Endangered Greater One-horned Rhinoceros Carry High Levels of Genetic Variation

ERIC DINERSTEIN*

Smithsonian/Nepal Terai Ecology Project
Conservation and Research Center
National Zoological Park
Front Royal, VA 22630, U.S.A.

GARY F. MCCRACKEN

Department of Zoology
Graduate Programs in Ecology and Ethology
University of Tennessee
Knoxville, TN 37996, U.S.A.

Abstract: *The population of* Rhinoceros unicornis *in the Chitwan Valley, Nepal, was reduced to an estimated effective population size (N_e) of 21–28 individuals (60–80 total animals) in 1962. Protein electrophoresis shows that heterozygosity remains very high in this population (H_o = 9.9%) despite its near extinction. We attribute this high heterozygosity to large N_e's prior to the population bottleneck, the recent occurrence of the bottleneck, and long generation time. These results illustrate the importance of considering historical demography and life history parameters when evaluating the possible genetic effects of bottlenecks in wild populations. They also offer support to recent arguments that the erosion of genetic diversity attributed to bottlenecks may be overemphasized.*

Resumen: *La población de* Rhinoceros unicornis *en el Valle Chitwan en Nepal, fue reducida a un tamaño de población efectiva estimada (Ne) en 21–28 individuos (60–80 total de animales) en 1962. La electroforésis de proteínas muestra que la heterosidad permanece muy alta en esta población (H_o = 9.9%) a pesar de estar a punto de extinguirse. Atribuimos esta heterosidad al gran Ne's anterior al cuello de botella en la población, a la reciente ocurrencia del cuello de botella y al largo tiempo generacional. Estos resultados ilustran la importancia de considerar el historial demográfico y los parámetros biológicos cuando se estén evaluando los posibles efectos de cuello de botella en las poblaciones silvestres. Los resultados también apoyan las recientes discusiones de que la erosión de la diversidad genética, debido a los cuellos de botella, puede estar siendo sobreenfatizada.*

Introduction

The genetic consequences of near extinction are a major concern in conservation (Franklin 1980; Frankel & Soulé 1981; Schonewald-Cox et al. 1983; Allendorf & Leary 1986; Allendorf 1986; Lande & Barrowclough 1987). The concern arises because severe reductions in population size (bottlenecks) result in losses of heterozygosity, allelic diversity, and polymorphism. This may lower the fitness of individuals and jeopardize the long-term survival and evolutionary potential of their populations (Franklin 1980; Allendorf 1986). The lack of genetic diversity in populations of numerous rare and

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** Present address: World Wildlife Fund, 1250 24th St. NW, Washington, D.C. 20037.*

endangered species has been attributed to bottlenecks (Bonnell & Selander 1974; Pemberton & Smith 1985; O'Brien et al. 1987; O'Brien et al. 1985; O'Brien & Evermann 1988), and because of the attention given to bottleneck effects the preservation of genetic diversity has been a focus in designing recovery plans for some species (Lande 1988).

However, the conclusion that a population carries low diversity because it has experienced one or more bottlenecks is inferential. Genetic analysis of all species cited in the above references was conducted only after their populations had been reduced; therefore, cause and effect between low diversity and small population size has not been demonstrated. We can estimate present levels of variability, but low diversity at present does not indicate how much diversity a population had in the past.

Although the consequences of losing genetic diversity are serious for normally variable populations, several authors have questioned whether the genetic effects of bottlenecks are being overemphasized in conservation literature. Bottlenecks must be very small and repeated or sustained over several generations for major erosion of heterozygosity (Nei et al. 1975; Chakraborty et al. 1980; Allendorf 1986; Lande & Barrowclough 1987). Moreover, the probability of extinction is high when population size is very small (Goodman 1987; Lande 1988; Pimm et al. 1989). Thus, the loss of genetic diversity due to bottlenecks may be less of a problem than current literature suggests because most small populations probably will go extinct before losing a substantial portion of their existing variability (Lande 1988; Pimm et al. 1989).

