GENETIC VARIATION AND ITS MANAGEMENT APPLICATIONS IN EASTERN U.S. FERAL HORSES

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Abstract: We used electrophoretic and immunologic techniques to analyze blood samples collected from feral horses (*Equus caballus*) on 4 eastern United States barrier islands. Genetic variation measured in the island herds was similar to that reported in domestic horse breeds. No deficiency in mean multilocus heterozygosity at 10 protein loci (H = 0.319–0.400) was apparent. Mean number of alleles per locus ranged from 2.2 to 4.4 for 10 protein loci and from 2.7 to 4.8 for 6 red blood cell loci. Greatest genetic resemblance existed between Assateague Island, Virginia, and Cumberland Island, Georgia, horses (S = 0.830). The herd on Shackleford Banks, North Carolina, was least similar to the other feral herds (S = 0.745–0.793), but a small sample size (n = 4) could have affected our results. Overall, the feral herds did not appear to be genetically unique at the loci that we sampled. Local historic and economic values of feral horses might require maintenance of populations on some barrier islands. However, based on ecological and genetic criteria, we think that these populations should be reduced in size. We used models to estimate minimum effective population sizes for 3 island horse herds with different harem structures, age structures, or management regimes. We estimated that a minimum of 72 animals should be retained on Assateague Island National Seashore, Maryland; 122 animals on Cumberland Island, Georgia; and 155 animals on Chincoteague National Wildlife Refuge, Virginia, to limit genetic loss to <1% per generation.

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Domestic livestock introduced to barrier islands along the United States Atlantic Coast could alter natural habitats or compete with native species (Johnson et al. 1974). To a great extent, feral cattle, sheep, and goats have been removed from public lands on these islands. However, free-ranging feral horses remain on Assateague Island, Virginia and Maryland (Chincoteague Natl. Wildl. Refuge on the southern half and Assateague Island Natl. Seashore on the northern half); Shackleford Banks, North Carolina (Cape Lookout Natl. Seashore); Carrot Island, North Carolina (Rachel Carson Natl. Estuarine Res. Reserve); and Cumberland Island, Georgia (Cumberland Island Natl. Seashore). Currently, only the privately owned population on Chincoteague National Wildlife Refuge is managed routinely, through sale of excess foals at auction. Feral horses on Ocracoke Island, North Carolina (Cape Hatteras Natl. Seashore) are maintained in an enclosure.

Bratton (1988) suggested that feral animals be retained on public lands only if (1) they are genetically unique and have not been outbred within the past century, or (2) they pose no threat to endemic or endangered species or ecosystems. Our study was designed to examine part of the first criterion—the level of genetic diversity within the barrier island horse populations—and to derive genetically based recommendations for minimum population size.

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METHODS

Sample Collection and Analysis

We took blood samples by venipuncture from 18 randomly selected adult female horses on Cumberland Island between November 1986

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and February 1987, after the animals were immobilized with a mixture of 8.5 mL etorphine hydrochloride (1 mg/mL) and 0.5 mL xylazine hydrochloride (200 mg/mL). Additional samples were obtained from fresh carcasses of 2 males (1 ad, 1 yrl) located during the 1987 breeding season and from 1 young island gelding maintained in captivity. Our sample size represented approximately 11% of the estimated population of 186 animals at the end of the 1986 breeding season (Goodloe 1991). Four animals on Shackleford Banks (2 ad M, 1 M subad, and 1 F subad) were immobilized and sampled in November 1986; we halted sampling after the 2 adult stallions died, and only 4% of the population (92 animals in 1984 [Rubenstein and Hohmann 1989]) was included in our study.

