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Abstract: The "cheetah paradigm" proposes that a low level of genetic variation has resulted in a high probability of extinction for this species, a connection that has recently been questioned. I do not wish to address this controversy further but to suggest that the extent of genetic variation observed in cheetahs, including the recent minisatellite and microsatellite data, is consistent with the equilibrium heterozygosity expected from the small effective population size that may occur because of metapopulation dynamics, that is, because of extinction and re-colonization of habitat patches. In other words, a severe, ancient population bottleneck or a series of ancient bottlenecks "over time, over space or both, with small populations being founded and surviving, while the larger parent populations died out" at the end of Pleistocene (10,000 to 12,000 years ago) are not the only explanations for the observed pattern of genetic variation in cheetahs. Alternative possibilities are presented.

Comment

Bottleneck(s) or Metapopulation in Cheetahs

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The "cheetah paradigm" proposes that a low level of genetic variation has resulted in a high probability of extinction for this species (O'Brien et al. 1983, 1985, 1987), a connection that has recently been questioned (Caro & Laurenson 1994; Caughley 1994; May 1994; Merola 1994). I do not wish to address this controversy further but to suggest that the extent of genetic variation observed in cheetahs, including the recent minisatellite and microsatellite data, is consistent with the equilibrium heterozygosity expected from the small effective population size that may occur because of metapopulation dynamics, that is, because of extinction and recolonization of habitat patches (Slatkin 1977; Maruyama & Kimura 1980; Hedrick & Gilpin 1996). In other words, a severe, ancient population bottleneck (O'Brien et al 1983) or a series of ancient bottlenecks "over time, over space or both, with small populations being founded and surviving, while the larger parent populations died out" (O'Brien et al. 1987) at the end of Pleistocene (10,000 to 12,000 years ago) are not the only explanations for the observed pattern of genetic variation in cheetahs. It should also be noted that there is no direct fossil evidence in Africa for extinctions (or population bottlenecks) of megafauna in general, or cheetahs in particular, at the end of the Pleistocene, as there is for megafauna in North America.

The original genetic data for which the cheetah bottleneck hypothesis was proposed was the low allozyme variation observed in the south African cheetah (*Acinonyx jubatus jubatus*) (O'Brien et al. 1983). In this initial survey 47 allozyme loci were all monomorphic, whereas in a follow-up study (O'Brien et al. 1987) the average heterozygosity over *A. j. jubatus* and *A. j. raineyi*, the east African subspecies, was 0.0072 (see comparisons to other species in Table 1). Heterozygosity for soluble proteins was somewhat lower in cheetahs (0.013) than in humans (0.024) (O'Brien et al. 1983) and heterozygosity for restriction fragment length polymorphisms (RFLPs) in the major histocompatibility complex was lower in cheetahs (0.059) than in humans (0.174) (Yukhi & O'Brien 1990). (In a related study, skin transplants between unrelated individuals were accepted in 11 or 14 attempts [O'Brien et al. 1985]). In contrast, substantial levels of genetic variation have recently been documented in cheetahs for mtDNA, minisatellites, and microsatellites (Menotti-Raymond & O'Brien 1993, 1995). For example, four mtDNA RFLP haplotypes were found in *A. j. jubatus*, and three different haplotypes were found in *A. j. raineyi*. The overall mtDNA nucleotide diversity was 0.0018, compared to 0.0035 in pumas (*Felis concolor*), the cheetah's closest relative. The level of heterozygosity in cheetahs is 0.435 for minisatellites (Menotti-Raymond & O'Brien 1993) and for microsatellites is 0.39 (Menotti-Raymond & O'Brien 1995; see Table 1 for comparisons to other species). Further, when the two cheetah subspecies are compared, 29% of the minisatellite fragments and 44% of the microsatellite alleles are unique to one of the subspecies. Because of the substantial variation observed for mtDNA, minisatellites, and microsatellites, Menotti-Raymond and O'Brien (1993, 1995) suggest that cheetahs have accumulated variation for these markers since the hypothetical bottleneck(s) because of their high mutation rates, whereas the low variation of allozymes remains because of the lower mutation rate for these loci.

An alternative to the bottleneck hypothesis for the observed pattern of genetic variation in cheetahs is that cheetahs have a metapopulation structure (Pimm et al. 1989; Gilpin 1991; see also O'Brien 1989). A number of factors, such as prey availability, predators, habitat struc-

Table 1. The observed heterozygosity for three types of genetic variants in cheetahs, pumas, and lions, and the theoretical equilibrium values for two effective metapopulation sizes.

