Zoo Biology 6:99–116 (1987)

RESEARCH ARTICLES

Evidence for African Origins of Founders of the Asiatic Lion Species Survival Plan

S.J. O'Brien, P. Joslin, G.L. Smith III, R. Wolfe, N. Schaffer, E. Heath, J. Ott-Joslin, P.P. Rawal, K.K. Bhattacharjee, and J.S. Martenson

Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland (S.J.O., J.S.M.); Lincoln Park Zoological Gardens, Chicago (N.S.); University of Illinois at Urbana-Champaign, Urbana, Illinois (E.H.); Sakkarbaug Zoo, Junagadh, Gujarat State (P.P.R.) and Wildlife Institute, Dehra Dun, U.P. (K.K.B.), India; Knoxville Zoological Park, Knoxville, Tennessee (G.L.S., R.W.); Chicago Zoological Park, Brookfield, Illinois (P.J., J.O.-J.)

The Asiatic lion (*Panthera leo persica*) exists in the wild as a single relict population of approximately 250 individuals in the protected Gir Forest Sanctuary in western India. In 1981, a species survival plan (SSP) for the Asiatic lion was established by the American Association of Zoological Parks and Aquariums to manage the 200+ descendants of Asiatic lions in captivity in western zoological facilities. This captive population was derived from seven founders. In order to compare the genetic structure of the Gir Forest population with that of the captive SSP population, a genetic survey of 46 electrophoretic allozyme systems resolved from extracts of lion blood was undertaken by using 29 SSP Asiatic lions and 28 wild-caught or captive-bred lions maintained at the Sakkarbaug Zoo in India but originally derived from the Gir Forest. The Gir lion population was found to be genetically monomorphic at each of 46 allozyme loci. This was in contrast to several African lion (*Panthera leo leo*) populations, which show moderate levels of allozyme variation at the same loci. The SSP lion population was polymorphic at three allozyme loci (*IDH1*, *TF*, and *PT1*) for alleles that were previously found only in African lion populations. Pedigree analysis of the genetic transmission of these three biochemical loci demonstrated that two of the five primary founder animals of the SSP Asiatic lion population (a breeding pair originally imported from the Trivandrum Zoo in southern India) were descendants of the African subspecies. Three other founder animals were pure Asian. A retrospective SSP pedigree analysis of two morphologic characters (prominent abdominal fold and pairing of infraorbital foramen) that are partially diagnostic for *persica* vs *leo* was consistent with this conclusion as well. The implications for the management of small captive populations of threatened species and of the Asiatic lion SSP population are discussed.

Key words: Asiatic lions, African lions, SSP, population genetics

Received for publication August 13, 1986; accepted September 15, 1986.

Address reprint requests to Stephen J. O'Brien , National Cancer Institute, Building 560, Room 21-105, Frederick, MD 21701-1013.

INTRODUCTION

As recently as 200 years ago the Asiatic lion (*Panthera leo persica*) occupied a wide range across southwestern Asia extending from Syria to northern India [Joslin, 1984]. Although several authors have suggested that the range of lions also included the Arabian peninsula [Talbot, 1959; Neff, 1983; Guggisberg, 1963, 1975; Hemmer, 1974; Kingdon, 1977; Zuber, 1978], a recent extensive analysis of over 150 hunting and sighting records by one of us (P. Joslin, personal communication) produced no evidence to support the recent occurrence of lions in this region. The advance of agriculture, the increased use of firearms, and other familiar companions of human population pressure brought the subspecies to extinction in Syria, Iraq, Iran, Afghanistan, and Pakistan in the latter part of the 19th century. The single wild population of Asiatic lions remaining today occupies a 1,400-square-kilometer area that includes the Gir Forest Sanctuary and the surrounding forest in the Gujarat State in western India. The Gir Forest lions are a relict population of Asiatic lions that has been isolated since the 1880s [Pocock, 1930; Joslin, 1984]. After two reports in the early 1900s put the number of lions at 20 or less, a complete prohibition of lion hunting was imposed in the Junagadh State [Caldwell, 1938]. In 1947, the Indian government declared the Gir Forest area a reserve and more recently upgraded part of the ecosystem to Wildlife Sanctuary and National Park status. The Gir lion population today includes approximately 250 individuals [Dharmarkumarsinhji, 1982].

Phenotypically, the Asiatic lion has a number of characteristic morphological features that have been considered as diagnostic for the subspecies, especially in contrast to the African lion subspecies, *Panthera leo leo* [Pocock, 1930, 1939]. The Asiatic lion has a prominent fold of skin that spans the length of the abdomen; the abdominal fold is seldom observed in African lions. African lion males are thought to have a larger and fuller mane than their Asian counterparts. Asiatic lion skulls often (but not always) have a bifurcation of the infraorbital foramen. Differences in morphological traits prompted the subdivision of Asiatic lions by Pocock [1930] into five zoogeographic subspecies. By means of similar morphological and zoogeographic distinctions, the African lions have been split into as many as 24 subspecies [Smithers, 1971]. The modern consensus opinion, however, would classify all African lions into a single subspecies, *P l leo*, and all Asiatic lions into *P l persica*.

In an attempt to assist in the preservation of the severely threatened Asiatic lion subspecies, a species survival plan (SSP) was developed in 1981 by the American Association of Zoological Parks and Aquariums to manage cooperatively a second captive population of Asiatic lions. An international studbook was organized and published, and a management plan was developed. A total of 333 lions was registered, and of these, 205 "Asiatic" lions are housed today in 38 zoological institutions in Asia, Europe, Australia, and North America [Smith, 1985]. The studbook and management plan were established retrospectively; that is, the lions that were held in institutions and derived from Indian populations were included in the studbook and in the management plan. The captive Asiatic lion population has proceeded well in terms of reproduction (compared, for example, to the cheetah [see Marker, 1985; O'Brien et al, 1985, 1986]) since its inception.

The SSP management program did, however, have two caveats which were distressing. First, the entire captive Asiatic population outside India was derived from

only seven founders, and hence the potential for inbreeding and exposure of damaging recessive traits was real. Second, although the origins of the founder lions could be confidently traced to Indian zoos in the late 1960s, a persistent, but unconfirmed concern was that the founder animals may have been African imports or descendants from the same. For example, two founder animals came to the United States from the Delhi Zoo in 1972. Because the Delhi Zoo occasionally obtained its stock from the Baroda Zoo, which was known to have received African-Asian hybrid animals previously, an inadvertent substitution of African or hybrid imports from the Baroda Zoo remained a possibility despite the records that stated that the lions were Asiatic and were derived from the Sakkarbaug Zoo (originally captured in the Gir Forest 2 years earlier).

A second founder pair was derived from the Trivandrum Zoo in southern India. The possibility of mistaken origins of these animals was amplified in 1984 when the director of the Trivandrum Zoo communicated to one of us (P.J.) that 18 of 23 lions presently in his collection were African-Asian hybrids.

