Case Study of a Population Bottleneck: Lions of the Ngorongoro Crater

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Abstract: *Lions in the Ngorongoro Crater, Tanzania, form a small and naturally isolated population. In 1962, the Crater lions suffered an epizootic that reduced the population to nine females and one male. An additional seven males apparently immigrated into the Crater in 1964–1965, but there has been no further immigration into the Crater in the past 25 years. By 1975, the population had recovered to its current level of 75–125 animals. All members of the current Crater population are descended from only 15 founders, and over the years there has been considerable variance in the reproductive success of both sexes. The Crater was probably colonized by lions from the nearby Serengeti ecosystem and the contemporary Crater lion population shows a significant lack of genetic diversity compared to the much larger Serengeti population. The detailed reproductive history of the Crater population was incorporated into a series of stochastic computer simulations that generated distributions of expected allele frequencies under different sets of initial conditions. The simulations suggest that the Crater population may have passed through previous bottlenecks before 1962 but that the level of heterozygosity in the breeding population has been declining since the mid-1970s, regardless of the population's genetic composition in the 1960s. High levels of*

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Resumen: *Los leones del Cráter del Ngorongoro, en Tanzania, forman una población pequeña y aislada naturalmente. En 1962, ésta población de leones sufrió de una epidemia que redujo la población hasta que quedaron nueve hembras y un macho. Aparentemente siete machos adicionales inmigraron al cráter en 1964–1965, pero no ha habido otras inmigraciónes en el cráter durante los últimos 25 años. Para 1975, la población se había recobrado hasta su actual nivel que oscila entre 75 y 125 animales. Todos los miembros de la actual población del cráter son descendientes de sólo 15 individuos fundadores y en el transcurso de los años ha habido considerable variación en el éxito reproductivo de ambos sexos. El cráter fue probablemente colonizado por los leones que habitan el cercano ecosistema de Serengeti y la población contemporánea de los leones del cráter muestra una significativa falta de diversidad genética en comparación con la población de Serengeti, que es mucho mayor. La detallada historia reproductiva de la población del cráter fue incorporada dentro de una serie de simulaciones al azar en computadora que generaron distribuciones de frecuencias esperadas de alelos bajo diferentes juegos de condiciones iniciales. Los simulacros sugieren que la población del cráter pudo haber pasado por previos "cuellos de botella" antes de 1962 pero que el nivel de heterozigosis en la población reproductiva ha venido declinando desde mediados de los setentas, sin considerar la composición genética de la población durante los sesentas. Los altos niveles de*

inbreeding are correlated with increased levels of sperm abnormality in lions and there is evidence that the reproductive performance of the Crater lions has decreased as a result of decreasing heterozygosity.

trecruzamiento consanguíneo están correlacionados con el incremento en los niveles de anormalidad en el esperma de los leones y hay evidencia de que la función reproductiva de los leones del cráter ha disminuído como resultado de la disminución de la heterozigosis.

Introduction

Although the increasing fragmentation of wildlife habitats has raised serious concern over the possibly deleterious effects of decreased genetic diversity within each "island" population (Ralls & Ballou 1986; Simberloff 1988), there has been little opportunity to measure this effect in natural populations of large vertebrates. Such species are so long-lived that there are few cases where the degree of genetic isolation can be verified for more than a generation (e.g., Bonnell & Selander 1974). We report here on one of the best-studied mammalian populations in the world, the lions of Ngorongoro Crater, Tanzania, and show how genetic surveys can be combined with long term genealogical data to estimate the extent and rates of genetic change within a small population. We provide data on the extent of genetic isolation of the Crater lion population, compare the level of genetic diversity in this population to that of the larger Serengeti population, test whether the loss of genetic diversity in the Crater lions could have resulted from a single bottleneck, and examine the relationship between heterozygosity and reproductive performance.

Geography of the Region

The Ngorongoro Crater is an extinct volcanic caldera located at the western edge of the Gregory Rift in northern Tanzania. The Crater Highlands are the source of the volcanic soil that formed the Serengeti plains immediately to the west (see Sinclair 1979). The Highlands also act as a barrier to the moisture in the prevailing winds off the Indian Ocean. Consequently, the Crater is flanked by dense forest to the north, east, and south but by a near desert to the west. The 250 km^2 Crater floor is thus a natural island of savanna habitat: the Crater floor is primarily open grassland, and the combination of rich soil and moderate rainfall sustains a remarkable abundance of nonmigratory plains herbivores. In contrast, the surrounding areas support far lower densities of the same species.

The eastern limit of the Serengeti ecosystem is located less than 20 km to the west of Ngorongoro. The Serengeti extends over a 25,000 km^2 area. Large herbivores dominate the entire region and the Serengeti probably contains the largest remaining populations of herbivores and carnivores in Africa. Although the migratory wildebeest, zebra, and gazelle are the most abundant species, there are also large populations of resident herbivores, notably giraffe, buffalo, topi, hartebeest, impala, and warthog.

In contrast, the Crater is clearly an ecological island that contains only a subset of the large mammalian species found in the Serengeti. Impala, topi, and giraffe have never been noted in the Crater and warthog have only been present for the past 20 years. Although present in the 1960s (Estes 1967), wild dog have been absent for nearly 20 years.

Lion Populations

Ngorongoro

THE POPULATION CRASH

Lions have inhabited the Crater floor at least since the end of the nineteenth century when the first Europeans settled in the area (Fosbrooke 1972). Lions were considered plentiful and hunting of lions was permitted on the Crater floor until the 1920s. No data are available on the size of the Crater lion population until after the Crater floor first became accessible to motorized vehicles in the 1950s. Over the period 1957–1961, the lion population was estimated to be about 60–75 individuals (Wright 1960; Fosbrooke 1963, 1972).

After exceptionally heavy rains in late 1961 and early 1962, an extraordinary outbreak of *Stomoxys calcitrans* biting flies decimated the lion population in April 1962 (Fosbrooke 1963, 1972). Development of *Stomoxys* larvae requires moist soil for 25 days and breeding conditions remained optimal for six consecutive months. Large swarms of adult *Stomoxys* became common throughout the Crater floor and the lions appeared to be a preferred host. The emaciated lions developed devastating skin infections and were unable to hunt their normal prey. By June 1962, the population had dropped to 10–15 animals.