We sampled the Ocracoke Island (15 samples) and southern Assateague Island (77 samples) populations in April 1986 and April 1987, respectively, when the herds were corralled for annual veterinary care. Two additional samples were collected from privately owned horses originally from feral stock on Ocracoke. About 75% of the Ocracoke population (9 M and 8 F) and 60% of the Chincoteague National Wildlife Refuge population (5 ad M, 64 ad F, 5 subad F, and 3 M foals) were sampled. We did not obtain samples from feral horses on the northern half of Assateague Island (i.e., Assateague Island Natl. Seashore); the northern and southern Assateague Island herds have been separated only since 1966, and some interchange of horses still occurs between the 2 herds (Keiper and Houpt 1984).

Whole blood from horses in the Assateague Island, Shackleford Banks, and Cumberland Island populations was shipped, within a week of collection, to the University of Kentucky for analysis. Only serum and lysed red cell samples were available from the Ocracoke Island horses. Standard starch and polyacrylamide gel electrophoresis (Sandberg 1974, Juneja et al. 1978) were used to assess genetic variability at the following 10 protein loci: α-1-β glycoprotein (*A1B*), albumin (*ALB*), esterase (*EST*), vitamin D binding protein (*GC*), glucosephosphate isomerase (*GPI*), α-hemoglobin (α-*HB*), 6-phosphogluconate dehydrogenase (*6-PGD*), phosphoglucomutase (*PGM*), protease inhibitor (*PI*), and transferrin (*TF*). Red cell alloantigens at 6 blood group loci (*A*, *C*, *D*, *K*, *P*, and *Q*) were detected with standard immunological procedures involving hemagglutination and complement-mediated hemolysis (Stormont and Suzuki 1964, Stormont et al. 1964). The loci we examined are used routinely in horse parentage analysis, and all are known to be polymorphic in horses. Therefore, our estimates of heterozygosity and mean allele number were directly comparable only to studies on domestic horse populations and not to those on other species that included sampling for monomorphic loci.

Genetic Evaluations

We used 2 measures of genetic diversity—heterozygosity and the number of alleles per locus—to estimate genetic variation in the 4 island populations. Effective number of alleles at a locus was calculated as the inverse of the squared allele frequencies (Hendrick 1983). We assessed allelic frequencies for protein polymorphisms and the *D* blood group by direct computation from genotypes. Ambiguous phenotypes are possible in the *D* system; however, we did not assume an allele was present unless it was clearly identified. Blood group allele frequencies were calculated for loci *C* and *K* by the square-root method and for loci *A*, *P*, and *Q* by maximum likelihood techniques (Spiess 1977). A locus was considered monomorphic when the most common allele occurred at a frequency >0.95.

Heterozygosity (h) at each locus for animals within a population was determined by direct count of individuals with 2 different alleles; mean multilocus heterozygosity (H) was calculated as the average of heterozygosity values for all loci sampled within a population. Heterozygosity was not determined for the red blood cell factors. We calculated inbreeding coefficients as the net difference between observed and expected numbers of heterozygotes at a locus, divided by the number of heterozygotes observed (Hartl 1981). Genetic similarities (S) among the feral horse populations and between feral and standard horse breeds were evaluated according to Rogers' (1972) procedure. Dendrograms of similarity coefficients were prepared using the unweighted pair group method with arithmetic averages (Sneath and Sokal 1973).

Statistical Analysis

We used Kruskal-Wallis nonparametric procedures to test differences between measures of mean multilocus heterozygosity and allele number between and among the feral populations. *T*-tests with arc sine transformation of data were

Table 1. Effective and actual number of alleles found at each locus in feral horse populations on 4 eastern United States barrier islands, 1986 and 1987.