It is improbable that pre- and post-bottleneck genetic surveys will be conducted for many wild populations, and the relationship between current genetic diversity and past demographic events will remain inferential in most cases. However, we can evaluate the strength of this inference by considering demographic features of populations (e.g., mating systems, dispersion and dispersal patterns) that affect levels of variation and by obtaining the best data available on historic population sizes. If we can estimate current and past effective population sizes (N_e), we can calculate expected erosion rates of pre-bottleneck genetic diversity (Lande & Barrowclough 1987) to assess the plausibility of the bottleneck scenario for explaining the levels of diversity observed in current populations. Even approximate estimates of N_e will allow calculations that could be important to the inferential arguments on which this controversy centers.

Here we use this approach to evaluate data on the genetic diversity observed in a wild population of the greater one-horned rhinoceros, *Rhinoceros unicornis*. *R. unicornis* is one of the world's most endangered large mammals and persists today in only two populations exceeding 80 individuals (Laurie 1978; Dinerstein & Wemmer 1988; Dinerstein & Price, in press; Fig. 1). Both of these populations approached extinction within this century, but have recovered substantially in recent years. The population in what is now Royal Chitwan National Park, Nepal, was reduced to an estimated effective size of 21–28 (60–80 total individuals) in the early 1960s but recovered to almost 400 animals by 1988 (Dinerstein & Price, in press). The population in Kaziranga National Park, Assam, India, was reduced to less than 100 individuals in ca. 1912 (Laurie 1978), but now has an estimated 1,500 animals (E. Martin, personal communication).

Methods

In 1986–87, we obtained blood and dermal tissue samples from 23 Chitwan *R. unicornis* (about 6% of the current total population) immobilized as part of a larger field study and translocation program (Dinerstein et al., 1990; Dinerstein & Price, in press). Plasma and red blood cells were separated and all samples frozen in liquid nitrogen. After their arrival in the laboratory, sam-

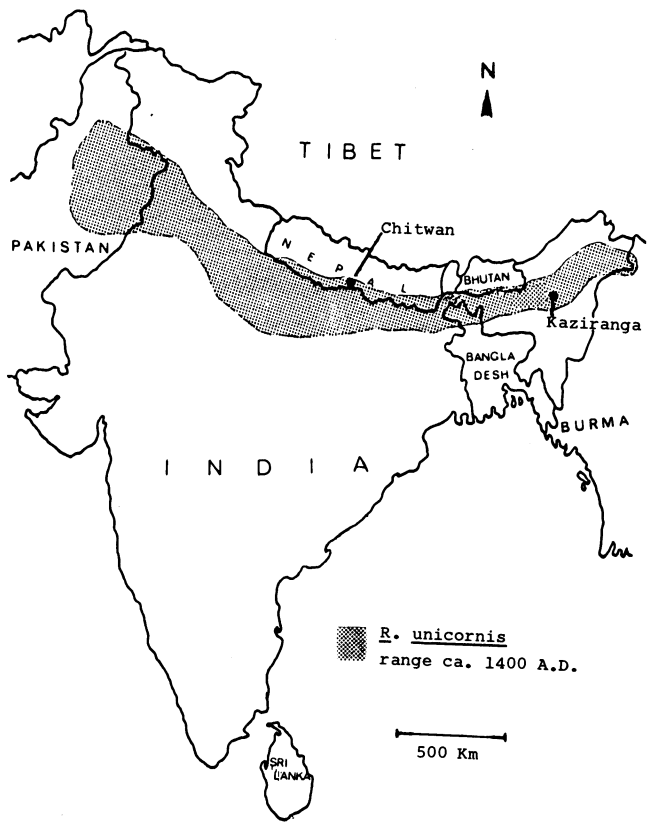

Figure 1. The geographic range of Rhinoceros unicornis, *ca. 1400* A.D., *with the locations of Royal Chitwan National Park and Kaziranga National Park indicated.*