Further fueling the suspicion of a potential African contribution to the SSP population was an erratic incidence of the diagnostic morphologic characters in captive lions. These observations, however, were inconclusive because many of the characters have a low penetrance (the incidence of morphologic expression in animals that contain the appropriate genotype among free-ranging lions). Further, the influence of diet and other aspects of captive rearing on the expression of these traits was difficult to assess. Thus, the presence of an abdominal belly fold or the absence of a full mane could not be used as conclusive evidence for an Asian origin of a captive-born lion or vice versa.

We present in this report the results of a genetic analysis of allelic enzyme (allozyme) loci in a group of 28 lions recently derived from the Gir lion population and held at the Sakkarbaug Zoo in India. We also surveyed 29 lions from the captive SSP population of Asiatic lions. The character and extent of genetic variation were compared between the two populations and to similar data on African populations from different locations. Three polymorphic loci (*IDH1, TF,* and *PT1*) were informative with respect to the discrimination of Asian versus African lions. The distribution of allelic variation in natural populations was compared to a pedigree analysis of the markers in the captive SSP population for each locus. In every case, the results implicated the African origin of the Trivandrum (India) Zoo founder pair and the Asian origin of the Delhi Zoo founders. A retrospective analysis of two informative morphological characters (abdominal fold and pairing of infraorbital foramen) supported this conclusion. The implications of the genetic interbreeding of Asian and African subspecies in the SSP program for future directions are discussed.

MATERIALS AND METHODS

Lion Populations

Heparinized blood was collected from the zoological facilities listed in Table 1. Sampled animals are identified by their International Studbook number [Smith, 1985]. Their familial relationships are presented in a pedigree for Sakkarbaug Zoo animals in Figure 1 and for the SSP animals in Figure 2. Both pedigrees are derived from the Asiatic lion studbook [Smith, 1985].

TABLE 1. Zoological institutions holding studied lions

Institution	Director	Veterinarian/curator	Studbook No. of animals studied
National Zoological Park Washington, DC	M. Robinson	M. Bush	133,167
San Diego Wild Animal Park San Diego	D. Myers	D. Janssen J. Dolan	111,114
Minnesota Zoological Garden Apple Valley, MN	S. Iserman	F. Wright	51,207,226
Philadelphia Zoological Garden Philadelphia	W. Amand	K. Hinshaw	87,49
Knoxville Zoological Park Knoxville, TN	G. Smith	R. Wolfe	37,52,108 128,134,197
Lincoln Park Zoo Chicago	L. Fisher	T. Meehan	6,12,22,28,89,91 137,168,169,333
Audubon Park & Zoological Gardens New Orleans	L.R. Forman	S. Winslow	33
Miller Park Zoo Bloomington, IL	R. Carney	—	113,191
Cheyenne Mtn. Zoo Colorado Springs	D. Allen	P. Calle	13
Sakkarbaug Zoo Junagadh, India	P.P. Rawal	C.N. Bhuva	284,285,286,289, 290,291,292,293, 296,297,298,299, 300,301,302,305, 307,308,309,310, 313,316,318,319, 321,322,323,327

Anesthesia

The lions sampled at the Sakkarbaug Zoo were restrained in a squeeze cage for bleeding, tattooing, and sedation. Six adult male lions were sedated for electroejaculation. Effective immobilization was achieved with dosages ranging from 5.0 to 6.5 mg of ketamine/kg (mean, 5.6 mg/kg) (Ketaset, Bristol Laboratories, Syracuse, NY) and 0.5 to 0.75 mg xylazine/kg (mean, 0.6 mg/kg) (Rompun, Bayvet Division, Miles Laboratories, Inc., Shawnee, KS). One of the six males could not be caught in a squeeze cage, and it was sedated in a small enclosure by using a blow pipe. Additional ketamine was supplemented as needed in 100- to 150-mg increments intravenously to maintain anesthesia.

Electrophoretic Procedures

Blood was separated into plasma, erythrocytes, and leukocytes as previously described [O'Brien, 1980; Newman et al, 1985; Ford, 1978]. Field collections were stored in liquid nitrogen freezers until their return to the National Institutes of Health where they were stored at $-70°$. Isozyme extracts were prepared by sonication and were subjected to aqueous gel electrophoresis as previously described [O'Brien, 1980; Newman et al, 1985]. Histochemical stains for 46 feline isozyme systems were applied, and the results were evaluated by using the criteria for genetic variations listed by Newman et al [1985].

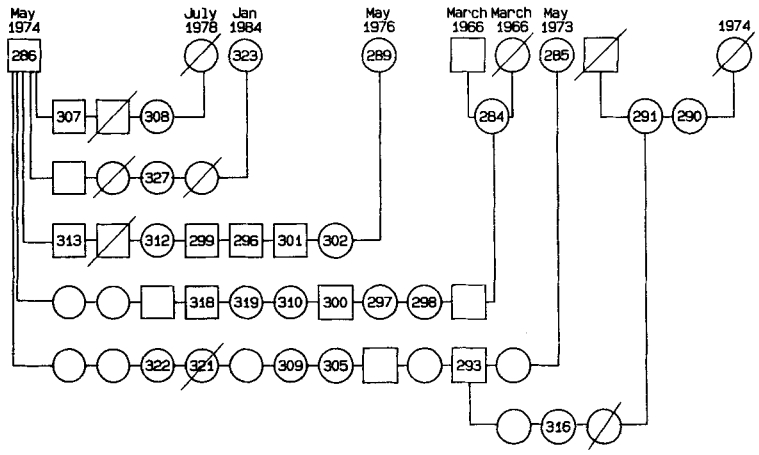

Fig. 1. Pedigree of Asiatic lions bred and held at the Sakkarbaug Zoo, Junagadh, India. Founder animals were captured from the Gir Forest population on the indicated date. Numbered animals are International Studbook numbers of lions sampled in this study.

