Interestingly, in contrast to the case for SiO_2, the Cl^- and SO_4^{2-} trends at the three cities from 1949 to 1980 are, with one understandable exception, consistent with each other (Table 1). There was an increase in Cl^- from 0.15 to 0.16 ppm per year at Chicago, Milwaukee, and Grand Rapids. There was an increase in SO_4^{2-} of 0.21 ppm per year at Chicago and 0.22 ppm per year at Milwaukee, but 0.38 ppm per year at Grand Rapids; the difference is probably due to a change in methods that resulted in an overestimate of 57 percent.

Thus municipal water analyses appear to document some trends, but the results continue to be equivocal on the question of whether Lake Michigan is experiencing progressive change in SiO_2, dissolved or total. (One must also question whether water samples collected in the nearshore zone can ever be representative of the water quality of the lake as a whole.) This is not to say that the Schelske-Stoermer hypothesis is wrong, but clearly it is undemonstrable in Lake Michigan, although it may have been demonstrated elsewhere (*13*).

This is an unfortunate conclusion in view of the fact that a few precautions could have rendered the Lake Michigan analyses an unparalleled environmental record. Although the changes in method at Chicago were made in a search for greater accuracy, had the analytical methods not changed in 1949 or had the methods used been calibrated against each other for a year (as was done for SO_4^{2-} at Grand Rapids), the record would now be of much greater value.

Although we have used these municipal data to evaluate claims for long-term trends in the limnology of Lake Michigan, the data were collected not for that purpose but rather to demonstrate compliance with water quality criteria. The difference is not trivial. These data demonstrate that there was no continuing concern over measurement control, which involves cross-calibration of techniques, evaluation of error, use of appropriate standards, and interlaboratory comparison. There was also no apparent effort made to maintain consistent sampling and laboratory procedures (*11*).

Because of the laboratory change in Chicago in 1948, it was incorrect initially to fit lines to those data, and, in fact, because of the obvious lack of measurement control, it is incorrect to search for long-term trends in any of the data. Furthermore, if the data are examined closely, there seem to be a number of discontinuities in addition to the 1948 changes (Fig. 1): (i) an abrupt reduction in SiO_2, SO_4^{2-}, and Cl^- data variability starting in 1935; (ii) abrupt shifts in Cl^- and SO_4^{2-} values in 1956; (iii) an abrupt increase in SO_4^{2-} values starting in 1965, coincident with an increase in the variability of the SiO_2 values; and (iv) an abrupt increase in Cl^- values in 1971. In one of these cases the discontinuity correlates with a known change in measurement procedure: in 1965, a new laboratory began performing the analyses (*11*). Because for many years no records were kept on changes in analytical procedures, we do not know whether the other discontinuities are so correlated. Therefore, because the 1948 and 1965 changes were in the laboratory and not in the lake, we believe that it is not safe to conclude anything about long-term trends in Lake Michigan from these data.

Those agencies doing analyses on any environmental parameter, year after year, for whatever reason, have a largely unrecognized obligation (and opportunity) to carry out long-term monitoring. This obligation includes a responsibility for measurement control. Analytical methods should not be changed unnecessarily, and any necessary changes should be cross-calibrated.

JOSEPH SHAPIRO
EDWARD B. SWAIN
Limnological Research Center, University of Minnesota, Minneapolis 55455

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11. From 1926 to 1980 six different water intakes at various depths and distances from shore were used. The South District Filtration Plant conducted the analyses until November 1964, at which time the Jardine Plant took over; the filtration plants occasionally took in water directly at the plant, a "shore intake"; from August 1956 to August 1963 approximately 1 mg of fluoride per liter of water was added (as H_2SiF_6) at one pumping station, Chicago Avenue, before analyses were made; and since 1926, at least five different methods of SiO_2 analysis have been used, alternating between measurements of dissolved and total SiO_2. To circumvent some of these problems, we analyzed just the data from the Chicago Avenue pumping station, which used the Harrison intake until 1936 when it was replaced by the Dever intake, 100 feet away. We corrected for the SiO_2 that accompanied the F^- additions by subtracting 0.5 ppm SiO_2 from those data.
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The Cheetah Is Depauperate in Genetic Variation