DETERMINATION OF THE POPULATION'S RECOVERY

Lions can be individually recognized from close-up photographs of their vibrissae spots (Pennycuick & Rudnai 1970), and we have used photographic records to reconstruct the population's recovery from the epizootic. We assembled a large collection of photographs taken

by tourists and scientists between 1959 and 1968 and collated the identity files of several independent research teams who studied the lions from 1969 onward. Because the Crater lions have been a favorite tourist attraction since 1959, they are extremely tame and tourists are often able to photograph them at close range. We received several hundred of these photos after soliciting old photographs in an article written for an East African wildlife magazine (Packer & Pusey 1987). Many of these were sufficiently clear to provide unambiguous identifications. In addition, we received comprehensive sets of excellent photographs taken by Richard D. Estes in 1963–1965, Henry Fosbrooke in 1963–1968, Joan Root in 1968, and Pierre DesMeules in 1969.

We received insufficient photographs to assess the composition of the *entire* Crater population prior to the epizootic. However, it was clearly larger in 1959–1961 than in 1963–1965, confirming that the Crater population had crashed in 1962. Several animals first photographed by tourists in 1959 were extensively photographed by Estes and Fosbrooke in 1963. These individuals could therefore be verified as survivors of the plague. Over the following years, the survivors were photographed with successive sets of cubs.

In 1969, DesMeules began a systematic identification file of all the Crater lions. DesMeules passed these records to John Elliott, who studied the Crater lions from 1970–1972 (Elliott & Cowan 1978). Elliott was followed by Jeannette Hanby and David Bygott in 1975–1978 (Hanby & Bygott 1987) and by Packer and Pusey from 1978 to the present. Bygott and Hanby's background data had formed the basis of our own studies (Pusey & Packer 1987; Packer et al. 1988). In 1989–1990, we linked the photographs from 1963–1968 to DesMeules and Elliott's identification files, and these in turn to Bygott and Hanby's records. We thereby completed the extension of our long term records back to January 1963.

These data reveal the composition of each pride and the approximate birth date of each individual born after the plague (age can be estimated from size, degree of speckling of the nose, dentition, and coat condition). Although we can deduce the natal pride of every lion born in the Crater since 1963, more precise records of maternal kinship date from 1969 to 1972 and from 1975 to 1989. During periods of regular behavioral observations, maternity can be deduced from the spatial associations and nursing behavior of females and cubs. DNA fingerprinting of the Serengeti lions reveals that such behavioral estimates were correct for 77 of 78 cubs (Gilbert et al., in press). In the seventy-eighth case the real mother was the putative mother's pride mate.

We can also specify candidate fathers for each cub. DNA fingerprinting in the Serengeti confirms that members of the resident coalition father all cubs born during their tenure in the pride (Gilbert et al., in press), and we have been able to deduce the paternal coalition for all surviving cubs born in the Crater after the plague.

POPULATION SIZE AND COMPOSITION: 1961–1989

Figure 1 shows the total population size in the Crater each year. Following the *Stomoxys* plague, the total population dropped to about 10 individuals but then grew rapidly and reached current levels by the mid-1970s. Figure 2 shows a "family tree" for all of the breeding lions in the Crater from 1962 until 1989. The Crater floor was largely repopulated by descendents of a group of four females that were themselves born in the Crater in 1957. Each successive cohort of cubs belonging to these four females subsequently became established breeders. In 1963 they had a cohort of three daughters and one son; in 1965 a cohort of four daughters and six sons; and in 1967 a cohort of nine sons. From 1970 until 1975, the cohort of nine sons bred with their three "half-sisters" from the 1963 cohort, with their four "sisters" from the 1965 cohort, as well as with various "nieces." Four of the five breeding prides currently on the Crater floor are directly descended from these four females; the fifth is descended from a second group of three females. A third surviving pride went extinct. Note that no new breeding prides have been established since 1973.

There have only been seven breeding males that could have entered the Crater from elsewhere during the past 27 years (Fig. 2). The first resident male in the Crater had been seen in the Crater floor prior to the

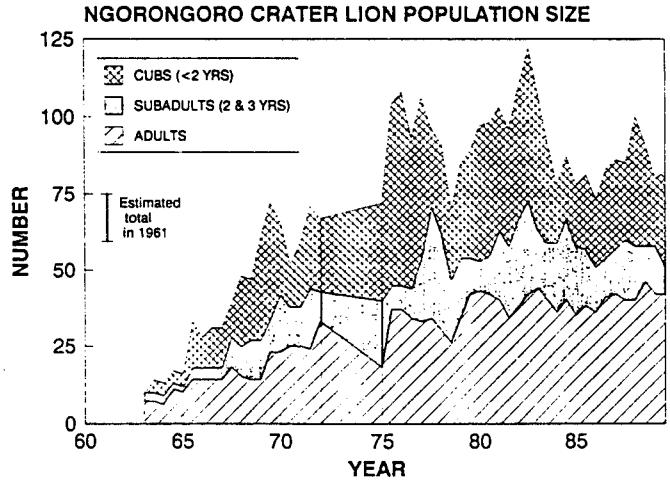

Figure 1. Population size and composition of the Ngorongoro Crater population. Data for 1961 are based on Fosbrooke's (1963) estimate. Subsequent data give the population size on January 1 and July 1 each year. For each date, the number of individuals in each age class is illustrated by the height of the respective hatched areas. Data for 1973 and 1974 are interpolated.