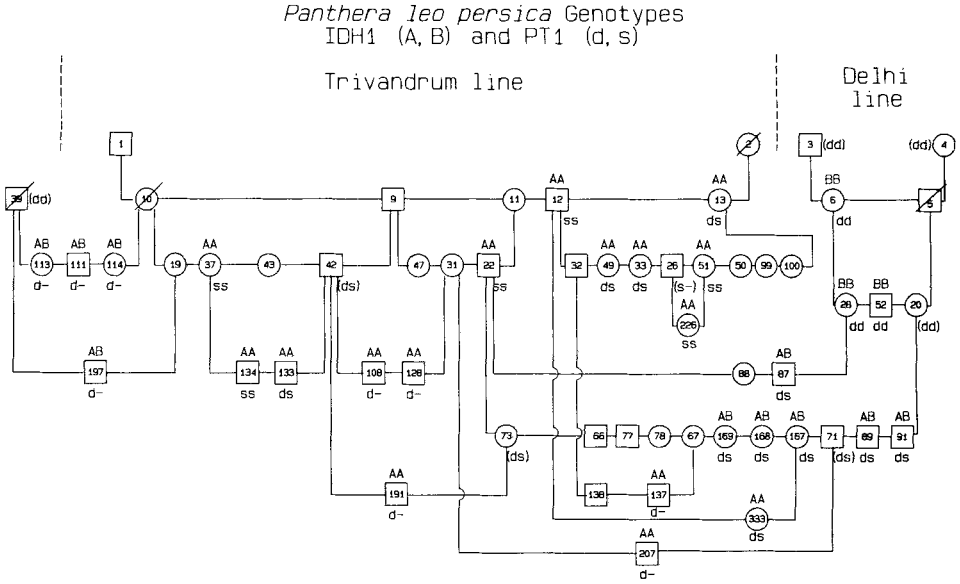

Fig. 2. Pedigree of Asiatic lion SSP animals studied in this report. Numbers indicate International Studbook numbers [Smith, 1985]. Upper case letters AA, AB, and BB indicate *IDH1* phenotypes (see Fig. 3), which are equivalent to their genotype. Lower case letters *dd*, *ds*, *ss* are genotypes of *PT1* (see Fig. 5). $PT1^d$ is dominant to $PT1^s$, so heterozygous *ds* or homozygous *dd* phenotypes and animals not typed, in parentheses, were deduced from the pedigree relationships. Genotypes in parenthesis were deduced from offspring or parents and were not directly typed. We cannot exclude the possibility that Delhi line animals SB3, 4, 6, 28, and 52 were heterozygous for the $PT1^s$ allele although the probability of this is quite low. The five founders imported from India were SB39, SB1, SB2, SB3, and SB4. Another pair, SB14 and SB15, and their offspring, SB106, SB126, and SB127, remain in India.

Transferrin (*TF*) and *serum protein-1* (*PT1*) were purified from lion plasma by using the procedures of Chen and Sutton [1967] and Sutton and Karp [1965]. Briefly, for 10 μl plasma, 10 μl of 0.2% ferric ammonium sulfate was added; then 40 μl of 0.6% rivanol (2-ethoxyl-6,9 diaminoacridine lactate) was added. The mixture was shaken by hand and then centrifuged with the aid of an Eppendorf microfuge for 60 sec. The yellow supernatant (15 μl) was loaded on an acrylamide gel, and following electrophoresis, the gel was stained with 1% Coomassie brilliant blue (G-250).

Semen Analysis

Semen was collected by rectal probe electroejaculation from five anesthetized Asian lions. A Lane Pulsator III Z Electroejaculator was used to deliver a 20-Hz electrical stimulus to the rectal probe in 3-sec pulses. The probe had three 130 × 3-mm electrodes spaced at 10-mm intervals. Spermatozoa concentrations were determined with a hematocytometer, and fractions were put in 2% glutaraldehyde for later examination under interference (×1,000) and phase-contrast (×1,000) microscopy. Primary abnormalities were identified as a tightly coiled tail and a pleomorphic sperm head, midpiece, or acrosome. Secondary abnormalities included a retained protoplasmic droplet, tailless normal head, and a bent midpiece, neck, or tail.

RESULTS

Genetic Monomorphism of Gir Lions and Polymorphism of SSP Lions

Heparinized blood samples were collected from 28 lions at the Sakkarbaug Zoo in March 1985. These lions were either captured from the Gir Forest or were their captive-born offspring. A pedigree of the Sakkarbaug lions is illustrated in Figure 1. Blood was fractionated by centrifugation into plasma, erythrocytes, and leukocytes and stored in liquid nitrogen. Isozyme extracts were prepared at the NIH and were subjected to gel electrophoresis followed by histochemical isozyme development with the use of standard techniques for 46 allelic isozyme loci. Blood from 29 captive-born lions from 13 western zoos that are included in the SSP was similarly processed and typed. The collaborating zoos and the International Studbook number of sampled lions is presented in Table 1.

The Gir (Sakkarbaug) lions were genetically monomorphic at each of 46 allozyme loci. This is in contrast to several African lion populations in which between 7% and 11% of their allozyme loci have more than one electrophoretic form (Table 2) [O'Brien et al, 1987b]. When we examined the Asiatic lions in the American SSP pedigree, we found three loci that were polymorphic: *isocitrate dehydrogenase-1* (*IDH1*), *transferrin* (*TF*), and a serum protein of unknown function designated *protein-1* (*PT1*). The electrophoretic pattern of each of these polymorphic systems is presented in Figures 3–5.

Two of the loci that were polymorphic in SSP lions, *IDH1* and *TF*, were also polymorphic, and for the same alleles, in African lion populations. The African populations we studied were 1) American zoo lions from central and North Africa, 2) lions from the Serengeti Plains in Tanzania, and 3) lions from Kruger Park in South Africa [O'Brien et al, 1987b]. The *PT1* locus was monomorphic for the *d* allele in the Gir lions and for the *s* allele in the African lions (see below). The allelic frequency of each of the three loci in African lions, in Gir lions, and in the SSP lions is listed in Table 2.

TABLE 2. Distribution and allele frequency of polymorphic allozyme loci in lions

Population	No. lions scored	Allozyme locus[a] *IDH1*	*TF*	*PT1*	Percent polymorphic loci	Average Heterozygosity[b]
African						
Kruger Park South Africa	15	A = 1.0	a = 0.65 b = 0.35	s = 1.0	7	0.023
Serengeti ecosystem Tanzania, East Africa	27	A = 0.72 B = 0.28	a = 0.52 b = 0.28	s = 1.0	11	0.038
African Zoo lions (Atlas)	18	A = 1.0	a = 0.72 b = 0.28	s = 1.0	7	0.030
Asian						
Indian lions (Gir Forest)	28	B = 1.0	a = 1.0	d = 1.0	0.0	0.0
Indian lions (SSP-studbook)	29	A = 0.72 B = 0.28	a = 0.93 b = 0.07	s = 0.45 d = 0.55	7	0.021

[a]Allozyme designations: *IDH1*, isocitrate dehydrogenase-1; *TF*, transferrin; *PT1*, serum protein-1.
[b]Estimates of the percent polymorphic loci and average heterozygosity are based on an electrophoretic survey of 46–50 allozyme loci described elsewhere [Newman et al, 1985; O'Brien et al, 1987b].

The presence of three polymorphic isozyme alleles in the SSP lions, which were absent in Gir lions but present in African populations, was our first indication that the SSP lions may have included some African genetic material in their background. This finding prompted a pedigree analysis of each of three allozyme loci within the SSP population plus a retrospective analysis of morphological traits.