ples were prepared for protein electrophoretic studies as described in McCracken and Wilkinson (1988). Horizontal starch-gel electrophoresis following the techniques of Selander et al. (1971) was used to examine 29 presumptive, protein-encoding loci; 17 loci from dermal tissue (Aat, Es-1, 2, 3, 4, Fum , G3pdh, Gpi, Lap-1, 2, Ldh-1, 2, Me, Pgm-1, 2, Pmi, and Sod), 7 from red blood cells (Dia-1, 2, G6pdh, Hb, Mdh, Pep, 6Pgd), and 5 general proteins (Gp-1, 2, 3, 4, 5) from blood plasma. Aat, Mdh, Me, and Pmi were resolved using tris maleate buffers; Dia-1, 2, G6pdh, Gp-1-5, and Hb using lithium hydroxide buffers; Es-1-3, Fum, G3pdh, Lap-1, 2, and Ldh-1, 2 using tris-citrate buffers (pH 8.0); Es-4 and 6Pgd using tris versene borate buffers; and Gpi, Pep, Pgm-1, 2, and Sod using Poulik discontinuous buffers (Selander et al. 1971). Protein stain recipes are from Selander et al. (1971) and Harris and Hopkinson (1978).

Results

Nine of the 29 presumptive loci examined were polymorphic. Genotype frequencies at each polymorphic locus conformed to Hardy-Weinberg expectations (Levene 1949). Allele frequencies for each of the polymorphic loci are listed in Table 1, and Figure 2 illustrates the variability seen at three of the loci. The overall heterozygosity (H_o) measured from this suite of loci (Hedrick et al. 1986) was 9.9 ± 4.5%.

Table 1. Allele frequencies at the polymorphic loci examined in *Rhinoceros unicornis.

Polymorphic Loci			
Locus	*Allele*	*Frequency*	*No. of Individuals Examined*
Es-3	a	0.05	21
	b	0.59	
	c	0.36	
Es-4	a	0.84	22
	b	0.16	
G6pdh	a	0.50	20
	b	0.50	
Gp-3	a	0.11	19
	b	0.89	
Gpi	a	0.80	23
	b	0.20	
Hb	a	0.93	23
	b	0.07	
Ldh-1	a	0.48	23
	b	0.52	
6Pgd	a	0.02	23
	b	0.04	
	c	0.94	
Pgm-2	a	0.17	23
	b	0.83	

* *Dia-1 also was variable with a single rare allele at 0.02.*

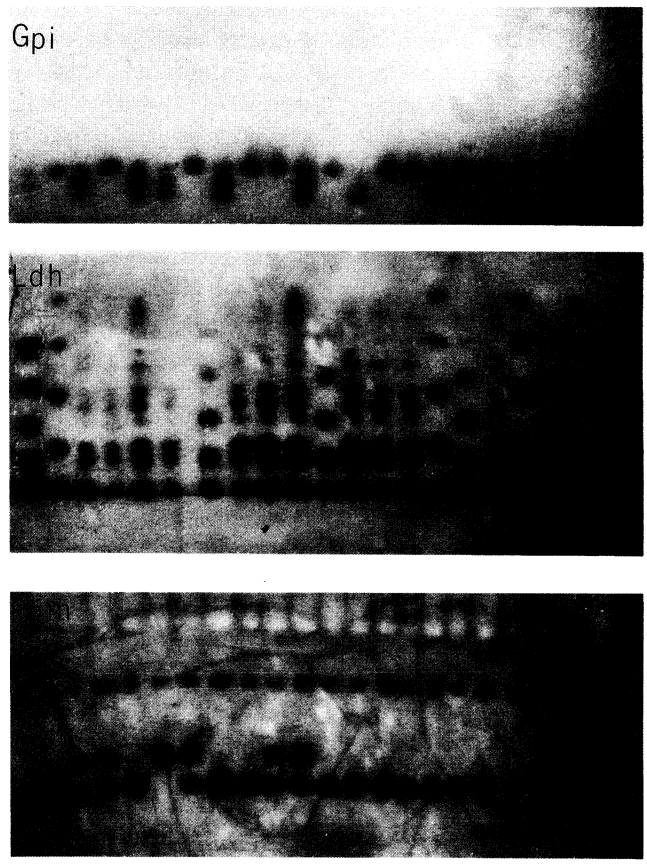

Figure 2. Electrophoretic variation observed in Rhinoceros unicornis *at Gpi, Ldh, and Pgm.*