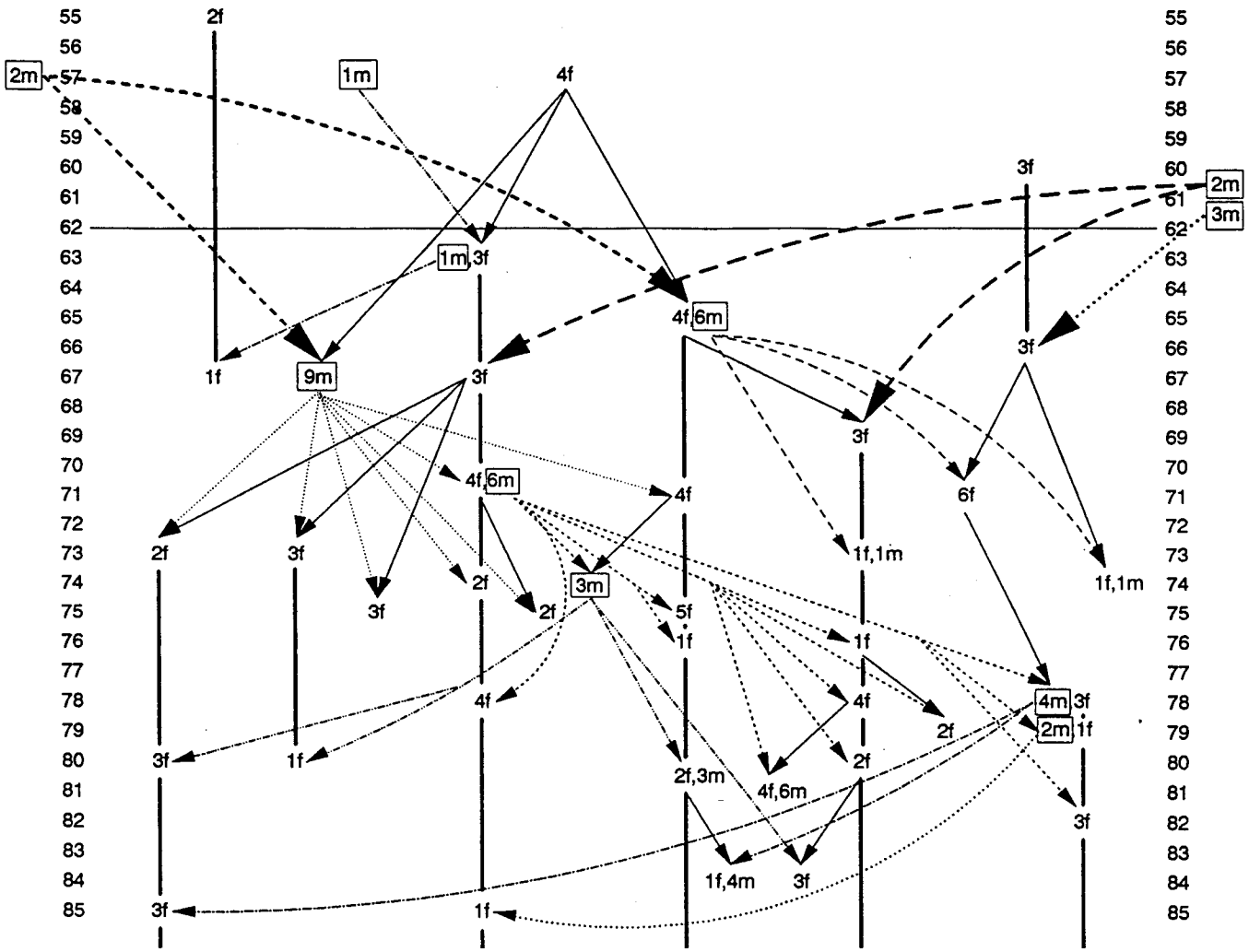

Figure 2. Family tree for the Ngorongoro Crater lion population after the 1962 epizootic. The tree includes only those females that survived until the age of four and males that became resident in a Crater pride. Numbers and letters indicate the number of females (f) and males (m) born in the same cohort during a particular year. The horizontal line in 1962 indicates the Stomoxys *plague; animals listed above this line are those that repopulated the Crater after the plague. Heavy vertical solid lines indicate female lineages where daughters were recruited into their mothers' prides. Lineages that end before 1985 have gone extinct. Diagonal solid arrows indicate cohorts of daughters that left their mothers' prides to start a new pride on the Crater floor. Boxes designate male coalitions that fathered offspring that survived to breeding age. Coalitions believed to have entered the Crater from elsewhere are listed to the left or right of the years of their birth; their progeny are indicated by heavy dotted arrows. The progeny of coalitions known to have been born in the Crater are indicated by narrow dotted arrows.*

plague, so he was also presumably born there. The next three sets of resident males in the Crater were probably immigrants: they were not seen prior to the plague (although our records from this period are sparse) and also were of an age that suggests they had not been exposed to the plague. These all bred during the period shortly after the epizootic when there were relatively few males born on the Crater floor. However, after 1969 all breeding males have been born in the Crater. In 1970 the first two large cohorts of Crater-born males reached maturity and since that time successive sets of large Crater-born coalitions have been effective in preventing any immigration of males from outside the Crater. Small nomadic coalitions have occasionally entered the Crater over the past 15 years, but these have not been able to establish breeding status in the face of competition from the larger native coalitions. Prey availability in the Crater floor is the highest in Africa (van Orsdol et al. 1985) and cub survival in Ngorongoro is therefore far higher than elsewhere (Packer et al. 1988). Thus cohorts of young Crater males are often very large.

In recent years, two Crater-born coalitions have fa-

thered a disproportionate number of offspring: most members of the current population are descended from the group of nine males born in 1967 and the group of six born in 1970 (Fig. 2). Paternity data from the Serengeti reveal that only two males per coalition father almost all of the offspring in their pride regardless of the size of the coalition (Packer et al., in press). Thus, even though the two large Crater coalitions totaled 15 males, it is possible that a total of four males fathered most of the offspring sired during their respective tenures.

In summary, the current Crater population is descended from a founding population of seven females and eight males with no further immigration after 1969. There has been extensive exchange of males between prides of recent common ancestry and there has also been considerable variance in reproductive success of both males and females.

Serengeti

The Serengeti has long been famous for its large population of lions (Schaller 1972). Lions appear to have been plentiful in the Serengeti since the nineteenth century, despite periodic rinderpest epizootics between 1890–1962 that greatly reduced the Serengeti ungulate populations and even though hunting of lions was permitted in the entire Serengeti region until 1937. Schaller estimated the Serengeti population to total about 2000 in the late 1960s, and we currently estimate the population to be about 3000 (Hanby & Bygott 1979; Pusey & Packer 1987).