Isocitrate Dehydrogenase-1 (IDH1)

IDH1 displays two electrophoretic forms, $IDH1^{A}$ and $IDH1^{B}$ [Fig. 3] and is codominant in its transmission; thus, heterozygotes are resolved from the two homozygous phenotypes. The Gir lions (Fig. 1) were genetically monomorphic for $IDH1^{BB}$. A pedigree of the SSP Asiatic lions with individual *IDH1* phenotypes is presented in Figure 2. It seems clear that the pure direct descendants of Delhi line founders (studbook numbers 3 and 4: SB3 and SB4) are homozygous $IDH1^{BB}$, while the founders of the Trivandrum line, SB1 and SB2, were homozygous $IDH1^{AA}$. Offspring of crosses between the two lines were invariably heterozygous $IDH1^{AB}$. Four offspring of founder SB39 × Trivandrum line animals were $IDH1^{AB}$, suggesting that SB39 was $IDH1^{BB}$ as was the Delhi lion pair.

Since the 28 Gir Forest lions were genetically monomorphic for $IDH1^{B}$, these results suggest that the founders of the Trivandrum line were pure African or possibly African-Indian hybrids, while the Delhi founder and SB39 were pure Asiatic lions. A second explanation for these results is that our sampling of Sakkarbaug lions simply missed a polymorphism for *IDH1* by chance in our sampling of the Gir Forest population. Although it is not possible to exclude this hypothesis, the probability of such an event is very unlikely. The Sakkarbaug lion population consisted of nine founder animals, each captured from different regions at different times over a span of 18 years (Fig. 1). The probability of not recovering a polymorphic allele present at a given frequency in a population from a sample of 1, 5, or 9 individuals is estimated in Figure 6. By way of example, if the frequency of the $IDH1^{A}$ allele in the

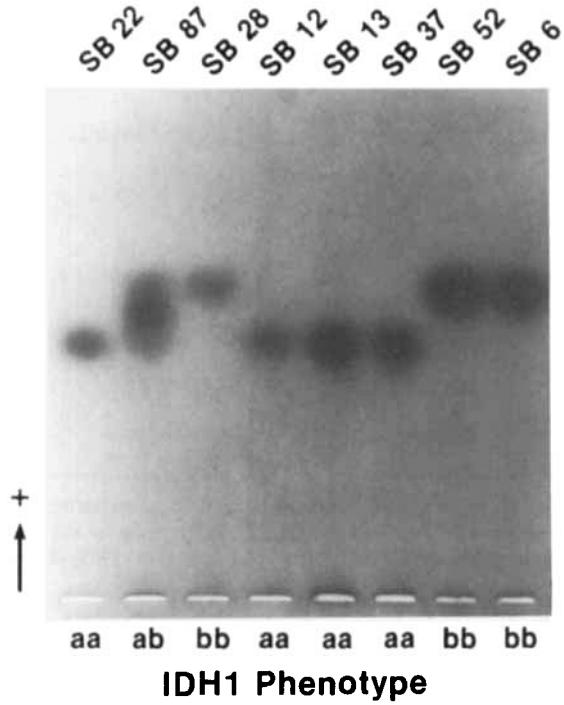

Fig. 3. Phenotypes of soluble *isocitrate dehydrogenase-1* in lions. All lions from the Sakkarbaug Zoo were homozygous *BB*.

Gir population was 0.72 (which it is in the Serengeti lions and also in the SSP animals, see Table 2), the chance of selecting nine individuals from the population all homozygous for $IDH1^B$ equals $(0.28)^{18} = 1.1 \times 10^{-10}$.

Transferrin (TF)

The second polymorphic locus in the SSP lions was *TF*. The TF^B allele is found in each of the African populations but not in the Gir lions, which were all TF^A (Table 2). The pedigree analysis presented in Figure 4 shows that the TF^B allele only appears in one family of the Trivandrum lineage. All of the other lions typed (see Fig. 2) were TF^A. Thus, direct offspring of the three founders implicated to be Asiatic by the *IDH1* typing (SB3, SB4, and SB39; Fig. 2) were also homozygous for the Asiatic TF^A genotype. The Trivandrum line descendants carried the TF^B allele heretofore only seen in African animals.

Serum Protein-1 (PT1)

The *PT1* locus was discovered as a protein that, like transferrin, remains soluble after the addition of rivanol to plasma. Among lions, two phenotypes were observed: a single (*s*) slow band and a double (*d*) band pattern, which includes the single band plus a faster band (Fig. 5). The 28 Gir lions all expressed the $PT1^d$ phenotype, while 77 African lions from four populations (Table 2; Fig. 5) were all $PT1^s$. The *PT1* phenotypes of the SSP lions are also included in Figure 2. Examination of the *PT1* transmission in the SSP pedigree indicates that $PT1^d$ is controlled by a dominant autosomal gene, while $PT1^s$ is recessive. In support of the above-stated observations

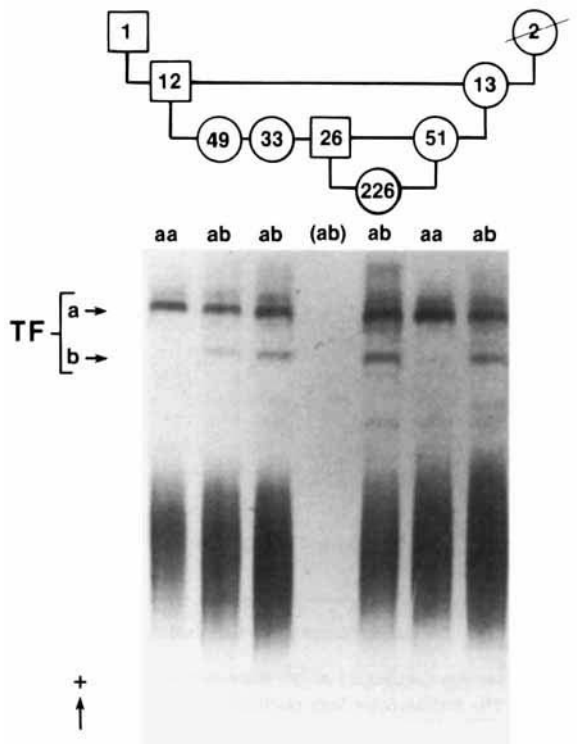

Fig. 4. *Transferrin* phenotypes of SSP lions descended from founders SB1 and SB2. All of the other lions in the pedigree were homozygous for the *A* allele (see also Fig. 2).

with *IDH1* and *TF*, the Delhi founders and SB39 are genetically identical with the Gir lions ($PT1^{dd}$), while the Trivandrum animals expressed the $PT1^{s}$ allele, which confirms the presence of an African heritage.