Discussion

The heterozygosity documented in the Chitwan population of *R. unicornis* is in striking contrast to the much lower heterozygosities that have been reported for populations of other species that have experienced near-extinction (Bonnell & Selander 1974; Pemberton & Smith 1985; O'Brien et al. 1987; O'Brien et al. 1985; O'Brien & Evermann 1988). This observed heterozygosity is also at the extreme high end of values observed in over 140 other mammal species which have been examined using similar techniques, and an exception to the generalization that large mammals have low heterozygosity (Nevo 1978; Wooten & Smith 1985). To account for these results we must address two questions (1) How did such high levels of genetic variability accumulate in these mega-mammals prior to their reduction in numbers? and (2) how has this variability persisted through the bottleneck? In our attempt to answer these questions we will outline the historical demography of the Chitwan population of *R. unicornis*

and consider life history characteristics that are relevant to the loss or preservation of genetic variability.

In the fifteenth century, before extensive human settlement within its range, *R. unicornis* occurred along the flood plains, oxbows, and feeder streams of major rivers from northwestern Burma, across the Gangetic plain, to the Indus River Valley in northern Pakistan (Fig. 1) (Laurie 1978). *R. unicornis* can be abundant in their prime habitat, the tall grasslands along major rivers. Average densities in the Kaziranga National Park currently approach 4 ind/km^2 (Martin et al. 1987) and in Chitwan, peak densities reach 13.3 ind/km^2 (Dinerstein & Wemmer 1988; Dinerstein & Price, in press). The area of prime habitat within *R. unicornis*'s historic range was approximated as a 4-km-wide band along major rivers. Conservatively, 35,800 km^2 of such habitat existed. This area multiplied by current Chitwan densities in prime habitat provides a minimal total population estimate of 476,140 individuals, ignoring individuals in less-than-prime habitat. *R. unicornis* also are highly vagile; even within their very restricted current range, individuals have moved linear distances of over 60 km within a year (G. Singh, personal communication). This vagility, coupled with their wide distribution and probable high density, all suggest that, in ca. 1400, *R. unicornis* could easily have had N_e's of tens of thousands. High levels of genetic diversity can accumulate in populations of this magnitude provided that the large effective population size is sustained over many generations (Soulé 1976; Nei 1987). Fossils of *R. unicornis* date from the Middle Pleistocene, and fossils also demonstrate a broader prehistoric distribution for this species than is estimated for ca. 1400 (Laurie et al. 1983). Therefore, it is likely that *R. unicornis* persisted in very large numbers for at least 100,000 rhinoceros generations. It also seems probable that much of the accumulated genetic diversity in this species was distributed throughout its range, with little or no structuring among regions.

By the late nineteenth century extensive land clearing and hunting fragmented their range and eliminated *R. unicornis* from all areas but the Chitwan Valley, lowland Bhutan, the Teesta Valley, West Bengal, and the Brahmaputra Valley in Assam, India (Blanford 1888). In the Chitwan Valley at least 1,000 individuals of *R. unicornis* persisted until about 1950, when poaching and land clearing after malaria eradication caused their decline to an estimated low of 60–80 survivors in 1962 (Laurie 1978; H. Mishra, personal communication). Precise calculation of N_e during this period is problematic because we lack necessary life table statistics and full information on the variance in individual reproductive success (Lande & Barrowclough 1987). However, we do have sufficient information from field studies to approximate N_e. Of 251 Chitwan rhinoceros monitored in 1984–1988, 87 were breeding females and 51 breeding-age males. All mature females produce one calf approximately every 4 years, and variance in female reproductive success appears low. Throughout the study period, only 28 of the 51 adult males showed evidence of breeding activity (Dinerstein & Price, in press). The remaining 23 adult males never attained dominance, were not allowed to approach estrous females, and showed none of the behavioral and morphological characteristics obvious in breeding males. Assuming discrete generations and that the variance in progeny number equals the mean number produced per individual (excluding nonreproductives), we calculate that $N_e = 85$, or about 35% of the total population (Lande & Barrowclough 1987). *R. unicornis* clearly violates both of the above assumptions, but we cannot presently evaluate the net effects of these violations on N_e. Therefore, we use $0.35 \times N$ (N = total population size) as the best estimate of N_e. Our conclusions will not be qualitatively affected even if this estimate is incorrect by a factor of two (see below).