Long term records exist for a 2000 km^2 area in the southeastern part of the Serengeti National Park (Schaller 1972; Bertram 1975; Hanby & Bygott 1979; Packer et al. 1988). In 1966, 8 prides occupied this area. Currently there are 17 prides and an additional seven solitary females resident in the same area (Schaller did not tally solitaries). At least 12 of these 17 prides are descended from Schaller's 8 prides, but two have entered the study area from adjacent areas. The population increased by nearly 50% in the 1970s in response to two factors: (1) the rapid expansion of the herbivore populations following the eradication of rinderpest in 1963, and (2) favorable rainfall patterns that resulted in more continuous availability of migratory herbivores in the southeastern part of the Serengeti for five consecutive years in the 1970s (Hanby & Bygott 1979; Packer et al. 1988). For each of the past 14 years this area has contained about 200 resident lions.

There is considerable migration of male lions into and out of this area (Schaller 1972; Pusey & Packer 1987). Of the 118 resident males born in 1973 or later (when the natal origins of lions in this entire area could be deduced), 81 (69%) entered the study area from elsewhere. Genetic fingerprinting in this population confirms that most mating partners are unrelated to each other (Packer et al., in press). Thus the Serengeti lions effectively comprise a single large, panmictic population.

Genetics of the Two Lion Populations

In an earlier study, O'Brien et al. (1987) found that 17 Crater lions showed a diminished level of allozyme heterozygosity compared to 26 Serengeti lions. We report here on data that include additional lions from both the Crater and Serengeti. To provide representative samples from the two populations, we restrict our analysis to a subset of animals from each population that excludes all parents, offspring, or siblings of any individual in the sample. Table 1 shows the allele frequencies for each polymorphic locus in this new sample. Four polymorphic loci were detected in the Crater lions and seven in the Serengeti. Consistent with the earlier study, the Crater individuals show significantly lower levels of heterozygosity than those in the Serengeti (.88 heterozygous enzyme loci per individual in Ngorongoro versus 1.47 per individual in the Serengeti, $z = 2.309$, $P = .017$, two-tailed, Mann-Whitney). The average heterozygosity (h) of the Crater lions is 2.2% compared to 3.3% in the Serengeti.

Recently, Yuhki and O'Brien (1990) found that the Ngorongoro lions show a striking loss in restriction fragment length variation in the class I genes of the major histocompatibility complex (MHC). The MHC is one of the most polymorphic gene complexes in mammals (Klein 1986) and the Crater lions retain only one-third

Table 1. Polymorphic allele frequencies.

	Enzyme Locus[a]						
Population	*ADA*	*IDH1*	*GOT*	*GPT*	*MDH2*	*MPI*	*TF*
Serengeti (n = 79)	a = .79 b = .19 c = .02	a = .74 b = .26	a = .99 b = .01	a = .85 b = .13 c = .03	a = .99 b = .01	a = .99 b = .01	a = .49 b = .51
Ngorongoro (n = 17)	a = .85 b = .15	a = .94 b = .06	a = 1.0	a = .91 b = .03 c = .06	a = 1.0	a = 1.0	a = .56 b = .44

[a] *Allozyme variation was detected using starch or acrylamide electrophoresis as described in O'Brien et al. (1987).*

the level of MHC polymorphism observed in the Serengeti lions. A loss in DNA variation in the MHC in the Crater correlates with the allozyme data and is consistent with a history of restricted population size. However, the drop in MHC polymorphism could also have resulted from selection during the epizootic in the Crater: the MHC system is considered to play a critical role in the development of immune defenses (Zinkernagel et al. 1985; Klein 1986; O'Brien & Evermann 1988).

Relationships of Adjacent Populations

To evaluate the reduced genetic diversity of the Crater population, it is necessary to determine its affinities to neighboring populations. The comparisons presented above contrasted the Crater lions with the Serengeti lions, but did the Crater population originate in the Serengeti? Besides the Serengeti, the only other population adjacent to the Crater inhabits the floor of the Gregory rift. This population extends to Lake Manyara National Park, 30 km to the southeast of the Crater.

The habitat between Ngorongoro and Manyara consists of dense forest and land that has been cleared for agriculture, and the rift escarpment acts as a barrier between subspecies of wildebeest. Significant exchange of lions between these two populations is therefore unlikely. In contrast, the habitat between the Serengeti and Ngorongoro Crater contains an abundance of prey each rainy season and the Ngorongoro and Serengeti wildebeest populations are known to mingle (Kruuk 1972). Two sets of male lions have been observed to move between the Serengeti and Ngorongoro. One of these was a pair of Crater males that became resident in the Serengeti (Pusey & Packer 1987) and regularly returned to the Crater when prey was scarce. The second was a nomadic male in the Crater that was first seen in the Serengeti.

Morphologically, the Crater lions appear more similar to the Serengeti lions: males in both populations have fuller manes than the Manyara males and both sexes have shorter faces. The morphological characteristics of the Manyara lions are more similar to lions from the east of the Rift valley.

Although our sample size from Manyara is very small, genetic data also suggest a greater affinity between the Crater and Serengeti populations. First, the allele frequencies for two blood enzyme loci in Manyara are very different from either the Crater or Serengeti. All five Manyara lions were homozygous for the TF b allele and the four animals that could be tested for ADA carried the b allele at a frequency of .625 (cf. Table 1). Note however that all five Manyara animals are from a single pride.

Second, DNA fingerprinting analysis indicates a greater genetic distance between the Crater and Manyara. Within each population, there is a strong positive relationship between the degree of kinship of any two individuals and their extent of variable number of tandem repeat (VNTR) "band-sharing" (Gilbert et al., in press). Band-sharing between any two individuals is $2S_{ab}/(F_a + F_b) \times 100\%$, where S_{ab} is the total number of DNA fragments showing similar molecular weight and intensity carried by both individuals, F_a is the number of fragments resolved in individual "a," and F_b is the number of fragments resolved in individual "b." On average, any two members of the Serengeti population that are unrelated to each other share 49% of their VNTR bands and the most distantly related members of the Ngorongoro population share 47% (Gilbert et al., in press).

Six lions from four different prides in Ngorongoro Crater were compared with five animals from five prides in the Serengeti and two animals from one Manyara pride. VNTR band-sharing was higher between prides within each population than between populations, and the mean degree of band-sharing between each Crater lion and the Serengeti lions (37%, S.D. = 8%) was significantly higher than between the Crater and Manyara lions (27% ± 5%; $P = .032$, $n = 6$, sign test).