Abstract. *A sample of 55 South African cheetahs (*Acinonyx jubatus jubatus*) from two geographically isolated populations in South Africa were found to be genetically monomorphic at each of 47 allozyme (allelic isozyme) loci. Two-dimensional gel electrophoresis of 155 abundant soluble proteins from cheetah fibroblasts also revealed a low frequency of polymorphism (average heterozygosity, 0.013). Both estimates are dramatically lower than levels of variation reported in other cats and mammals in general. The extreme monomorphism may be a consequence of a demographic contraction of the cheetah (a population bottleneck) in association with a reduced rate of increase in the recent natural history of this endangered species.*

The cheetah (*Acinonyx jubatus*) is the world's fastest mammal (achieving speeds of up to 112 km per hour) (*1*) and probably the most specialized of felids. Unlike other feline species, the cheetah has semiretractile claws, a long slender skeleton, and a number of derived anatomical characters related to its adaptations as a high-speed sprinter. Cheetahs are highly successful predators whose primary interference competitors are species such as the spotted hyena (*Crocuta crocuta*), the lion (*Panthera leo*), and the African wild dog (*Lycaon pictus*) (*2–4*). Their numbers are sparse (estimates range between 1500 and 25,000 in

Africa today) (5–8), the species is endangered, and a tendency for population deceleration has been evident in recent years (5). We present an estimate of the extent and character of biochemical genetic variation in samples of two geographically isolated South African cheetah populations. The estimate is derived from two conventionally studied groups of genes: 47 allozyme (allelic isozyme) loci and 155 soluble proteins resolved by two-dimensional gel electrophoresis.

Since the application of electrophoretic techniques to assessment of genic variation in natural populations, more than 250 biological species have been examined (9–11). In general, appreciable genetic variation has been found in most of the surveys, with frequencies of polymorphic loci (P) ranging from 0.15 to 0.60 and average heterozygosities [the frequency of heterozygous loci (H) over all loci in all individuals in a population] (12) ranging from 0.0 to 0.26 (9–11). This amount of genetic variation has been considered normal and typical for panmictic (random bred) biological species. With only a few exceptions, the mammalian populations surveyed have considerable amounts of allozyme genic variation (average $P = 0.147$; average $H = 0.036$) (10).

An isozyme sample was derived from blood and lymphocytes collected in January 1981 from 50 cheetahs maintained at the De Wildt Cheetah Breeding and Research Center (National Zoological Gardens of South Africa, Pretoria). The De Wildt cheetah population consisted of wild-caught and first generation captive-bred offspring from two apparently isolated geographic regions: (i) the northern region of the Transvaal Province of the Republic of South Africa and (ii) South-West Africa (Namibia). Twenty-seven cheetahs were derived from the Transvaal population, four from South-West Africa (Namibia), and 19 were captive-born hybrids between parents from different regions. In addition, blood samples from five captive South African cheetahs maintained in two United States zoos (St. Louis Zoo and Henry Doorly Zoo, Omaha) were included in the survey.

Crude extracts of erythrocytes from the 55 cheetahs and lymphocytes from ten of them were subjected to gel electrophoresis and histochemically stained for 40 gene-enzyme systems (13). Because certain enzyme stains detect more than one gene product (for example, soluble and mitochondrial malate dehydrogenase), the sample of loci represents 47 distinct gene products. These genes were selected solely because they could be resolved by electrophoretic techniques in our laboratory (14–17). The enzyme loci studied were homologous to those studied in the domestic cat (16), the mouse (14, 15), and man (17). The entire cheetah sample was invariant at each of the 47 loci (18, 19), and the mobility of each enzyme was identical in extracts from cheetahs of the Transvaal and South-West Africa and from hybrid and zoo cheetahs.

Included in the 47 loci were 18 allozymes previously defined as "polymorphic cluster" genes (17). Certain homologous gene enzyme loci have a tendency to be monomorphic in surveys of mammalian populations (about 60 percent of the standard markers examined); others tend to be polymorphic in several different species (about 30 percent of the standard markers). This conservation of the tolerance of genetic polymorphism is apparently more characteristic of a particular locus than that of the vertebrate species or of the genome. None of the 18 polymorphic cluster markers tested was polymorphic in the cheetah sample.