PT1 is the first biochemical locus that is homozygous for one allele ($PT1^{d}$) in Asiatic lions and for another ($PT1^{s}$) in African lions. Because the *d* allele is dominant,

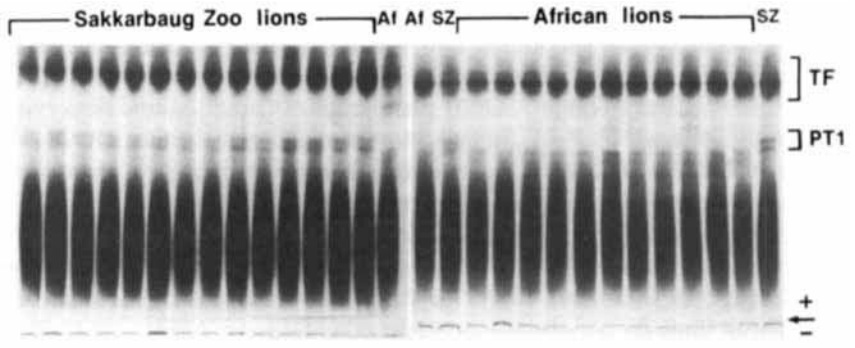

Fig. 5. *Protein-1*, *PT1*, phenotypes in African and Asian lions. Pedigree analysis demonstrated that $PT1^{d}$ is dominant to $PT1^{s}$. Thus, it is not possible to discriminate *dd* from *ds* individuals except by pedigree analysis (see Fig. 2).

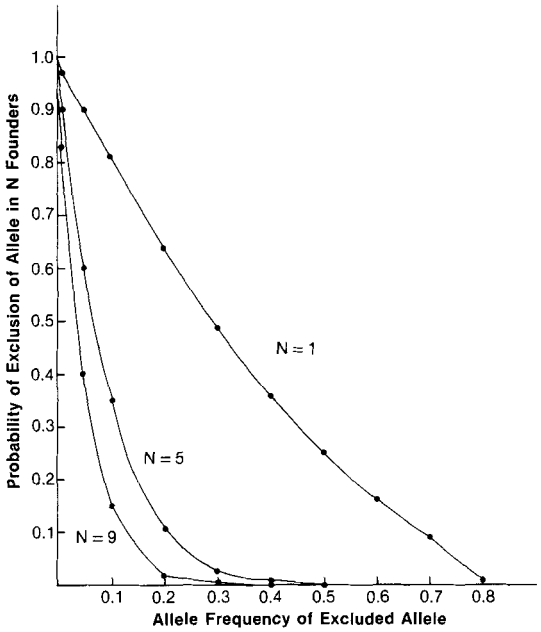

Fig. 6. Estimate of the probability (ordinate) of not retaining an allele of indicated frequency (abcissa) in a sample of N founders. The Sakkarbaug lion population was derived from nine founders (see Fig. 1).

it is not possible to discern *dd* from *ds* individuals without access to a pedigree analysis. Thus, the SSP pedigree presented in Figure 2 does permit resolution of the mode of inheritance of this locus as well as provide more compelling evidence for the presence of African-specific alleles in the Trivandrum blood line.

Pedigree analysis of the Trivandrum lineage revealed the presence of the PTI^{d} allele, which is clearly restricted to Asiatic lions in our species survey (Fig. 5; Table 2). We interpret this result as strong evidence that one of the Trivandrum-line founder animals (SB1 or SB2) was an African-Asiatic hybrid. The other partner was likely pure African, although it also could have been a hybrid animal.

Abdominal Fold

The incidence of an abdominal fold was reexamined in representative populations of lions from Asia, Africa, and within the SSP captive population (Fig. 7). The results are summarized in Table 3. Of 51 photographs of adult Asiatic lions (published and personally collected by P.J.), 50 had prominent abdominal folds. During a 3-year field study in the Gir Forest, no exceptions to the occurrence of a prominent belly fold in over 40 adult male lions were observed. Each of the 28 adult Asian lions held at the Sakkarbaug Zoo (Fig. 1) in 1985 had a prominent abdominal fold. An examination of published photographs of 160 African lions revealed only six with prominent abdominal folds. In summary, 98.0% of Asiatic lions have the abdominal fold and 96% of African lions do not possess a recognizable fold.

Twenty-seven of the SSP lions were reexamined with respect to the presence or absence of the abdominal fold (Table 3). Of the four animals that were direct, living

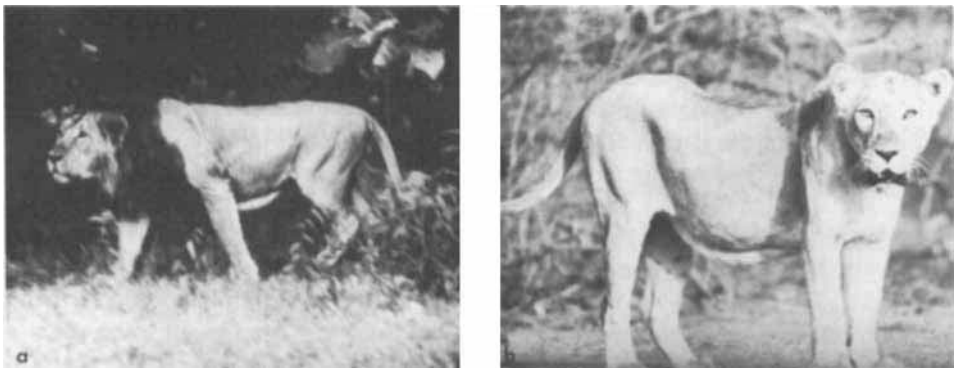

Fig. 7. Abdominal fold in adult male Asiatic lion (**a**) and in adult female Asiatic lion (**b**).

drum-line animals examined, 11 did not have an abdominal fold and one did. Hybrid animals between Trivandrum × Delhi or Trivandrum × SB39 were a mixture that did or did not possess the fold or possessed it to a lesser degree. The incidence of transmission did not fit the inheritance of a single gene model and suggests that the development of an abdominal fold is a quantitative trait controlled by several genes. Further, the nonrandom occurrence of the abdominal fold in all Delhi animals and its near absence in pure Trivandrum animals support the conclusions of the molecular data—namely, that the Trivandrum line is largely, but not entirely, African in its ancestry, while the Delhi line is bona fide *P l persica*.

Infraorbital Foramen

One feature that clearly distinguishes the Gir lions from all other felids, including African lions and Asian lions found in parts of Asia outside the Gujarat State, is

TABLE 3. Incidence of abdominal fold

	Abdominal fold		
Origins	Present	Absent	Slight or questionable
Asiatic lions—published photos examined	22[a]	0[a]	0[a]
Asiatic lions—Gir Forest Field Study and Sakkarbaug Zoo unpublished photos (Joslin)	28[a]	1[a]	0[a]
African lions—published photos examined	6[a]	147[a]	7[a]
Studbook No. for Asiatic lions[b]			
Pure Delhi line	52,6,20,28	None	None
Pure Trivandrum line	13	31,37,43,47, 49,50,99,100 108,128,134	None
"Hybrid" (Trivandrum × [SB39])	None	113,197	114
"Hybrid" (Trivandrum × Delhi)	66	77,87	67,78,88 138,191

[a]No. of lions.
[b]See Figure 2.

the dividing of the infraorbital foramen into two distinct orifices (Fig. 8). This character was first reported by Pocock [1939]. Pairing of the infraorbital foramen can occur on either the right side or left side or both sides of the skull. We observed pairing in 16 of 36 Asiatic lion skulls (44%) in collections of the Bombay Natural History Museum, the British Natural History Museum, the Field Museum of Natural History, and the Sakkarbaug Zoo. Pairing has never been observed in African lion skulls.