Although the genetic, morphological, and demographic data are clearly limited, they all consistently indicate that the Crater population is more closely allied to the Serengeti lions than to the Manyara lions. We therefore suggest that the original Crater population was founded by Serengeti lions, and that immigrants entering the Crater after the *Stomoxys* plague were also from the Serengeti.

Modelling the Loss of Genetic Diversity in Ngorongoro: Is One Bottleneck Enough?

The genealogical reconstruction of the Ngorongoro population (Fig. 2) permits computer simulations of the changes in allele frequencies that have occurred since the population crash in 1962. By generating an expected pattern of genetic drift that would result from this pedigree and comparing the output with our data on actual gene frequencies in the contemporary population (Table 1), we can estimate the genetic composition of the founding population in the Crater. We use these simulations to test three alternative models about the founders and to estimate changes in levels of heterozygosity through time.

For each of the 15 founding animals, the genotypes at each polymorphic allozyme locus are randomly assigned according to three alternative hypotheses (see below). The computer then simulates the subsequent reproductive history of the population according to the observed pedigree. Each allele is assumed to be selectively neutral and loci are assumed to assort indepen-

dently. Where maternity is not known with certainty, maternity is assigned randomly to one of the potential mothers. For each litter, paternity is awarded with equal probability to one of two males resident in the pride at conception and litter mates are full siblings (Packer et al., in press). Finally, the simulated genetic composition of the 17 descendant lions sampled in 1984–1987 is recorded. After 1000 runs for each model, the distributions of simulated gene frequencies are compared to the empirical data in Table 1.

Each simulation results in a unique genealogy, but over a series of runs most of the variance in final gene frequencies results from random variation in initial gene frequencies. Therefore, the uncertainties in our genealogical data do not greatly affect the predicted gene frequencies. For a more complete description of stochastic simulations that utilize incomplete pedigrees see Starfield (in press).

We consider three alternative models of the genetic composition of the 15 founders of the post-*Stomoxys* population.

1. *Unrelated founders.* The founders were all unrelated to one another and hence constituted a random sample of the Serengeti population. Each of these 15 individuals was randomly assigned each allele with a probability identical to the allele frequency observed in the Serengeti population (Table 1).
2. *Founders siblings.* The founders were members of six kin groups from the Serengeti that were unrelated to the other kin groups. Each founding group was composed entirely of full siblings, and the 12 parents of these six sets of siblings were a random sample of Serengeti lions. The parents were randomly assigned each allele as in (1).
3. *Survivors homozygous.* The seven male immigrants were members of three kin groups from the Serengeti as in (2), but all eight of the survivors of the plague were homozygous for the allele at each locus that is most common in the Serengeti. Thus the eight survivors are assumed to descend from a population that had undergone previous bottlenecks.

Models (1) and (3) represent upper and lower bounds on the initial genetic diversity of the recovering Crater population. Model 1 represents the maximum genetic diversity available to the founding population, whereas Model 3 assumes that all diversity in the ancestral Crater population had been lost by previous episodes of genetic drift. The most plausible outcome of such extreme drift would be the retention of the most common Serengeti allele at each locus.

Model 2 is based on a more realistic pattern of kinship than Model 1. Demographic and genetic data show that female pride mates are typically close relatives, as are most male coalition partners (Packer et al., in press). Model 2 differs from Model 1 only in that it starts with 12 founders rather than 15 and extends the pedigree back an additional generation. Consequently, Model 2 generates slightly greater variance in the distribution of expected allele frequencies.

We tested these hypotheses by examining the expected frequencies of each allele at each locus. Of the seven loci that are polymorphic in the Serengeti, only four (ADA, GPT, IDH1, and TF) contained alleles that are sufficiently common to be informative in these tests. Note that because the observed allele frequency in TF in the Serengeti is so close to .5, it is equally likely that a high degree of genetic drift could fix either allele. For Model 3, we therefore ran two alternative simulations of the TF locus. In the first the survivors of the *Stomoxys* plague were all homozygous for the a allele and in the second they were homozygous for the b allele.

Outcomes from a hypothetical set of runs are presented in Figure 3. In the example the "observed" data show an allele frequency of .325 and there are four different frequency distributions generated by simulation. The means of two distributions are exactly the same as the observed, whereas the means of the other two distributions are much higher; two distributions show a much higher variance than the other two. In Figure 3b, the distributions are plotted as cumulative probabilities to allow a simultaneous comparison of their means and variances. The vertical line shows the observed allele frequency for the hypothetical locus. If a particular distribution mostly consists of allele frequencies that are close to the observed data, then the cumulative curve quickly climbs from 0–1 at the vertical line and intersects it at .5. A poor fit consists of a cumulative plot that crosses the vertical line very close to 0 or 1; a greater variance results in a shallower slope at the vertical line.

Outcomes of the simulations for each of the four loci are presented in Figure 4. The observed allele frequencies are taken from Table 1 and the frequency distributions generated by the three models are plotted as cumulative probabilities. Of the four loci, the observed frequencies of the respective alleles in the ADA, GPT, and TF loci were most frequently mimicked by simulations based on an initial population of sibling groups (Model 2). However, the observed frequency of the IDH1 a allele is much closer to fixation, and this extreme skew was most often produced by the model where the survivors were homozygous (Model 3). A population founded entirely by Serengeti animals (Models 1 or 2) would suffer a lower risk of fixation of the most common Serengeti allele than would a founding population containing eight homozygotes for that allele.

