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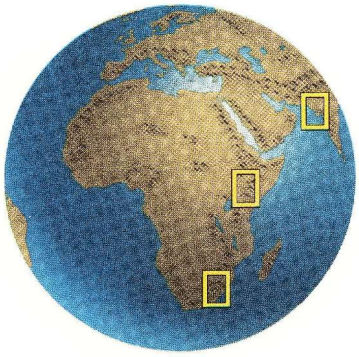
Biochemical Genetic Variation in Geographic Isolates of African and Asiatic Lions



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Electrophoretic variation of 46 to 50 allozyme loci were typed in four African and one Asiatic (Indian) lion populations. The African populations revealed moderate amounts of genetic variation compared with other cat species. The lions from the Ngorongoro Crater, a small isolated "island" population within the Serengeti ecosystem in Tanzania, had a reduced level of variability which was a precise subset of the larger founder population of the Serengeti plains. The Asiatic lion population from the Gir Forest Sanctuary in the state of Gujarat in western India is a relict population of less than 250 individuals which descended from a much more widely distributed subspecies early in the 19th century. The Gir lions were genetically monomorphic at each of 46 typed loci, suggesting a drastic population bottleneck followed by inbreeding in their recent history. The allozyme genetic distance estimates between African lion populations and between Asian and African subspecies were low and comparable with the distance values between conspecific mouse populations or between human racial groups. These results suggest that the two subspecies shared a common ancestor recently, estimated at between 50 000 and 200 000 years before the present.

As a formidable predator the primacy of the lion *(Panthera leo)* is evident in its natural ecosystems and in the early drawings on cave walls made over 15 000 years ago; it has been recognized throughout human civilization as a symbol of power and strength. The lion is the only cat that has a cooperative social organization based upon the metastable pride unit. Lion prides consist of one to 18 adult females plus a coalition of one to seven adult males that live in definable territories (Bertram 1975, Packer 1986, Schaller 1972). The social organization of lions has been the object of extensive behavioral and demographic studies in the Gir Forest Sanctuary in India (Dharmarkumarsinhji 1982; Joslin 1971, 1984), the Kalahari Desert in South Africa (Owens & Owens 1984), and, most notably, in the East African Serengeti ecosystem which has been under continuous demographic and behavioral observation for 20 years (Bertram 1975, 1979; Hanby & Bygott 1979; Packer 1986; Pusey & Packer in press; Schaller 1972).

The earliest lionlike species occurred approximately 1.8 million years ago, as evidenced by Villafranchian deposits of lions in Europe (Figure 1) during the early Pleistocene (Savage & Russell 1983). The oldest true

lions *(P. l. spelaea)* first appeared about 600 000 years ago and were distributed throughout Europe, across Asia, and into Alaska (Kurtén 1968, Neff 1983). The European cave lions, as *P. l. spelaea* are called, were massive creatures, some 25% larger than today's lions. Aristotle mentions this fossil subspecies but it eventually became extinct 100 to 300 years B.C. A second lion (sub) species, *P. atrox*, resided in North and South America during the upper Pleistocene, but also became extinct within historic times (Merriam & Stock 1932, Neff 1983). Large numbers of *P. atrox* fossils have been described throughout the United States and in several cases *P. atrox* fossils occur in the same deposits as fossils of *Smilodon,* the American Pleistocene saber-toothed tiger, suggesting sympatric coexistence of the two extinct felid species.

Although the lion achieved a terrestrial range greater than any land mammal (except man and his domestics and commensals), the free-