Another approach used to estimate genic variation in populations is the separation at high resolution of abundant soluble proteins in two dimensions on polyacrylamide gels (2DE) (20–25). The procedure involves two-stage electrophoresis: first, soluble proteins from crude tissue extracts are separated on the basis of isoelectric points in a pH gradient produced by a mixture of ampholytes in an aqueous column gel; second, the proteins in the column gel are separated on the basis of molecular weight in a slab denaturing gel containing sodium dodecyl sulfate (SDS) (20). The 2DE technique may resolve a different group of proteins from the enzymes studied in allozyme surveys. Many of the enzymes that are detected by their enzymatic activity are present in too low a concentration in biological tissues to be among the relatively abundant proteins observed in the 2DE gel assays (21–25).

Six primary skin fibroblast cell lines were established, labeled with ^{14}C amino acids, and subjected to 2DE gels and autoradiography. The six cheetahs included one from the St. Louis Zoo, one from the Henry Doorly Zoo, and four from unrelated South African cheetahs from the Blijdorp Zoo, Rotterdam. The

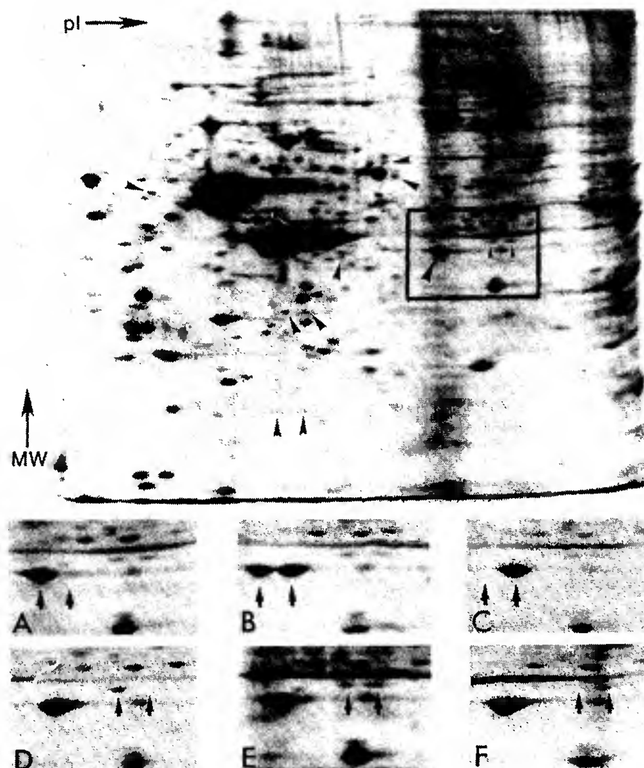

Fig. 1. Two-dimensional autoradiogram of cheetah fibroblast proteins. Molecular weight (MW) range, 15,000 to 200,000; pH range, 4.5 to 7.0. Skin explants were digested with 0.5 percent collagenase and 0.25 percent trypsin, serially collected, and cultured in RPMI 1640 tissue culture medium supplemented with 10 percent heat-inactivated fetal bovine serum. Subconfluent monolayers were rinsed with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline, placed on amino acid–deficient RPMI 1640 for 6 to 8 hours, incubated with ^{14}C-labeled protein hydrolyzate (Amersham, 100 Ci; 50 μCi/ml) for 18 hours, and rinsed three times with phosphate-buffered saline. Two-dimensional electrophoresis was carried out as described by O'Farrell (20) with modifications (28). Cells were freeze-thawed and proteins were extracted at 95°C in a solution of 2 percent SDS, 5 percent B mercaptoethanol, 20 percent glycerol, and 2 percent NP-40. For the first-dimension isoelectric focusing gel, a 4:1 ratio of pH 5 to 7 to pH 3 to 10 ampholytes (Bio-Rad) was used while a 10 percent uniform SDS–acrylamide gel was used for the second dimension. Gels were dried and used to expose Kodak XAR-2 x-ray film for 10 days. Entire gel of one cheetah has polymorphic systems indicated by arrows. The magnified regions [box; (A) to (C), (D) to (F)] illustrate the three dosage-dependent phenotypes for two of the systems in different individual cheetah samples. The isoelectric point (pI) is shown by the arrow at the top.