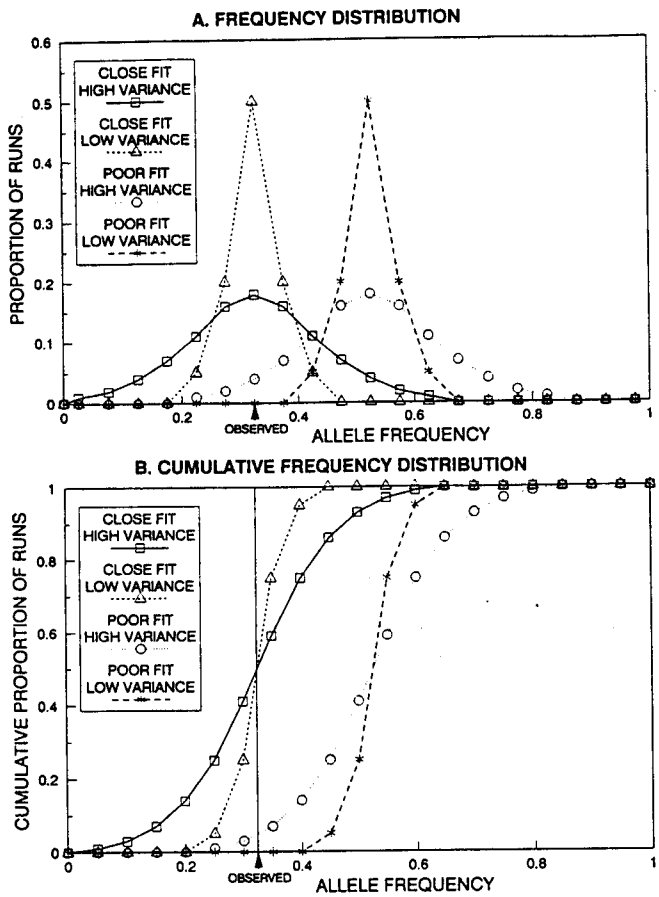

Figure 3. Hypothetical distributions of allele frequencies generated by repeated simulations. A. Frequency distribution. Four different distributions are presented: two with a mean that is the same as found in the "observed" data and two with a higher mean; two distributions have a high variance and two have a low variance. B. Cumulative frequency distribution. The vertical line shows the "observed" allele frequency. Note that the two cumulative distributions with the same mean as the observed cross the line at .5 and that the distribution with the lower variance has a steeper slope at the intersection.

Unfortunately, the observed allele frequencies of all four loci were generated by more than 5% of runs in all three models, and we cannot reject with confidence any of the alternative hypotheses concerning the initial genetic composition of the Crater population.

Nevertheless, for all four loci the observed frequencies are intermediate between those most often predicted by a model of sibling founders (model 2) and those predicted by a model of homozygous survivors (model 3). (Although Models 1 and 2 generated very similar distributions, Model 2 consistently gave a better fit to the observed data than Model 1.) It is therefore plausible that the surviving population already lacked some genetic diversity due to previous bottlenecks, but that this relict population was not completely homozygous.

Expected Changes in Heterozygosity After 1962

Figure 5 shows the expected average heterozygosity for all of the breeding adults in the Crater population alive each year after the *Stomoxys* plague, based on the assumptions in Models (2) and (3). If the Crater population was founded entirely by sibling groups from the Serengeti (Model 2), then average heterozygosity would have remained constant until the first relatively inbred Crater-born progeny reached maturity in the mid-1970s. Heterozygosity would have declined by about 10% between the early 1970s and the late 1980s (a very similar pattern is predicted by Model 1). On the other hand if the survivors of the plague were completely homozygous (Model 3), then the levels of heterozygosity would have initially increased dramatically as the result of male immigration from the Serengeti, but subsequently declined by about 10% due to inbreeding. Note that the average level of heterozygosity in the breeding population would have declined by about 10% since the early 1970s regardless of the initial genetic composition of the population.

If the Crater had been through previous severe bottlenecks, then the *Stomoxys* plague may have had the rather surprising effect of increasing levels of heterozygosity in the Crater lion population: by removing most of the breeding males from the previous population, the plague created conditions whereby new males could enter the population from elsewhere. Once the surviving females had bred successfully, the large coalitions of Crater-born males prevented any further immigration, and heterozygosity in the Crater population would have subsequently declined due to inbreeding.

Relationship Between Heterozygosity and Reproductive Performance

The Crater population shows considerably lower levels of heterozygosity than the Serengeti. However, comparisons of fertility and survival between the two populations are complicated by the very different patterns of prey availability in the two areas: the Crater lions have access to a consistently high biomass of prey, whereas the Serengeti lions are regularly subjected to serious food deprivation due to the extensive seasonal migration of their preferred prey (Schaller 1972; Kruuk 1972). Therefore cub mortality is considerably lower in the Crater than in the Serengeti (Packer et al. 1988).

Nevertheless, two lines of evidence suggest that lowered levels of heterozygosity impair reproductive performance in the Crater lions. First, males in Ngorongoro