Locus[a]	Possible no. alleles[b]	Assateague		Cumberland		Ocracoke		Shackleford	
		Effect	Actual	Effect	Actual	Effect	Actual	Effect	Actual
Protein									
A1B	3	1.0	2[c]	1.0	1[c]	1.1	2[c]	1.3	2
ALB	3	1.4	2	1.8	2	2.0	2	1.6	2
EST	6	1.7	3	1.2	3	2.9	3	2.1	3
GC	2	1.5	2	1.6	2	1.9	2	1.3	2
GPI	4	1.1	2[c]	1.7	2	1.5	3	1.0	1[c]
α-HB	4	2.0	3	1.9	2	2.0	3	2.1	3
6-PGD	3	1.2	2	1.4	2	1.2	2	1.0	1[c]
PGM	3	1.6	2	1.0	1[c]	1.0	2[c]	1.0	1[c]
PI	20	9.6	17	4.3	8	1.7	4	3.6	4
TF	12	4.3	9	2.9	4	2.9	4	1.7	3
$\bar{X}$		2.5	4.4	1.9	2.7	1.8	2.7	1.7	2.2
% polymorphic loci			80.0		80.0		80.0		70.0
Red cell									
A	11	4.5	8	2.1	4			2.7	4
C	2	2.2	2	1.9	2			1.1	1[c]
D	12	4.6	10	5.0	8			2.2	3
K	2	1.3	2	1.0	1[c]			1.7	2
P	3	2.4	3	1.7	3			2.5	3
Q	5	3.0	4	2.3	4			2.4	3
$\bar{X}$		3.0	4.8	2.3	3.5			2.1	2.7
% polymorphic loci			100.0		83.3				83.3
Total % polymorphic loci			87.5		81.2		80.0		75.0

[a] α-1-β glycoprotein (*A1B*), albumin (*ALB*), esterase (*EST*), vitamin D binding protein (*GC*), glucosephosphate isomerase (*GPI*), α-hemoglobin (*α-HB*), 6-phosphogluconate dehydrogenase (*6-PGD*), phosphoglucomutase (*PGM*), protease inhibitor (*PI*), and transferrin (*TF*).
[b] Based on the number of formally named alleles detectable by the techniques we used.
[c] Monomorphic locus (frequency of most common allele >0.95).

applied to compare our measures of heterozygosity with values reported for other horse populations.

RESULTS

Heterozygosity and Allele Number

All 16 loci analyzed were polymorphic in at least 1 of the feral horse populations, and 10 were polymorphic across all populations (Table 1). The greatest degree of polymorphism was observed in samples collected from the Assateague Island herd. Monomorphic loci were observed from this population only at the *A1B* and *GPI* loci, which also were monomorphic in several of the other feral herds. The reduced number of polymorphic loci found in the Shackleford Banks population probably was the result of our small sample size ($n = 4$) rather than limited genetic diversity within the herd.

The actual number of alleles recorded at polymorphic loci varied from a low of 2 alleles at several loci to 17 at the *PI* locus in the Assateague Island population (Table 1). The effective number of alleles, which is a measure of the number of alleles that contributed to heterozygosity at each protein locus, ranged from 1.2 to 9.6 alleles per polymorphic locus. Mean number of alleles per locus was not significantly different among populations ($P = 0.553$ for red cell loci and $P = 0.549$ for protein loci), although greater allele counts were documented from populations with larger sample sizes. Overall, 78% of the alleles described at these loci in domestic horses were located in the feral populations (Table 1). We did not detect any alleles that had not been described previously in horses.

The 4 populations of island horses tended to share the same predominant or fixed allele at each polymorphic or monomorphic locus (Tables 2 and 3). In general, these corresponded to the predominant alleles in domestic horse breeds. At 2 loci, *α-HB* and *C*, the predominant allele varied among the populations, but predominant alleles recorded for 1 population at these loci occurred at relatively high frequencies in all other populations. In contrast, most common alleles at the *PI* and *D* loci for the Shackleford Banks population occurred only at low fre-

Table 2. Observed proportions of heterozygotes (*h*) and allelic frequencies (*p*) for 10 protein loci from feral horse populations on 4 eastern United States barrier islands, 1986 and 1987.