autoradiographic patterns (Fig. 1) were analyzed by computer-assisted scanning densitometry (*26–28*). Five of the 155 sampled proteins (3.2 percent) exhibited qualitative polymorphism within the sample (Fig. 1). Each polymorphism involved a mobility shift due to a charge change, and all the putative polymorphisms exhibited gene dosage dependence in heterozygotes, which is consistent with a genetic basis (*26, 29*). The estimated average heterozygosity for all loci from the sample of six cheetahs was 0.013.

A number of studies in man, mouse, and *Drosophila* have suggested that the 2DE technique yields estimates of genic variation in these species that are two to ten times less than estimates derived from isozyme data (Table 1). Thus, Smith *et al.* (*23*) and McConkey *et al.* (*24*) both reported that less than 1 percent of the tested human loci were variant, with H values less than 0.01. However, another 2DE survey of human populations (*26*) revealed substantially greater polymorphism than has been previously reported. The reasons for the discrepancy may be technical (*26*). Nonetheless, the cheetah sample we tested showed two to three times less variation than that observed in the human survey performed in the same laboratory (Table 1).

Table 1. Proportion of loci estimated to be polymorphic and the proportion of genome estimated to be heterozygous in selected species.

Species	Populations (*N*)	Individuals (*N*)	Loci (*N*)	Polymorphic loci (%)	Average heterozygosity	Reference
			Allozyme			
Drosophila	43*	> 100	24	43.1	0.140	(*10*)
Mus musculus	2	87	46	20.5	0.088	(*15*)
Felis catus	1	56	55	22.0	0.076	(*16*)
Homo sapiens	Many	> 100	104	31.7	0.063	(*43–45*)
Acinonyx jubatus	2	55	47	0.0	0.0	
			2DE			
Drosophila	1	20	54	11.1	0.04	(*21*)
Mus musculus	1		72	4.1	0.02	(*22*)
Homo sapiens	3	34	83–400	1.2	≤ 0.006	(*23–25*)
Homo sapiens	1	28	185	10.8	0.024	(*26*)
Acinonyx jubatus	1	6	155	3.2	0.013	

*Forty-three species.

The cheetah is unusual but not the only mammalian species with low levels of variation. The northern elephant seal (*30*), the moose (*31*), the polar bear (*32*), and the Yellowstone elk (*33*) have been reported to have diminished levels of variation. Bonnell and Selander (*30*) found no variation at 24 loci in northern elephant seals and attributed the monomorphism to a bottleneck in population size due to decimation of the species by hunters in the late 19th century. An early low estimate (*31*) of genic variation in the moose ($P = 0.04$; $H = 0.0006$) was shown to be a marked underestimate due to sampling error by the same investigators in an extensive study of 734 moose collected from 18 locales in Scandinavia ($P = 0.13$; $H = 0.020$) (*34*). A more extensive survey of allozyme variation in elk revealed appreciable genetic variation (*35*).

The evolutionary interpretation most compatible with all the data would suggest that the cheetah has experienced a severe population bottleneck followed by inbreeding in its recent history. It is well accepted that population bottlenecks have the qualitative effect of reducing the amount of variation of natural populations because of the combined forces of natural selection and genetic drift (*36–38*). If we assume that at one time the ancestors of modern cheetahs had levels of genic variation similar to those of other mammals, a severe recent (within 100 generations) population bottleneck (for example, due to intensive poaching and decimation by African cattle farmers) would account for the present paucity in genetic variation. Another possibility is that of a more ancient (possibly 1000 generations ago) bottleneck followed by a rather low intrinsic rate of increase (*38*). It is not possible to determine the specific timing of the proposed bottleneck of the cheetah, and it may be that both ancient and recent bottlenecks have been experienced by the species.