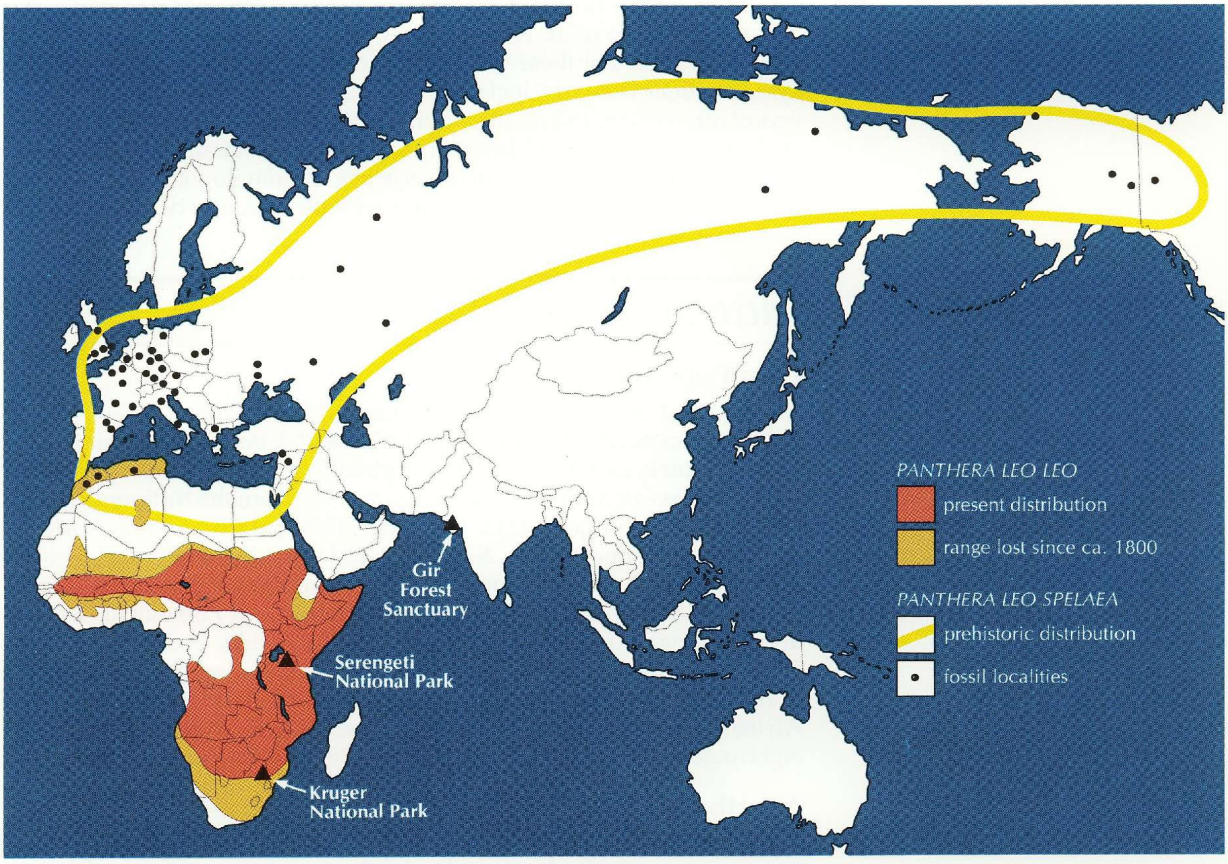

Figure **1.** *Ranges of* Panthera leo.

ranging lion exists today only in Africa and in a restricted population of approximately 250 individuals in the Gir Forest Sanctuary, Gujarat, western India (Figure 1). Myers (1975) estimated the number of lions in Africa at approximately 200 000. The decline in geographic range near the end of the late Pleistocene in Europe has been suggested to result from the gradual formation of dense forests, where lions do not do very well, but more recently the decline is clearly the consequence of range destruction by human development (Guggisberg 1975, Kingdon 1977, Nowak & Paradiso 1983).

Subspecies classification of lions has been largely subjective and often based on too few specimens to be rigorous. For example, Smithers

(1971) listed 24 African subspecies but Pocock (1930) listed five Asian or Indian subspecies. The consensus among modern authors places all the African populations in a single subspecies, *P. l. leo,* and the remnant Asian population as the second zoogeographic subspecies, *P. l. persica* (Guggisberg 1975, Neff 1983, Nowak & Paradiso 1983). The Asian subspecies has several morphologic distinctions that more or less characterize *P. l. persica,* including reduced mane in males, a recognizable abdominal fold, and pairing or bifurcation of the infraorbital foramen (Joslin 1971; O'Brien, Joslin et al. in press; Pocock 1930).