		Assateague (n = 77)		Cumberland (n = 21)		Ocracoke (n = 17)		Shackleford (n = 4)	
Locus[a]	Allele	*h*	*p*	*h*	*p*	*h*	*p*	*h*	*p*
A1B		0.039[b]		0.000[b]		0.059[b]		0.250	
	F		0.019				0.029		0.125
	K		0.981		1.000		0.971		0.875
ALB		0.299		0.381		0.471		0.000	
	A		0.175		0.333		0.471		0.250
	B		0.825		0.667		0.529		0.750
EST		0.351		0.190		0.529		0.750	
	F		0.104		0.023		0.294		0.125
	G		0.143		0.071		0.294		0.250
	I		0.753		0.906		0.412		0.625
GC		0.312		0.429		0.294		0.250	
	F		0.792		0.738		0.618		0.875
	S		0.208		0.262		0.382		0.125
GPI		0.078[b]		0.381		0.412		0.000[b]	
	F		0.039		0.286		0.029		
	I		0.961		0.714		0.795		1.000
	S						0.176		
α-HB		0.558		0.286		0.647		0.750	
	AII		0.013				0.029		0.250
	BI		0.538		0.619		0.382		0.125
	BII		0.448		0.381		0.588		0.625
6-PGD		0.182		0.286		0.176		0.000[b]	
	F		0.896		0.810		0.912		1.000
	S		0.104		0.190		0.088		
PGM		0.338		0.000[b]		0.059[b]		0.000[b]	
	F		0.234				0.029		
	S		0.766		1.000		0.971		1.000
PI		0.857		0.667		0.471		1.000	
	F		0.006						
	G						0.029		
	H		0.013		0.048				
	I		0.052						0.375
	K		0.071						
	L		0.123		0.167				
	L_2		0.039		0.023				
	N		0.097						
	O		0.013						
	P		0.006				0.088		0.250
	Q		0.013		0.023				
	R		0.110		0.023				
	S		0.156		0.287		0.147		0.250
	T		0.052		0.095				
	U		0.169		0.334		0.735		
	V		0.039						0.125
	X		0.026						
	Z		0.013						
TF		0.779		0.571		0.882		0.500	
	D		0.253				0.117		0.125
	E		0.006						
	F_1		0.026		0.071				
	F_2		0.338		0.405		0.471		0.750
	H_1		0.039						
	H_2		0.006						
	M		0.026						
	O		0.104		0.119		0.088		
	R		0.201		0.405		0.324		0.125
H^c		0.379		0.319		0.400		0.350	

[a] α-1-β glycoprotein (*A1B*), albumin (*ALB*), esterase (*EST*), vitamin D binding protein (*GC*), glucosephosphate isomerase (*GPI*), α-hemoglobin (*α-HB*), 6-phosphogluconate dehydrogenase (*6-PGD*), phosphoglucomutase (*PGM*), protease inhibitor (*PI*), and transferrin (*TF*).

[b] Monomorphic locus (frequency of most common allele >0.95).

[c] Mean multilocus heterozygosity index.

Table 3. Allelic frequencies for 6 red cell (alloantigenic) loci from feral horse populations on 3 eastern United States barrier islands, 1986 and 1987.

Locus	Allele	Assateague	Cumberland	Shackleford
A	Null	0.347	0.142	0.050
	a	0.029		
	adf	0.204	0.657	0.347
	adg	0.077	0.052	
	b	0.217	0.150	0.478
	bc	0.007		
	c	0.078		0.125
	e	0.041		
C	Null	0.545	0.387	
	a	0.455	0.613	1.000[a]
D	*ad*	0.072	0.050	0.625
	bc	0.122	0.200	
	cefg	0.014		
	cegi	0.007		
	cg	0.061	0.200	
	d	0.034		0.250
	de	0.176	0.125	
	dfk	0.101	0.075	
	dgh	0.277	0.025	0.125
	dk	0.135	0.325	
K	Null	0.862	1.000[a]	0.707
	a	0.138		0.293
P	Null	0.482	0.736	0.409
	a[b]	0.425	0.238	0.466
	b[c]	0.093	0.025	0.125
Q	Null	0.467	0.606	0.558
	abc	0.056	0.078	
	b	0.201	0.206	0.304
	c	0.276	0.111	0.139

[a] Monomorphic locus (frequency of most common allele >0.95).
[b] Includes *Pac*, *Pacd*, and *Pad*.
[c] Includes *Pb* and *Pbd*.