A limited number of Asiatic lion skulls from the Field Museum of Natural History in Chicago was from the offspring of SSP animals. Eleven skulls were examined, six of which were from the offspring of SB5 × SB6 in the Delhi line (Fig. 2); the other five were presumed to be derived from this lineage since the dates of their death preceded the arrival of Trivandrum lions in Chicago. Three of the 11 skulls exhibited pairing of the infraorbital foramen, consistent with the Asiatic origin of the Delhi-derived lions. Skulls from other portions of the SSP pedigree were unavailable for analysis.

Mane Size

Several early authors noted that the Asiatic lion had a sparse mane or in some cases was maneless. This observation has been widely quoted, and has given rise to a misunderstanding among those who have not seen adult Gir lions. The fact that the type specimen in the British Museum, captured in the 1830s from the Gujarat State in Western India, was a young male with a scanty mane helped to perpetuate this misconception. More recently, Guggisberg [1963], Mazak [1968], and Pocock [1939] have pointed out that the Gir lions have a well-developed mane. Nonetheless, there are some differences between the Asiatic lion manes and certain African lion manes. Asiatic lions have only moderate mane development between the ears, while African lions sometimes, but not always, have sufficient mane development in this region to obscure the ears entirely. No Asiatic lions have been observed to have a mane of this type.

A study of 148 African lion photographs taken in the wild and documented in the literature revealed 118 (80%) lions with manes that resembled those of Asiatic lions, while 30 (20%) had manes that were sufficiently full around the ears so as to almost completely hide the ears. The continuous variation of mane development with

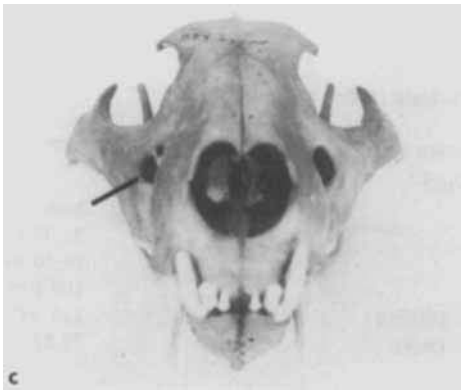

Fig. 8. Variations in pairing of the infraorbital foramen in the Asiatic lion.

age, its restriction to males only, and its broad variation in African animals make mane development a relatively poor character for subspecies identification.

Seminal Traits of the Asiatic Lion

The extensive genetic monomorphism observed in the Gir lion sample was reminiscent of the genetic impoverishment we had observed previously in the African cheetah [O'Brien et al, 1983, 1985, 1986]. That species is also characterized by an extreme degree of morphologically abnormal spermatozoa in ejaculates collected from both captive and free-ranging animals [Wildt et al, 1983, in preparation]. We have hypothesized that the spermatozoal abnormalities of the cheetah are a consequence of recessive genetic factors that became homozygous and therefore were expressed in the recent history of the species. Since the Gir lions have comparable low levels of allozyme variation, it becomes important to examine certain reproductive parameters in Gir lions.

Sperm samples were collected from each of five Asiatic lions at the Sakkarbaug Zoo and were examined for the concentration of motile spermatozoa. Samples were fixed in 2% glutaraldehyde and were later examined under phase-contrast microscopy for the incidence of spermatozoal abnormalities. The results obtained from the four animals that produced sperm (one animal, SB293, was virtually azospermic) are presented in Table 4. One striking result is a rather large incidence of morphological defects. An average of 79% of the spermatozoa in each ejaculate was morphologically abnormal. This value is rather high compared to other species, such as the bull or the dog, in which 20–30% of abnormal sperm is nearly always associated with infertility. [Larson, 1980; Chenoweth and Ball, 1980; Chandley et al, 1975; Johansson and Rendel, 1968]. In fact, this value is more than the cheetah (average = 71%), or four African lion populations similarly studied (range = 25–61% abnormal sperm) [Wildt et al, in preparation].

DISCUSSION

The population of Asiatic lions that survives in the Gir Forest Sanctuary is genetically monomorphic at each of 46 allozyme loci. This is in contrast to four African lion populations and other felid species with the single exception of the cheetah, *Acinonyx jubatus* [O'Brien et al., 1985, 1986, 1987a,b]. Like the cheetah, the Gir lions display a high incidence of morphologically abnormal spermatozoa in contrast to other captive and free-ranging African lions [Wildt et al, in preparation]. The Gir lion population may thus represent another example of a severely endangered species that has suffered a population bottleneck or a series of bottlenecks followed by inbreeding in their recent history. The admittedly small sample size (N = 4) and the fact that the semen samples from Sakkarbaug lions were collected only once from sexually inactive males make definitive interpretation of the spermatozoal data difficult. The well known deleterious effects of seasonality [Pickett, 1980], age, sexual activity, serial electroejaculation sampling, and captivity could not be adequately controlled [Pickett, 1980]. Nonetheless, it is tempting to speculate that the spermatozoal abnormalities seen in the Gir lions may be a consequence of forced homozygosity of genes affecting sperm development, as has been suggested in similar abnormalities found in deliberately inbred mice and livestock [Wyrobek, 1979; Salisbury and Baker, 1966].

TABLE 4. Seminal traits of Asiatic lions

Studbook No.	Total sperm/ ejaculate ($\times 10^{-6}$)	Morphologically abnormal spermatozoa (%)							
		Protoplasmic droplet	Tailless head	Acrosomal defect	Midpiece defect	Bent tail	Coiled tail	Tail around head	Total percent abnormal sperm
307	36	21	14	30	2	5	9	1	82
300	58.5	11	17	25	3	13	2	1	72
296	528	33	5	23	4	11	4	0	80
299	88	28	7	28	6	6	6	0	81

A major conclusion of this study is that the North American populations of captive Asiatic lions are composed of descendants of five founder lions, three of which were pure Asian and two of which were African or African-Asian hybrids. This conclusion is predicated on the assumption that the Sakkarbaug Zoo sample of 28 animals (Fig. 1) available to us is representative of the remaining Gir Forest population in India. Although the Sakkarbaug Zoo sample was itself established by a small founder event (nine founders), we feel that the data are compelling for several reasons. First, we have shown that even in a sample of nine animals that the probability of *not* detecting a moderately polymorphic allele (as in our *IDH1* example) is vanishingly small ($P < .0001$). Second, the unexpected alleles seen in the SSP pedigree, but not in the Sakkarbaug Zoo animals, were in every case the same alleles seen previously in African populations [O'Brien et al, 1987b]. Third, genetic analysis of the SSP pedigree of 29 typed animals produced no exceptions to implicating two (and only two) individual founders (SB1 and SB2) as African; and both came from the same zoo, Trivandrum. Conversely, the other three animals looked precisely like the Gir Forest/Sakkarbaug Zoo sample with no pedigree contradictions. Fourth, in addition to *IDH1*, two additional biochemical loci, *TF* and *PT1*, raised the same suspicion, also revealed alleles previously seen in African animals, and independently implicated the same two founder lions as being of African lineage. Fifth, one of the biochemical loci, *PT1*, was fixed for different alleles in *P l leo* and in *P l persica*. The only polymorphism and/or heterozygotes ever observed were in the SSP lion pedigree. Further, electrophoretic typing of *PT1* in the SSP pedigree animals confirmed the subspecies hybridization in the precise pattern indicated by the other two markers.