In the present study the extent and character of genetic variation in five populations of lions have been estimated using electrophoretic techniques. The populations were free-ranging lions from the Kruger National Park in South Africa, and from the Serengeti National Park and the Ngorongoro Crater in Tanzania, East Africa; a group of 28 wild-caught or captive-born lions from the Gir Forest Sanctuary in western India; and a group of 18 African lions, including nine Atlas lions from American zoos. For these groups 46 to 50 enzyme structural gene (allozyme or allelic isozyme) loci were typed and compared with similar surveys of other feline and mammalian species (O'Brien 1980; O'Brien, Gail et al. 1980; O'Brien, Goldman et al. 1983). Genetic distance estimates based on the extent of genetic divergence of homologous gene enzyme systems were determined and compared with estimates between the lion and other species of great cats, *Panthera.*

Materials and Methods

Lion Populations

Heparinized blood samples were collected from captive lions from the following zoological facilities: Asiatic lions *(P. l. persica)* held at the Sakkarbaug Zoological Gardens but originally derived from the Gir Forest in Gujarat, western India; Atlas lions *(P. l. leo)* from the National Zoological Park, Washington, D.C. (Hemmer 1974); African lions from the Henry Doorly Zoo, Omaha, Nebraska, and from the Detroit Zoo, Detroit, Michigan; and African lions derived from Kruger National Park in Transvaal, South Africa, collected at Johannesburg Zoological Gardens and National Zoological Gardens of South Africa, Pretoria. Samples from free-ranging lions were collected following chemical immobilization at the following locations: Kruger National Park, Transvaal, South Africa; Serengeti National Park, Tanzania; and Ngorongoro Crater, Ngorongoro Conservation Area, Tanzania (Figure 2).

Anesthesia

Free-ranging lions in Kruger National Park and in the Serengeti ecosystem were immobilized using a projectile dart delivered by a carbon dioxide pistol. The dart contained 500 mg of CI744, a combination of tiletamine hydrochloride and zolazepam hydrochloride (Telazol, Warner Lambert Co., Ann Arbor, Mich.). Supplemental doses in 100-mg increments were administered to maintain anesthesia.

Electrophoresis

Blood was separated into plasma, erythrocytes, and leukocytes as previously described, and stored at −70° (Ford 1978, Newman et al. 1985, O'Brien 1980). Field collections were stored in liquid nitrogen freezers until their return to the National Cancer Institute. Isozyme extracts were prepared by sonication and subjected to aqueous gel electrophoresis as

previously described (Newman et al. 1985, O'Brien 1980). Histochemical stains for 50 feline isozyme systems were applied and evaluated using the criteria for genetic variants given by Newman et al. (1985). Genetic distance estimates were computed based on the formula of Nei (1972) with computer assistance using the BIOSYS program kindly provided by R. Swofford, University of Illinois.

Results

Blood samples from lion populations in three distinct African regions and from the single remaining Asian (Indian) population were subjected to electrophoretic analysis of as many as 50 allozyme loci. The specific isozyme systems were selected on the basis of technical resolution from more than 50 isozyme protocols used in the author's laboratory in

Figure **2.** *David Wildt evaluates a semen sample while donor looks on, Ngorongoro Crater.*

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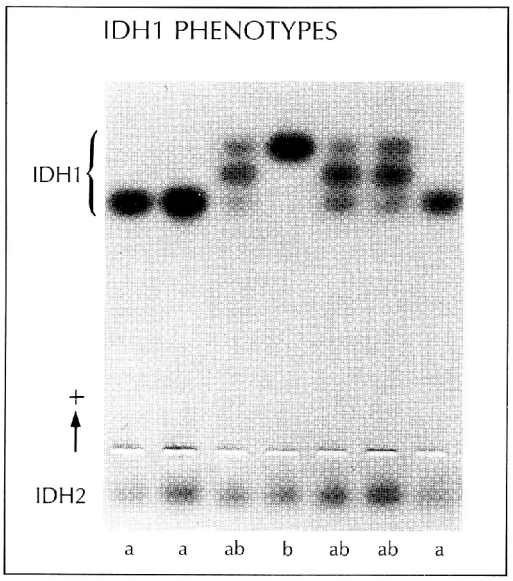
studies involving somatic cell genetic analysis of somatic cell hybrids made with domestic cat cells (O'Brien & Nash 1982; O'Brien, Simonson et al. 1982). Since the utility of these systems in cell genetic analysis does not depend on allozyme polymorphism, the bias of their being generally polymorphic is minimized.