The rate of decay of heterozygosity resulting from small population size is known to approximate $1/(2N_e)$ per generation (Allendorf 1986; Lande & Barrowclough 1987). Our estimate of average generation time in free-ranging *R. unicornis* is ca. 12 yrs., which is lower than that calculated for other rhinocercatids (T. Foose, personal communication). Because prior to 1950, Chitwan maintained a population of no less than 1,000 individuals ($N_e > 350$), we can calculate that the population should have lost no more than 6.4% of its original heterozygosity during the approximately 46 *R. unicornis* generations going back to 1400 A.D. After 1950, the rate of loss of heterozygosity would have accelerated. However, because only about three *R. unicornis* generations have elapsed since the population's precipitous decline, and because recovery has been rapid (i.e., in 1962, $N_e = 21$–28; in 1975, $N_e = 95$; in 1988, $N_e = 133$; Dinerstein & Price, in press), we also calculate that further erosion of heterozygosity should not have exceeded an additional 3%. Therefore, the current population in Chitwan should retain approximately 90% of the heterozygosity present when *R. unicornis* was still widespread and common. If N_e's were actually half our estimates, approximately 82% of the original heterozygosity should as yet be preserved, whereas if N_e's were twice our estimates, over 95% should still be present. These estimates of the heterozygosity preserved are probably conservative because (1) the Chitwan population undoubtedly exceeded 1,000 individuals between 1400 A.D. and recent times; and (2) our estimate of length of generation time is probably too low.

Although Chitwan *R. unicornis* retain high heterozygosity we observed relatively low allelic diversity, with three alleles at two loci and two alleles at all other polymorphic loci (Table 1). High allelic diversity is an expected result of sustained large N_e's (Chakraborty et al. 1980; Nei et al. 1975), and loci with multiple alleles are

common in studies of organisms with apparently large N_e's (e.g. Ayala et al. 1972; McCracken 1984). Many of these alleles are at low frequencies and contribute little to overall heterozygosity (Allendorf 1986; Chakraborty et al. 1980; Nei et al. 1975; Lande & Barrowclough 1987; Fuerst & Maruyama 1986). Rare alleles are lost quickly during bottlenecks (Allendorf 1986; Fuerst & Maruyama 1986), and this could explain the relatively low allelic diversity observed in *R. unicornis*. However, with a sample of 23 individuals we expect to see only about 20% of all alleles at frequencies of 0.001–.01, and about 70% of those at frequencies of 0.01–.05. Therefore, small sample size may preclude our detecting any loss of allelic diversity resulting from reduced population size.

Finally, our results are in contrast to the only other published electrophoretic study of *R. unicornis*. In a recent paper Merenlender et al. (1989) report no observed variation among three individuals derived from the Kaziranga population. As the authors suggest, more individuals from that population must be examined to determine if the Chitwan and Kaziranga populations vary in the amount of genetic variation present.

Conclusion

We conclude that high heterozygosity persists in this population of *R. unicornis* because the population size remained large prior to 1950, the genetic bottleneck occurred recently, and average generation time is long. The observation of high genetic variability in the Chitwan rhinoceros population was surprising initially, and is in contrast to the results of several other studies on genetic variability in rare and endangered species. However, our results can be explained by considering the distributional history, demography, and life history parameters of these animals. We believe that this study illustrates the need for considering these factors when appraising possible genetic impacts of population bottlenecks in other species.

Chitwan *R. unicornis* provide an example of a population that almost went extinct while still carrying high genetic diversity. From this perspective, we believe that the situation with Chitwan rhinoceros will prove not to be exceptional. Given the accelerating rate of extinction (Meyers 1979), other threatened species like *R. unicornis* that were until recently common and widespread, may yet retain a substantial proportion of their original heterozygosity.

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