Ecological support for the bottleneck model is evident from the rather drastic climatic changes that occurred worldwide in the late Pleistocene (*39*). Many large specialized carnivores became extinct during this period, including at least four species of *Acinonyx* and as many subspecies of *Acinonyx jubatus* (*39, 40*). The modern cheetah is clearly in the process of a severe range contraction. Its distribution was worldwide (Africa, Asia, Europe, and North America) as recently as 20,000 years ago (*40*). Yet today the species is restricted to isolated populations in South Africa and East Africa with numbers as few as 1500 to 5000 (*5, 6*). Furthermore, both demographic and reproductive data of present populations suggest that their rate of increase is rather low if not an outright decline (*8*). It is conceivable that harsh environmental changes could have eliminated widely dispersed cheetahs of the past and that present populations are slowly recovering from these events.

An apparently significant consequence of the bottleneck followed by inbreeding is evident from a concurrent analysis of cheetah spermatazoa (*41*). The same cheetahs we studied were shown to have sperm counts (sperm per cubic centimeter of ejaculate) ten times lower than related Felidae species and to have 70 percent of their sperm morphologically aberrant. Such extremely unusual sperm characteristics have only been seen previously in inbred livestock and in inbred mice (*42*).

It is rather striking that despite the low levels of variation the cheetah has been able to compete and survive in the wild. The irony, however, of the persistence of the monomorphic cheetah is its apparent level of vulnerability. By whatever mechanism, a successful and specialized species has been produced with little genetic plasticity, one which could be the least adaptive in a time of perturbation of the ecological niche. It is tempting to speculate that similar circumstances that have produced precipitous monomorphism followed by niche perturbation might explain extinction of successful species in the past.

STEPHEN J. O'BRIEN
DAVID E. WILDT
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701
DAVID GOLDMAN
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20205
CARL R. MERRIL
Laboratory of General and Comparative Biochemistry, National Institute of Mental Health
MITCHELL BUSH
National Zoological Park, Smithsonian Institution, Washington, D.C. 20008

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Insulin Receptor Antiserum and Plant Lectins Mimic the Direct Effects of Insulin on Nuclear Envelope Phosphorylation

Abstract. *Insulin directly inhibits protein phosphorylation in isolated rat liver nuclear envelopes. In the present studies, an antiserum to insulin receptor as well as the plant lectins concanavalin A and phytohemagglutinin mimicked insulin action in isolated nuclear envelopes. These studies suggest that insulin and agents that mimic it may directly regulate nuclear functions.*

Insulin has multiple effects on target cells, including regulation of membrane transport, enzyme activation, and RNA levels (*1*). Many of these effects appear to be mediated by changes in phosphorylation and dephosphorylation reactions (*2*) that can be demonstrated in isolated subcellular fractions (*3*). However, the exact site (or sites) of insulin action is unknown.

Table 1. Combined effects of insulin, antiserum to insulin receptors, and Con A on ^{32}P incorporation into rat liver nuclear envelopes. Values are the mean ± standard error of three separate experiments.

Addition	^{32}P incorporated (percent of control)
Insulin ($10^{-11}M$)	64 ± 0.6
Antibody to receptors (1:10,000)	66 ± 0.4
Con A (10 μg/ml)	68 ± 1.8
Insulin ($10^{-11}M$) plus antibody to receptors (1:10,000)	71 ± 1.6
Insulin ($10^{-11}M$) plus Con A (10 μg/ml)	70 ± 1.9

Since insulin is internalized by target cells (*4*) and since nuclear envelopes have specific binding sites for insulin (*5*, *6*), it was thought that insulin regulates RNA levels by directly interacting with the nucleus. Recently, three direct effects of insulin on nuclei and nuclear envelopes were demonstrated. First, Schumm and Webb reported that insulin increases the efflux of messenger RNA (mRNA) from isolated nuclei (*7*). This effect was confirmed both in our laboratory (*8*) and the laboratory of Agutter (*9*). Second, we showed that insulin activates nuclear envelope nucleoside triphosphatase (NTPase) (*10*), an enzyme located at or near the nuclear pore complex. Since NTPase controls the transport of mRNA through the nuclear pore complex and into the cytoplasm (*11*), this finding suggests that the regulation of NTPase may be one mechanism whereby insulin controls mRNA metabolism. Third, we showed that insulin decreases ^{32}P incorporation into nuclear envelope proteins, including those proteins at the nuclear pore complex which presumably contain the NTPase (*12*). This effect of insulin,