The allelic frequencies of seven allozyme loci were polymorphic in one or more of the lion populations (Table 1). Gels that illustrate the electrophoretic pattern of three of these allozyme polymorphisms are shown in Figures 3 to 5. Gels for *GPI*, *GPT*, and *MPI* are in Newman et al. (1985). The remaining enzyme loci were monomorphic and fixed for the same allele in each of the lion populations. Estimates of the frequency of polymorphic loci (P) and the average heterozygosity (H), the incidence of heterozygous loci over all loci in the population, are presented in Table 2. Each polymorphism conformed to a Hardy–Weinberg equilibrium as tested by χ^2 analysis.



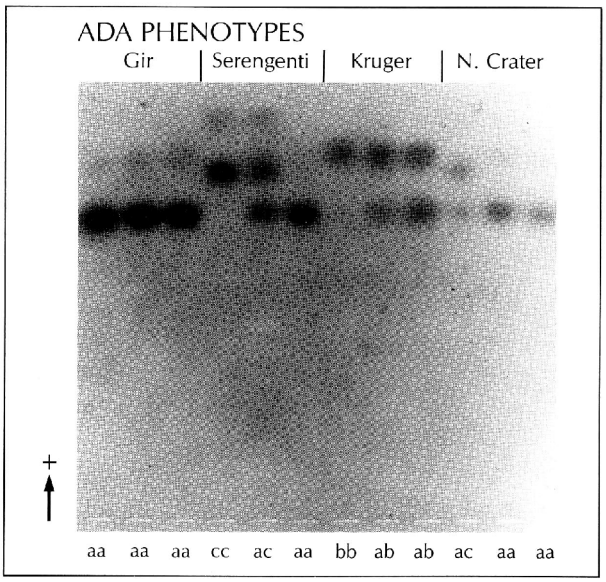


Figure 3. Electropherogram of isocitrate dehydrogenase-1 (IDH1) *allozymes. 4. Electropherogram of* adenosine deaminase (ADA) *allozyme.*

The amount of variation detected was different in separate populations. Compared with other felids (O'Brien, Roelke et al. 1985), the Serengeti lions exhibited a moderate amount of variation. For example, the domestic cat has a P value of 0.21 and an H of 0.082 while the cheetah has P and H values both equal to 0.0 (O'Brien, Goldman et al. 1983; O'Brien, Roelke et al. 1985). Within lions, however, the Serengeti population showed the greatest variation. This population is composed of approximately 3000 lions and has most likely been a large healthy population at least since the rinderpest epizootic of the 1890s which decimated the wildebeest population and probably altered the lions' prey base substantially (Sinclair 1979).

The Ngorongoro Crater group is a stable isolated population of 100 animals on the edge of the East African Serengeti ecosystem, which is isolated from immigration but not from emigration (Pusey & Packer in press). The population was presumably established from the larger surrounding Serengeti lion populations which also include a contiguous population of lion prides near Lake Manyara. Today the Ngorongoro lions subsist as an effective "island" population. Crater lions were shown to have less than 40% of the variation present in the parent population from the surrounding Serengeti ecosystem (Table 2). In fact, two loci

polymorphic in the Serengeti lions — glucose phosphate isomerase *(GPI)* and glutamate-pyruvate transaminase *(GPT)* — are monomorphic in the crater lions and two other loci — adenosine deaminase *(ADA)* and isocitrate dehydrogenase-1 *(IHD1)* which are highly variable in the Serengeti — are close to fixation in the crater lions (Table 1).

Mannose phosphate isomerase *(MPI)* and transferrin *(TF)* were also polymorphic in African lions. The following loci were monomorphic and fixed for the same electrophoretic allozyme allele in each population: *ACP1, ACP2, AK1, ALB, CA2, APRT, CAT, CPKB, DIA1, DIA4, ESA1, ES10, GOT2, GSR, GUSB, GLO, HBB, HEXA, HK1, HK2, IDH2, LDHA, LDHB, MDH1, MDH2, ME1, NP, PEPA, -B, -C, -D, PGD, PGM1, -2, -3, PK, PP, SOD1,* and *TPI.* Previously reported allozyme variation in lion populations was based exclusively on Atlas and African zoo lions in Table 1 (Newman et al. 1985). In that report, allozyme variants of *IDH1, G6PD,* and *TF* were not found. In this study, improved methods for

