeder and the only nine breeders that were at the San Diego Zoo during the study period. The likelihood of choosing by chance all nine cheetahs that eventually bred in a random sampling of 11 animals from 25 available male animals is statistically improbable ($p = 0.0000269$), raising to question whether the selected male subjects were included with a bias in terms of reproductive performance.

Finally, implicit in the conclusion we question here is the notion that cheetah ejaculates with an average of 70% abnormally developed spermatozoa are effective in reproduction or at least "good enough" to sustain a reproductively healthy population. Four lines of evidence directly contradict this inference. First, physiological assessment of cheetah spermatozoa has demonstrated that pleiomorphic sperm are incapable of fertilization, of oocyte or zona pellucida penetration (Howard *et al.*, 1990). Second, felid species have a gradation in the frequency of pleiomorphic spermatazoa, and there is a direct correlation between the incidence of successful fertilization of homologous ova and the frequency of normospermic (morphologically normal) sperm per ejaculate (Wildt, 1994). Third, in studies designed to evaluate the morphologically normal sperm present in teratospermia ejaculates, separated "normal sperm" were significantly impaired in both binding and penetration of homologous species' ova compared to normal sperm from nonteratospermia species (Howard *et al.*, 1990, 1993). For example, domestic cats, which have approximately 30% incidence of teratospermia, penetrate and fertilize 80% of cat ova, whereas normal sperm from pumas and cheetahs, which have the highest levels of teratospermia among feline species, bind and penetrate an average of 11% and 19% of homologous ova, respectively. Fourth, in a recent reproductive survey of 60 captive male cheetahs, five with a long unexplained failure to breed were each shown to produce more than 90% spermatozoal abnormalities per ejaculate, considerably higher than successful cheetah breeders (Wildt *et al.*, 1993). Each of these points offers persuasive evidence that genetically associated teratospermia negatively affects reproductive performance in cheetahs as in other mammalian species.

5. The cheetah's immune system is sound . . . no different from other cat species.

Recent reports have examined the ability of cheetah lymphocytes to recognize microbial antigens to which they had been exposed. A demonstration of lymphocyte proliferation plus "a wide variation in the level of responses to relevant infectious agents" led Miller-Edge and Worley (1991, 1992) to conclude that the cheetah's immune system was not contributing to the cheetah's intrinsic peril. The authors suggested further that the cheetah's immune system is neither compromised nor disarmed.

The new results of these studies are useful but not contradictory to the interpretation of that we inferred. Our earlier findings indicated that the cheetah's immune response is not "compromised or disarmed"; rather, it is genetically monomorphic or monotonous. That the cheetah's immune system does function is evident from many studies, including those from our laboratory, detecting antibody production in response to feline immunodeficiency virus and feline infectious peritonitis virus (Heeney *et al.*, 1990; Brown *et al.*, 1993). The cheetah's problem is not an impaired or nonfunctional immune system, rather one with little genetic plasticity (O'Brien *et al.*, 1985; O'Brien and Evermann, 1988). Pathogens evolve genetically, and their primary selective pressure is the host species' immune defense apparatus. When an individual member of an outbred, polymorphic host species becomes afflicted by an adapted virulent pathogen, other genetically distinct members of the population have a reasonable chance of being resistant. In an inbred monomorphic host population, the same adapted pathogen would likely infect and cause disease in all members. The potential for a widespread infectious disease plague is greatly increased in inbred populations or species. Familiar examples of the phenomena are evident in inbred mice, livestock, and even in the genetically depleted American Indians (Black, 1992). Expecting the cheetah to have an inherent immune deficiency (Miller-Edge and Worley, 1991, 1992) was neither indicated nor predicted by our studies.

CONCLUSIONS

The accumulated data on the cheetah's genetic and physiological status provide a remarkable example of how one endangered species has survived and recovered from near extinction. The prognosis is not entirely bad; in fact, the cheetah's long persistence and population growth over thousands of generations has aspects of a success story, particularly if natural habitat can be protected as a refuge for surviving cheetahs. But genetics and reproduction are only a part of any conservation story, as demography, ecology, nutrition, disease, and behavior must also be considered in developing

successful management plans. Claims that there is "nothing inherently wrong" with cheetahs based on breeding and immunologic studies seem inflated and require closer inspection of the published scientific evidence, particularly by the popular media, who in a few cases have promoted these points uncritically. Fortunately, the genetics and conservation community have debated the "cheetah controversy" in some depth (May, 1995; O'Brien, 1994*a–c*; Lewin, 1996; Sanjayan and Crooks, 1996) and by and large affirmed the general conservation significance of the data presented in Table I. A valuable lesson I learned from Professor Wallace, a value for hard clean empirical data, has provided an effective measure to prevail over rhetorical polemics driven by disciplinary chauvinism, missinterpretation, and even political manipulation of the cold hard facts.

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