Finally, a retrospective analysis showed that two morphological characters (abdominal skin fold and pairing of infraorbital foramen) exhibit high genetic penetrance in bona fide subspecies specimens. These characters were in contrast to another widely quoted but less reliable character, mane size. Prominent abdominal folds and the occurrence of paired infraorbital foramen (both specific for Asiatic lions) were present in the SSP lions from Asiatic background (the Delhi line) but were present very infrequently in the pure Trivandrum or hybrid animals. Thus, three molecular and two morphological characteristics that discriminate African from Asian subspecies independently converged on the conclusion that the SSP lions are comprised of both pure Asiatic and Asiatic-African hybrid animals.

The Asiatic lion breeding program is one of the most thoroughly researched and organized management plans of the SSP program. This is due to extensive efforts to reconstruct precisely the relationships and origins of animals in the International Studbook [Smith, 1985]. A testimony to that precision is the absence of a single unexplained parentage in the entire sample of three biochemical markers here presented. By way of comparison, in human pedigrees disputed parentage (by gene marker analysis) occurs approximately 10% of the time.

The SSP lions breed rather well compared to other species, even though they are derived from but a few founders, and, until recently when the Trivandrum and Delhi lines were mixed, consisted of animals with high inbreeding coefficients. If all of the animals had been derived from the genetically depauperate Gir lion animals, optimal reproduction might not have been observed owing to inbreeding depression [Ralls et al, 1979; Templeton and Read, 1984; Marker and O'Brien, in preparation;

Templeton et al, 1986; O'Brien et al, 1986a; Soule et al, 1986]. It seems possible that a portion of the breeding success of the Asiatic lion SSP can be attributed to the inadvertent subspecies hybridization that we have described.

Perhaps, then, there is a serendipitous lesson here for the management of a small captive population of endangered species. We have shown elsewhere that the molecular genetic distance ["D", Nei, 1972] between African and Asiatic lions is low, approximately 0.013 [O'Brien et al, 1987b]. This value is comparable to the distance between conspecific mouse populations or human racial groups [Nei and Roychoudhury, 1982; O'Brien et al, 1982]. When translated to evolutionary time, the genetic distance says that the African and Asiatic lions shared a common ancestor as recently as 100,000 years ago [Cavalli-Sforza and Bodmer, 1971]. This is not enough time for significant reproductive barriers to have evolved, and as such, there is no genetic evidence for separating subspecies, at least in captivity; in fact, there might actually be a reproductive advantage to interbreeding them [see Templeton et al, 1986].

CONCLUSIONS

Our results show that the present population of lions maintained as the Asiatic lion SSP consists of a mixture of pure African, pure Asian, and African-Asian hybrid lions. What then is the ideal strategy for propagation of lions in captivity? Our results show clear genetic differences (both molecular and morphological) which would support the subspecies designation of *P l leo* and *P l persica*. Nonetheless, the two subspecies have shared common ancestors recently enough to exclude confidently the development of reproductive isolation mechanisms. The existing option of a "generic lion" population with homogenized subspecies may be sufficient for species propagation and would save some space, but is it necessary? Perhaps not. Since lions are among the most popular zoo animals throughout the world, the space does not seem overly limiting. Given adequate space, the separate establishment and future propagation of both African lion and Asiatic lion populations with sufficient monitoring as has occurred for the SSP lions here discussed would provide a unique opportunity to follow the consequences of the genetic losses of the Asiatic lions compared to African lions. Furthermore, a variety of subspecific genetic adaptations that may have evolved in the two subspecies would be preserved en bloc in separate populations. Except for the admittedly limited spermatozoal evidence presented here, there is no specific evidence for reproductive or demographic impairment of Asiatic lions from the Sakkarbaug Zoo. Appropriation of coordinate effort to establish separate subspecific populations in captivity, each to be managed from reproductive, genetic, and survivorship perspectives, would appear to optimize the goals of the Species Survival Plan for this species.

ACKNOWLEDGMENTS

We are grateful to the National Geographic Society, the US Fish and Wildlife Service Office of International Affairs, the Friends of the National Zoo, and to the staff of participating zoological institutions listed in Table 1 for support of collecting expeditions described in this report. Special thanks go to the Sakkarbaug Zoo, the Gujarat State government, and the Indian Central government for their cooperation in making the collection of Asiatic lion material possible. Tissues were collected from

lions in full compliance with specific Federal Fish and Wildlife Permits (CITES; Endangered and Threatened Species; Captive Bred) issued to the National Cancer Institute, National Institutes of Health, principal officer Stephen J. O'Brien, by the US Fish and Wildlife Services of the Department of the Interior.

REFERENCES

Caldwell, K. The Gir lions. JOURNAL OF THE SOCIETY FOR THE PRESERVATION OF THE FAUNA OF THE EMPIRE 34:62-65, 1938.

Cavalli-Sforza, L.; Bodmer, W. THE GENETICS OF HUMAN POPULATIONS. San Francisco, W.H. Freeman and Company, 1971.

Chandley, A.C.; Edmond, P.; Christie, S.; Gowans, L.; Fletcher, J.; Frackiewicz, A.; Newton, M. Cytogenetics and infertility in man. I. Karyotype and seminal analysis: Results of a five-year survey of men attending a subfertility clinic. ANNALS OF HUMAN GENETICS 39:231-254, 1975.

Chen, S.-H.; Sutton, H.E. Bovine transferrins: Sialic acid and the complex phenotype. GENETICS 56:425-430, 1967.

Chenoweth, P.J.; Ball, L. Breeding soundness evaluation in bulls, pp 330-339 in CURRENT THERAPY IN THERIOGENOLOGY. D.A. Morrow, ed. Philadelphia, W.B. Saunders, 1980.

Dharmarkumarsinhji. The Gir lions. SANCTUARY 2:326-341, 1982.

Ford, W.L. The preparation and labelling of lymphocytes, pp. 23.1-23.22 in HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, Third Ed. D.M. Weir, ed. Oxford, England, Blackwell Scientific Publications, 1978.

Guggisberg, C.A.W. SIMBA. Philadelphia, Chilton Books, 1963.

Guggisberg, C.A.W. WILD CATS OF THE WORLD. New York, Taplinger Publishing Co., 1975.

Hemmer, H. Untersuchungen zur Stammesgeschichte der Pantherkatzen (Pantherinae). Teil III. Zur Artgeschichte des Lowen *Panthera* (*Panthera*) *leo* (Linnaeus 171-8). VEROFFENTLICHUNGEN DER ZOOLOGISCHEN STRATSSAMMLUNG MUNCHEN 17:167-280, 1974.