	Allozymes	*Minisatellites*	*Microsatellites*
Observed			
Cheetahs	0.0072	0.435	0.39 (0.56)[a]
Pumas	0.018–0.067	0.579	0.61
Lions	0.037	0.481	0.66
Theoretical			
$N_e = 200$	0.0008[b]	0.380[c]	
$N_e = 2000$	0.0079[b]	0.757[c]	

[a] *The observed value in parentheses for microsatellite variation in cheetahs excludes the three loci that are monomorphic in cheetahs and polymorphic in lions, pumas, and house cats.*
[b] *Calculated using* $u = 10^{-6}$ *and expression (1).*
[c] *Calculated using* $u = 10^{-3}$ *and expression (2).*

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ture, and social organization, appear to be important in making the distribution of cheetahs patchy (Caro 1994), consistent with a metapopulation structure. The dynamics of a metapopulation, because of frequent extinction and recolonization, can by themselves result in a quite small effective population size (Hedrick & Gilpin 1996; Whitlock & Barton, 1996). In such a metapopulation the census population number may be an order of magnitude or more greater than the genetic effective population size. The effective size of a metapopulation can be particularly small when there is frequent extinction and recolonization of subpopulations, when the number of recolonization founders is small, and when there is little gene flow at times other than recolonization (Hedrick & Gilpin 1996).

The observed heterozygosity for allozymes, minisatellites, and microsatellites is given in Table 1 for cheetahs, the puma, and the large Serengeti population of lions (*Panthera leo*). To illustrate the impact on the amount of standing (or equilibrium) variation for allozymes resulting from a small effective metapopulation size, the expected equilibrium heterozygosity can be calculated for neutral variation using the infinite allele model:

$$H_e = \frac{4N_e u}{4N_e u + 1}, \quad (1)$$

where u is the mutation rate per gamete per generation and N_e is the effective metapopulation size (Kimura & Crow 1964). The mutation rate for a given allozyme locus is approximately 10^{-6} per gamete per generation (Voelker et al. 1980). For the expected heterozygosity for minisatellites and microsatellites, a more appropriate expression is that for the stepwise mutation model (Slatkin 1995):

$$H_e = 1 - \frac{1}{(1 + 8N_e u)^{1/2}} \quad (2)$$

(Ohta & Kimura 1973). The mutation rates for minisatellites (Jeffreys et al. 1988, 1991) and microsatellites (Dallas 1992; Weber & Wong 1993) are quite high and are thought to be approximately 10^{-3} per gamete per generation. Assuming that the effective metapopulation size is either 200 or 2000 and the expressions and mutation rates are as given above, then the expected heterozygosity is given in Table 1 for the two categories of genetic variation.

Obviously, these parameters for mutation rates and effective metapopulation size can give levels of heterozygosity consistent with the low observed levels of variation for allozymes and the much higher observed variation for minisatellites and microsatellites. In other words, the low allozyme mutation rate results in low theoretical allozyme heterozygosity, and the high minisatellite and microsatellite mutation rates result in a high equilibrium genetic variation for these loci. These calculations do not depend at all on a bottleneck but require only that the effective metapopulation size of cheetahs is small enough to result in the low level of allozyme heterozygosity and that the mutation rate for minisatellites and microsatellites is high enough to result in a substantially higher equilibrium heterozygosity (the different expressions used make only small differences in the predictions).

The cheetah also appears to have genetic variation for fitness-determining loci because there is evidence of inbreeding depression for juvenile survival in captive animals (Hedrick 1987; Caughley 1994; Wielebnowski 1996). But because the additive genetic variance for many quantitative traits created per generation by spontaneous mutation is approximately 10^{-3} times the environmental variance (Lande 1975; Lynch 1988), a mutation rate the same magnitude as that for minisatellites and microsatellites, the observation of inbreeding depression in cheetahs is not unexpected.

The bottleneck and metapopulation hypotheses may potentially be differentiated with detailed molecular data. First, a bottleneck 10,000 to 12,000 years ago (approximately 2000 generations ago) should allow time for accumulation of a number of rare alleles for minisatellites and microsatellites, so that the distribution of allele frequencies should be close to neutrality expectations. On the other hand, metapopulation dynamics, which assume frequent colonization events of a few individuals and thereby result in a continual low effective population size, should result in the presence of fewer rare alleles than neutrality expectations. Second, genetic drift in the founder events in a metapopulation should continually generate disequilibrium between linked loci. In contrast, approximately 2000 generations from the bottleneck should be adequate to generate linkage equilibrium except for the most tightly linked loci and the most recent mutants. Third, the approaches suggested to investigate bottlenecks in human populations (Rogers & Harpending 1992) and that proposed to determine effective population size from DNA sequences (Milligan et al. 1994) may allow differentiation between these hypotheses.

The implications for conservation of a bottleneck(s) or a metapopulation explanation are difficult to state explicitly. If a severe bottleneck(s) occurred, it is possible that detrimental alleles could have been fixed, thereby lowering fitness (Hedrick 1994) and also possibly indicating past demographic instability in the species. On the other hand, if metapopulation dynamics are responsible for the pattern of genetic variation, then we would assume that there may have been repeated founder events and that subpopulations that became fixed for detrimentals would more likely have gone extinct. It is possible, however, that in both scenarios there could be variation for detrimentals, that some variation could be remaining after a bottleneck, and that in a metapopulation different subpopulations could have different detri-

mental alleles so that the metapopulation overall could be variable. The major difference in conservation strategy is probably the recognition that local extinction (or near extinction) would not be unexpected under a metapopulation scenario although it may be a greater cause for concern if there were past population-wide bottlenecks.

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