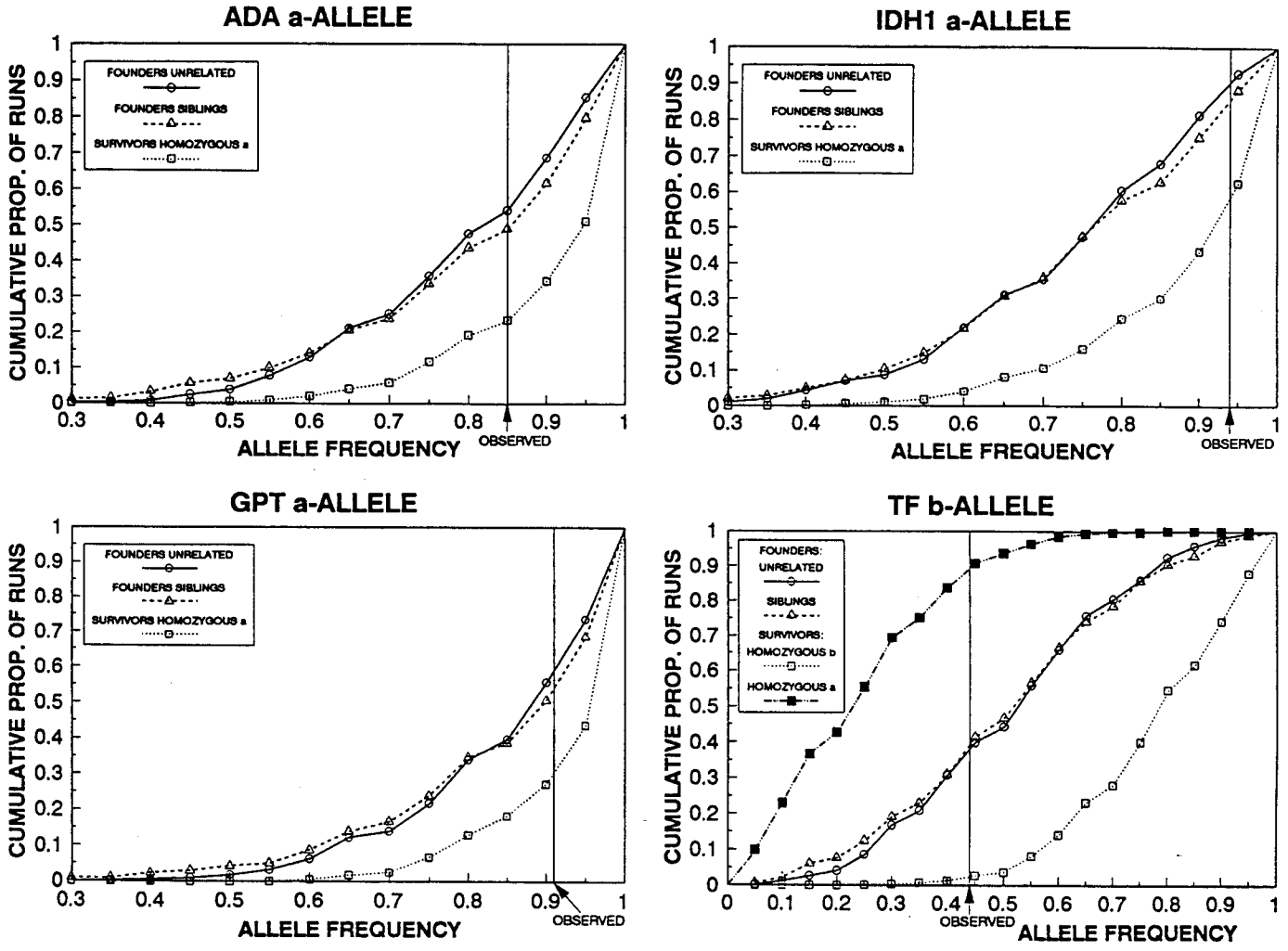

Figure 4. Predicted allele frequencies of four polymorphic enzymes. Outcomes of the simulations are plotted as cumulative probabilities and the vertical line in each graph shows the observed allele frequency for each locus (see text).

have a significantly higher proportion of abnormal sperm than Serengeti males and the highly inbred Asiatic lions of the Sakkarbaug Zoo have even higher levels of sperm abnormality (Wildt et al. 1987; Brown et al., in press). In other felids, studies of in vitro fertilization show that individuals with high levels of sperm abnormality penetrate domestic cat eggs at significantly lower levels (Howard et al, 1991).

Second, the estimates of average heterozygosity shown in Figure 5 are closely correlated with annual reproductive rates *within* the Crater population. We consider the estimates in Figure 4 to reflect changes in overall levels of genomic heterozygosity and we make no claims for heterozygote advantage at any particular locus.

Our measure of annual reproductive rate is based on the per capita production of yearlings. This is found by calculating the number of yearlings produced per adult female per year over a two-year period. The data are divided into two-year blocks because this is the interbirth interval for females with surviving cubs (Pusey & Packer 1987). We focus on yearlings because most cub mortality occurs in the first 12 months (Bertram 1975; Packer et al. 1988), and although our demographic data are inadequate to record all births, they are sufficiently accurate to detect all cubs that survive to one year of age (see Packer et al. 1988). Note that this measure of productivity is a composite of two factors: adult fecundity and cub survival until the age of 12 months; our data are therefore inadequate to measure which component is responsible for changes in the productivity of the Crater population. Complete data are available for yearling production except during the hiatus between studies in 1973–1974 and for two prides during 1963–1966. Data from 1963–1966 are therefore based on a single pride.

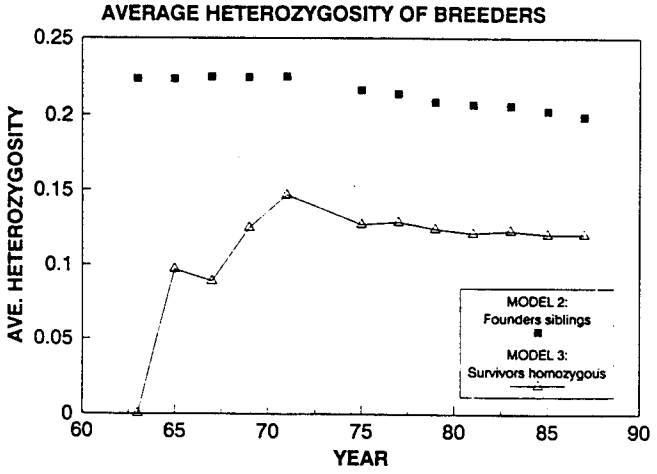

Figure 5. Estimated annual average heterozygosity of the breeding animals in the Crater population. In each simulation, the average heterozygosity was calculated each year for all living animals that were ≥4 years of age during that year. Data are averaged over two-year intervals to facilitate comparisons with Figure 6.

The annual per capita production of yearlings ("productivity") has varied dramatically over the past 25 years (Fig. 6). Our previous population analyses (which only included 10 years data) suggested that three factors contributed to the annual production of surviving cubs in the Crater (Packer et al. 1988): (1) the age of each female, (2) the proportion of females that experience male takeovers, and (3) the number of adult females resident on the Crater floor.

The reproductive rates of females are highly dependent on their age: reproductive rates are at a peak at

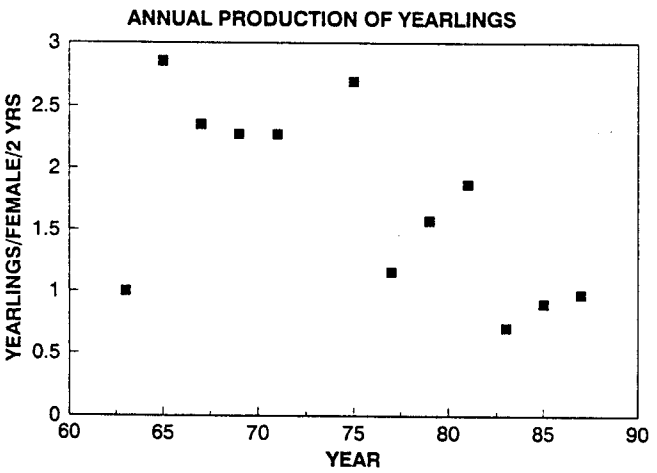

Figure 6. Annual production of yearlings in the Crater. Each point represents a two-year period. No data are available from 1973–1974.