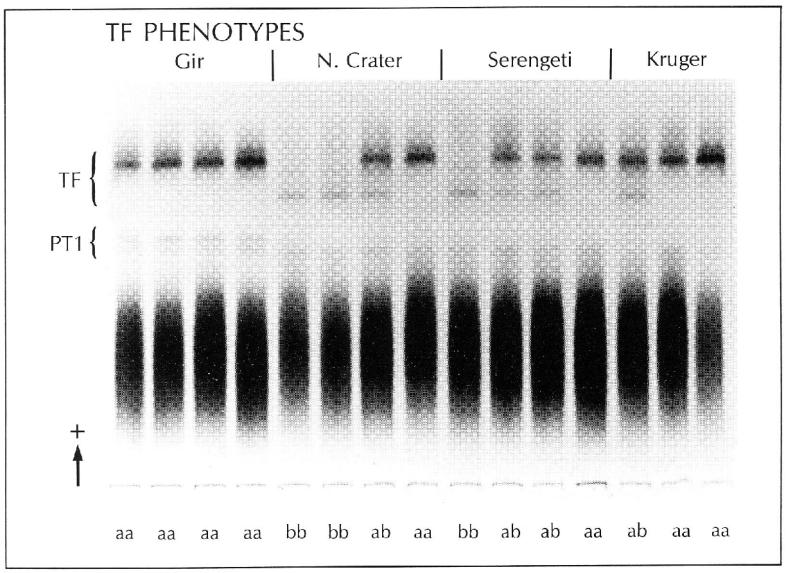


Figure **5.** *Electropherogram of* transferrin (TF) *allelic variants.*

resolving *TF* revealed variation at the *TF* locus in the African zoo lions; *IDH1* and *G6PD* were monomorphic. Newman et al. (1985) also reported allozyme variants in red cell *diaphorase, DIAB,* and in *ESA.* Both of these polymorphisms were tested in extensive pedigree analyses (unpublished observation) and were shown to be nongenetic variation related to extract storage. These loci were therefore not included in the present set of electrophoretically typed enzyme systems.

The Kruger Park lions have one polymorphism — glucose-6-phosphate dehydrogenase *(G6PD)* — not seen in other lions, but the novel allele *($G6PD^b$)* is somewhat rare ($p = 0.07$). Otherwise the Kruger animals appear low in the overall amount of variation (Table 2); they are comparable with the Ngorongoro Crater population, having five of the seven polymorphic (for lions) loci genetically fixed or nearly fixed. The Kruger lion population was severely afflicted by an anthrax epizootic in 1960 (Pienaar 1961). This event may have caused genetic drift or founder effects to reduce overall genetic variation.

The Gir Forest (Sakkarbaug Zoo) lions were unusual among lions in that no variation was seen at any of the loci in a sample of 28 animals. The sampled animals include four wild-born founder animals and 24 offspring of nine (including the four sampled) original founders (Smith

1985). In all cases except the *IDH1* locus, the allele fixed in the Gir lions was the most common allele in the African populations.

Genetic distance (D) (Table 3) is a statistical calculation developed by M. Nei for estimating the degree of allelic substitutions at a group of loci between two populations (or species) based upon the electrophoretic mobility of soluble proteins (Nei 1972). D is the estimated average number of gene differences per locus between individuals from two populations. Under the constraints of certain assumptions relating to electrophoretic resolution and the relative rates of nucleotide substitutions, the genetic distance estimates increase proportionately with the amount of time elapsed since the populations shared a common ancestor.