Johansson, I.; Rendel, J. GENETICS AND ANIMAL BREEDING. Edinburgh, Oliver and Boyd, 1968.

Joslin, P. The environmental limitations and future of the Asiatic lions. JOURNAL OF THE BOMBAY NATURAL HISTORY SOCIETY 81:648-664, 1984.

Kingdon, J. EAST AFRICAN MAMMALS, AN ATLAS OF EVOLUTION IN AFRICA, Vol. 3. New York, Academic Press, 1977.

Larson, R.E. pp. 646-654 in CURRENT THERAPY IN THERIOGENOLOGY. D.A. Morrow, ed. Philadelphia, W.B. Saunders, 1980.

Marker, L. NORTH AMERICAN REGIONAL CHEETAH STUDBOOK. Winston, OR, Wildlife Safari Publications, 1985.

Marker, L.; O'Brien, S.J. Captive breeding of the Cheetah, *Acinonyx jubatus*, in North American Zoos (1871-1986). IN PREPARATION.

Mazak, V. DER LOWE. DAS PELZGEWERBE 3:3-28, 1968.

Neff, N. THE BIG CATS: THE PAINTINGS OF GUY COHELEACH. New York, Harry N. Abrams Publishers, 1983.

Nei, M. Genetic distance between populations. AMERICAN NATURALIST 106:283-292, 1972.

Nei, M.; Roychoudhury, A. Genetic relationships evolution of human races, pp 1-59. in EVOLUTIONARY BIOLOGY. M.K. Hecht; B. Wallace; G.T. Prance, eds. New York, Plenum Press, 1982.

Newman, A.; Bush M.; Wildt, D.E.; van Dam, D.; Frankehuis, M.; Simmons, L.; Phillips, L.; O'Brien, S.J. Biochemical genetic variation in eight endangered feline species. JOURNAL OF MAMMALOGY 66:256-267, 1985.

O'Brien, S.J. The extent and character of biochemical genetic variation in the domestic cat (*Felis catus*). JOURNAL OF HEREDITY 71:2-8, 1980.

O'Brien, S.J.; Gail, M.H.; Levin, D.L. Correlative genetic variation in natural populations of cats, mice and men. NATURE 288:580-583, 1980.

O'Brien, S.J.; Moore, J.L.; Martin, M.A.; Womack, J.E. Evidence for the horizontal acquisition of murine AKR virogenes by recent horizontal infection of the germ line. JOURNAL OF EXPERIMENTAL MEDICINE 155:1120-1132, 1982.

O'Brien, S.J.; Goldman, D.; Merril, C.R.; Bush, M.; Wildt, D.E. The cheetah is depauperate in biochemical genetic variation. SCIENCE 221: 459-462, 1983.

O'Brien, S.J.; Roelke, M.E.; Marker, L.; Newman, A.; Winkler, C.A.; Meltzer, D.; Colly, L.; Evermann, J.F.; Bush, M.; Wildt, D.E. Genetic basis for species vulnerability in the cheetah. SCIENCE 227:1428-1434, 1985.

O'Brien, S.J.; Wildt, D.E.; Bush, M. The African cheetah in genetic peril. SCIENTIFIC AMERICAN 254:84-92, 1986.

O'Brien, S.J.; Collier, G.E.; Benveniste, R.E.; Nash, W.G.; Newman, A.K.; Simonson, J.M.; Eichelberger, M.A.; Seal, U.S.; Bush, M.; Wildt, D.E. Setting the molecular clock in Felidae: The great cats, Panthera, in press, in TIGERS OF THE WORLD. R.L. Tilson, ed. Apple Valley, MN, Noyes Publications, 1987a.

O'Brien, S.J.; Martenson, J.S.; Packer, C.;

Herbst, L.; De Vos, V.; Joslin, P.; Ott-Joslin, J.; Wildt, D.E.; Bush, M. Biochemical genetic variation in geographic isolates of African and Asiatic lions. NATIONAL GEOGRAPHIC RESEARCH 3:114–124, 1987b.

Pickett, B.W. Factors affecting stallion management, pp. in CURRENT THERAPY IN THERIOGENOLOGY. D.A. Morrow, ed., Philadelphia, W.B. Saunders Co., 1980.

Pocock, R.I. The lions of Asia. JOURNAL OF THE BOMBAY NATURAL HISTORY SOCIETY 38:638–665, 1930.

Pocock, R.I. The fauna of British India, pp. in MAMMALIA, Vol. I. London, Taylor and Francis, Ltd., 1939.

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

Salisbury, G.W.; Baker, F.N. Nuclear morphology of spermatozoa from inbred and linecross Hereford bulls. JOURNAL OF ANIMAL SCIENCE 25:476–479, 1966.

Smith, G.L., III. INTERNATIONAL STUDBOOK OF THE INDIAN LION, *PANTHERA LEO PERSICA* (MEYER, 1826). Knoxville, TN, Knoxville Zoological Park, 1985.

Smithers, R.H.N. Carnivora, family Felidae, pp, in THE MAMMALS OF SOUTH AFRICA—AN IDENTIFICATION MANUAL. J. Meester; H.W. Setzer, eds. Washington, DC, Smithsonian Institution Press (Part 8.1), 1971.

Soule, M.; Gilpin, M.; Conway, W.; Foose, T. The Millenium Ark: How long a voyage, how many staterooms, how many passengers: ZOO BIOLOGY 5:101–113, 1986.

Sutton, H.E.; Karp, G.W., Jr. Adsorption of rivanol by potato starch in the isolation of transferrins. BIOCHIMICA ET BIOPHYSICA ACTA 107:153–154, 1965.

Talbot, L.M. A LOOK AT THREATENED SPECIES. London, Fauna Preservation Society, 1959.

Templeton, A.R.; Read, B. Factors eliminating inbreeding depression in a captive herd of Speke's gazelle. ZOO BIOLOGY 3:177–199, 1984.

Templeton, A.R.; Hemmer, H.; Mace, G.; Seal, U.S.; Shields, W.M.; Woodruff, D.S. Local adaptation, coadaptation, and population boundaries. ZOO BIOLOGY 5:115–125, 1986.

Wildt, D.E.; Bush, M.; Howard, J.G.; O'Brien, S.J.; Meltzer, D.; van Dyk, A.; Ebedes, H.; Brand, D.J. Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat. BIOLOGY OF REPRODUCTION 29:1019–1025, 1983.

Wildt, D.E.; Bush, M.; Packard, C.; Pusey, A.E.; Brown, J.L., O'Brien, S.J. The reproductive and genetic consequences of founding an isolated population of east African lions. IN PREPARATION, 1987.

Wyrobek, A.J. Changes in mammalian sperm morphology after X-ray and chemical exposures. GENETICS 92:105–119, 1979.

Zuber, C. ANIMALS IN DANGER. New York, Barrons Woodbury, 1978.