4–12 years of age (Packer et al. 1988). An increase in the frequency of male takeovers results in a decrease in cub survival. When an incoming coalition of males first enters a new pride, they kill all of the unweaned cubs and evict all of the older cubs (Bertram 1975; Packer & Pusey 1983; Pusey & Packer 1987). Female density was only weakly correlated with productivity of surviving cubs in the Crater, but similar trends were found in two Serengeti habitats, suggesting that reproductive rates might decrease with increasing density in this species. If a density-dependent effect truly operates in the Crater, it could only result from increasing levels of intraspecific aggression rather than from food limitation. Prey availability does not appear to affect reproduction in the Crater lion population: herbivore numbers have been relatively constant in Ngorongoro over the past 20 years, prey are abundant throughout the year, and we have never seen starving lion cubs in the Crater (Van Orsdol et al. 1985; Packer et al. 1988).

Multiple regression was used to determine the relative influence of three factors on annual yearling production: (1) female density, (2) proportion of females that experience male takeovers, and (3) heterozygosity. We controlled for female age by considering only the productivity of females 4–12 years in age. We based our estimates of annual average heterozygosity on the assumption of sibling founders (Model 2) because it gave the best overall fit to the observed isozyme data (note: the following results are essentially unchanged if annual heterozygosity is based on unrelated founders, Model 1). The set of independent variables that best predicted the annual production of yearlings included only two factors: exposure to takeovers and estimated heterozygosity (df = 11, adjusted r^2 = .621, F = 10.023, P = .0051; takeovers: t = 2.633, P = .0272; heterozygosity: t = 3.012, P = .0147). These effects are illustrated in Figure 7.

Although female density does not appear to have a significant effect on yearling production in this analysis, female density is negatively correlated with estimated heterozygosity (r^2 = .801, P = .0001): compare the increasing number of adults in the Crater shown in Figure 1 with the gradual decline in heterozygosity in Figure 5. The high degree of colinearity between these variables suggests that the results from a multiple regression analysis should be interpreted with caution.

Model 2 does not allow for any effects from previous bottlenecks. If the survivors were less heterozygous than assumed by Model 2 and instead showed the same low level of heterozygosity as the current Crater population, the significant relationship between heterozygosity and productivity would be even higher (t = 4.472, P = .015), whereas there would be no colinearity between estimated heterozygosity and density (r^2 = .002). We therefore suggest that annual reproductive

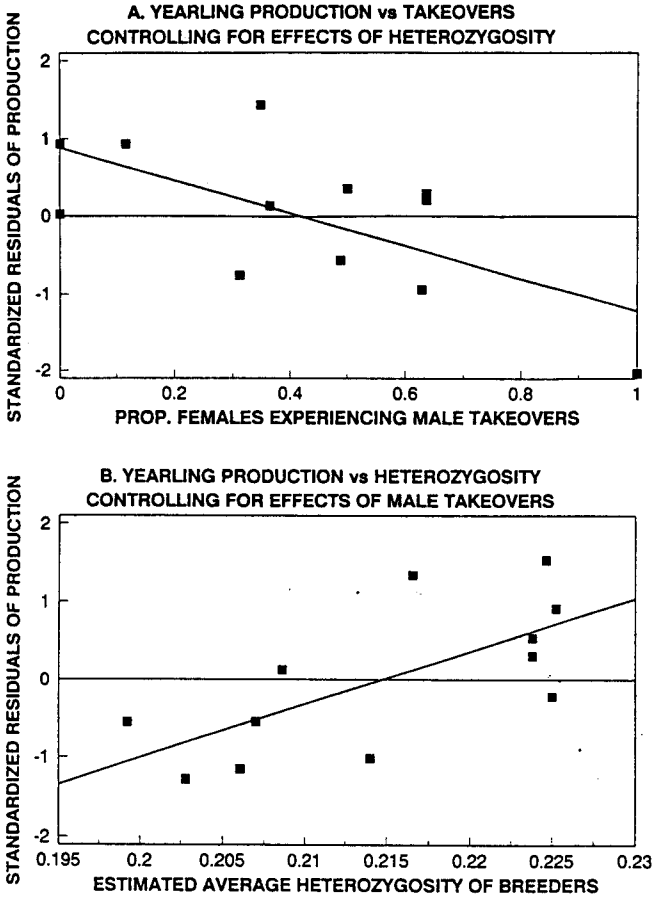

Figure 7. Factors with a significant effect on the annual production of yearlings in Ngorongoro Crater. A. Relationship between standardized residuals of yearling production and proportion of 4- to 12-year-old females exposed to male takeovers during each two-year interval, after removing the effects of heterozygosity. B. Relationship between production and heterozygosity, after removing the effects of male takeovers.

rates in the Crater population may indeed be affected by levels of heterozygosity.

Simulation methods permit estimates of the precise changes in the genetic composition of any small population for which there are data on the genetics of the contemporary population and an extensive history of the breeding and dispersal patterns of individual animals. With such estimates we can at least begin to address problems that may occur in many other small populations. Productivity of the Crater population has declined continuously since the mid-1970s (Fig. 6), and our simulations predict that average levels of heterozygosity in this population will continue to decline in the future in the absence of any male immigration. If productivity does depend on levels of heterozygosity, the Crater lion population may suffer a further reduction in reproductive rates. Similar problems may arise in other isolated populations of large vertebrates. We will continue to monitor the Ngorongoro Crater lion population for as long as possible.

Conclusions

We have been able to document the cause, extent, and consequences of a genetic bottleneck in a lion population that is naturally isolated. Male lions in the Crater suffer high levels of sperm abnormality, possibly as a result of reduced heterozygosity. Reproductive performance within this population has diminished over the years and this may be correlated with declining levels of heterozygosity. Both of these parameters may continue to decline in the future. Many other populations of large carnivores have become artificially subdivided in recent years and it is extremely important to contrast their performance with that of larger panmictic populations. With modern genetic techniques and computer simulations, it should be possible to undertake analyses similar to those presented in this paper. A principal advantage of simulations is that the models can be easily modified to conform to specific details in mating system and demography that are unique to each population.

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