Table 1. Polymorphic Allele Frequencies in Lion Populations

Population	Enzyme Locus*						
	ADA	*IDH1*	*GPI*	*GPT*	*G6PD*	*MPI*	*TF*
AFRICAN							
Kruger Park	a = 0.34 b = 0.61	a = 1.0	a = 1.0	c = 1.0	a = 0.93 b = 0.07	a = 1.0	a = 0.65 b = 0.35
Atlas	a = 0.61 b = 0.39	a = 1.0	a = 0.67 b = 0.33	a = 0.44 b = 0.56	a = 1.0	a = 1.0	a = 1.0
African (U.S. zoo)	a = 0.61 b = 0.28 c = 0.11	a = 1.0	a = 1.0	a = 1.0	a = 1.0	a = 0.72 b = 0.28	a = 0.72 b = 0.28
Serengeti	a = 0.74 c = 0.26	a = 0.71 b = 0.29	a = 0.96 b = 0.04	a = 0.78 b = 0.16 c = 0.06	a = 1.0	a = 1.0	a = 0.52 b = 0.48
Ngorongoro Crater	a = 0.92 c = 0.08	a = 0.97† b = 0.03	a = 1.0	a = 1.0	a = 1.0	a = 1.0	a = 0.44 b = 0.56
ASIAN							
Gir Forest	a = 1.0	b = 1.0	a = 1.0	a = 1.0	a = 1.0	a = 1.0	a = 1.0

**Allozyme variation was detected using starch or acrylamide gel electrophoresis. Abbreviations for typed loci follow the standard human nomenclature (McAlpine et al. 1985).*

†A single heterozygote out of 17 tested was heterozygous for IDH1 *in the Ngorongoro Crater population. Because of the low frequency this locus was not included in estimated percentage of polymorphic loci (Lewontin 1974).*

The genetic distance estimates between the various African lion populations were low (D = 0.004 to 0.031) and comparable with values obtained between conspecific mouse populations (D = 0.020) or between human racial groups (D = 0.022) (Nei & Roychoudhury 1982, Rice & O'Brien 1980). The closest values were obtained between the Serengeti and the derivative Ngorongoro Crater population and the central African lions. The largest extent of genetic differentiation from other African populations (although still slight) was seen in the Kruger Park lions (ave. D = 0.025). The Asiatic subspecies from the Gir Forest was strikingly similar to the African populations (ave. D = 0.013). This value was 12 times less than the average distance between the African lion and each of the four other *Panthera* species (ave. D = 0.153), and approximately equivalent to the distance computed between different subspecies of tigers (O'Brien, Collier et al. in press), cheetahs (O'Brien, Wildt et al. in press), and human racial groups (Nei & Roychoudhury 1982).

Discussion

The six lion populations revealed different amounts and patterns of biochemical genetic variation. Compared with other cat species (O'Brien 1980; O'Brien, Roelke et al. 1985), the African lion populations had

moderate amounts of variation ($p = 0.07$ to 0.11), while the Asiatic subspecies revealed none. The diminished amount of variation of *P. l. persica* may result from a founder effect in the recent history of the remnant population (< 200 individuals) in the Gir Forest Sanctuary in western India (Figure 1). In the 19th century this subspecies ranged through most of Asia Minor, Iran, and central India. The recent range contraction is consistent with a previous population bottleneck as has been hypothesized for the African cheetah *Acinonyx jubatus* (O'Brien, Goldman et al. 1983; O'Brien, Roelke et al. 1985).

Table 2. Proportion of Loci Estimated to be Polymorphic and Proportion of the Genome Estimated to be Heterozygous in Lion Populations

Population	Area of Ecosystem (km^2)	Estimated Population Size	Number of Lions Sampled	Number of Loci	Proportion Polymorphic Loci	Average Heterozygosity
AFRICAN (*P. L. LEO*)						
Kruger Park	21000	1500	15	50	0.07	0.023
Atlas, captive	*	—	9	50	0.07	0.031
African, captive	—	—	9	50	0.07	0.029
Serengeti ecosystem, free-ranging	25000	3000	26	46	0.11	0.038
Ngorongoro Crater, free-ranging	180	100	17	46	0.04	0.015
ASIAN (*P. L. PERSICA*)						
Gir Forest Sanctuary, captive	1400	250	28	46	0.0	0.0

**Atlas lions are derived from a population that lived north of the Morocco Mountains in North Africa. They are presently extinct in the wild.*

Table 3. Genetic Identity (I, bold face) and Distance (D, regular face) Estimates of Lion Populations*

Population	Kruger	Atlas	African	Serengeti	Ngorongoro	Gir
Kruger	——	**0.977**	**0.974**	**0.976**	**0.969**	**0.967**
Atlas	0.023	——	**0.987**	**0.988**	**0.981**	**0.987**
African (zoo)	0.026	0.013	——	**0.995**	**0.994**	**0.994**
Serengeti	0.024	0.012	0.005	——	**0.996**	**0.991**
Ngorongoro	0.031	0.019	0.006	0.004	——	**0.993**
Gir	0.033	0.013	0.006	0.009	0.007	——

**I equals the probability of allelic identity of any randomly selected genes at any locus in each of two test populations. D equals the average number of gene differences per locus between individuals from the two test populations. Algebraically*

$$I = J_{xy} \Big/ \sqrt{J_x J_y} \quad \text{and} \quad D = -\ln I$$

where J_{xy} is the arithmetic mean of $j_{xy} = \Sigma_i x_i y_i$ over all loci, J_x is the arithmetic mean of $i_x = \Sigma_i x_i^2$ over all loci, and x_i (or y_i) is the frequency of the ith allele in the first (or second) population (Nei 1972).

Comparison of the amount of genetic variation in the Ngorongoro population with the surrounding larger Serengeti ecosystem lion population revealed some important distinctions. The Ngorongoro lion population consists of approximately 100 animals inside an isolated crater (~ 180 km^2) adjacent to the Serengeti ecosystem. Lions occasionally leave the crater but no lions have entered since continuous individual identification began 12 years ago (Pusey & Packer in press). The Ngorongoro population retains only about 36% of the amount of variation present in the founder population from the Serengeti ecosystem (Table 2). The fewer loci that are variable in the Ngorongoro Crater lions are a subset of the loci that are also polymorphic in the Serengeti lions. Furthermore, two of the polymorphic loci in the crater (*ADA* and *IDH1*) are near fixation, so only one polymorphic locus (*TF*) has a similar allele frequency to that of the Serengeti population. The relatively reduced variation in the Ngorongoro population seems to represent yet another founder event which cost the derivative population a fair portion of its

genetic variation. This proposed bottleneck may have occurred at the inception of the population or as a result of the *Stomoxys* (blood-sucking fly) epizootic that reduced the Ngorongoro lion population from 70 to between 6 and 15 individuals in 1962 (Fosbrooke 1963).

The physiological consequences of a population bottleneck sufficient to reduce the extent of biochemical genetic variation have been discussed extensively in the authors' previous analyses of African cheetah populations (O'Brien, Goldman et al. 1983; O'Brien, Roelke et al. 1985; O'Brien, Wildt et al. 1986). Among the apparent aspects of inbreeding depression, this species displayed a rather high level of morphologically abnormal spermatozoa in both East African and South African populations (Wildt et al. 1983). A related study revealed similar structural abnormalities in spermatozoa as well as diminished average testosterone concentration in Ngorongoro Crater lions compared with free-ranging Serengeti lions (Wildt et al. unpublished manuscript). A limited sample of male Gir lions from the Sakkarbaug Zoo also revealed high levels (average, 79%) of spermatozoal abnormalities (O'Brien, Joslin et al. in press). These results tend to affirm the hypothesis that genetic diminishment of natural populations may have unfavorable physiological effects such as increased spermatozoal abnormalities.

The genetic distance between African lions was low (D = 0.016) as was the average distance between *P. l. leo* and *P. l. persica* (D = 0.014). These distances are nine times less than the genetic distance between any two of the five species of *Panthera* (D = 0.124) using the same loci (O'Brien, Collier et al. in press). The distances are actually less than the genetic distances calculated between human racial groups (D = 0.022; range = 0.01 to 0.029; Nei & Roychoudhury 1982). Since *Panthera* is estimated to have originated approximately 1.8 million years ago (Neff 1983, Savage & Russell 1983), the timing of separation of the lion subspecies could be estimated as $1.8 \times 10^6/9 = 200\,000$ years ago. An alternative estimate is based on the separation of Negroid and Caucasian racial groups circa 110 000 years ago (Nei in press, Nei & Roychoudhury 1982). Using this date as a calibration, the lion subspecies would share a common ancestor at $0.014/0.028 \times 110\,000 = 55\,000$ years ago. These estimates should be considered as tentative at best and probably as the oldest possible dates because the D values are pushed to the sensitivity of the molecular clock. Because of these low distance values and the failure to detect any subspecific allozyme markers, there is no evidence to suggest that sufficient time has elapsed in the history of these subspecies for specific genetic isolating mechanisms to have developed.

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