Table 4. Rogers' similarity matrix for feral horse populations on 4 eastern United States barrier islands compared to 15 recognized breeds.[a]

Origin	Assateague	Cumberland	Ocracoke	Shackleford
Assateague	1.000			
Cumberland	0.830	1.000		
Ocracoke	0.809	0.824	1.000	
Shackleford	0.793	0.745	0.782	1.000
Thoroughbred	0.780	0.817	0.735	0.714
Trotter	0.786	0.776	0.824	0.766
Pacer	0.828	0.833	0.868	0.757
Arabian[b,c]	0.810	0.836	0.764	0.745
Quarter Horse[b,c]	0.837	0.862	0.804	0.775
Morgan Horse[b]	0.836	0.855	0.811	0.773
Paso Fino[b]	0.862	0.840	0.784	0.736
Peruvian Paso[b]	0.827	0.815	0.766	0.747
Shire[d]	0.835	0.773	0.778	0.749
Belgian	0.827	0.807	0.791	0.774
American Saddlebred	0.814	0.809	0.825	0.760
Shetland Pony	0.828	0.743	0.811	0.790
Breton	0.770	0.750	0.802	0.704
Percheron	0.782	0.757	0.757	0.725
Tennessee Walker	0.818	0.863	0.851	0.732

[a] Similarity for Assateague, Cumberland, and Shackleford populations based on all 16 loci analyzed. Similarity for Ocracoke based on the 10 electrophoretic loci. All data are from samples tested at the University of Kentucky, except for those breeds with a reference indicated.
[b] Data from Bowling and Clark (1985), supplemented with University of Kentucky data.
[c] Data from Bell and Patterson (1987), supplemented with University of Kentucky data.
[d] Data from Nickel and Bowling (1987), supplemented with University of Kentucky data.

quencies or not at all in other populations. This probably is related to small sample size, although similar patterns of variation at these loci have been observed in other horse breeds.

Most alleles we detected were shared by ≥2 of the feral populations (Tables 2 and 3). Only 3 (*S* at the *GPI* locus in the Ocracoke population, *K* and *N* at the *PI* locus in the Assateague population) of the 17 alleles that we found only in a single feral population contributed substantially to genetic variability; the other 14 unique alleles occurred at frequencies <0.05. All protein loci were in Hardy-Weinberg equilibrium, except for the *EST* locus in the Ocracoke Island herd. Alleles at this locus that we could not detect, such as the silent *Es-O* allele, might have caused this deviation.

Mean multilocus heterozygosity (H) for the 10 protein loci was similar ($P = 0.84$) among the feral populations (Table 2), although the percentage of heterozygous individuals at each locus (h) varied among populations. Greatest contributions to heterozygosity were made by loci with >2 alleles, particularly *TF* and *PI* (Table 1). Inbreeding coefficients, which measure the reduction in heterozygosity compared to expected values at each polymorphic locus, generally were low ($F < 0.2$). The only exceptions occurred at the *ALB* locus in the Shackleford Banks population ($F = 1.0$), *α-HB* locus in the Cumberland population ($F = 0.394$), and *GC* locus in the Ocracoke population ($F = 0.377$).

Genetic Similarities

Greatest genetic similarity occurred between the Assateague Island and Cumberland Island populations ($S = 0.830$), and we observed close genetic resemblance between these herds and the horses of Ocracoke Island ($S = 0.809$ and 0.824, respectively) (Table 4). The Shackleford Banks horses were genetically least similar to the other feral populations ($S = 0.745$–0.793); this could be an artifact of small sample size but also could reflect true genetic differences.

Genetic similarities of the 4 island horse herds

to mainland breeds reflect their diverse genetic backgrounds (Table 4, Fig. 1); however, measures of genetic similarity are not proof of animal origin. The Assateague Island and Ocracoke Island populations are thought to have derived from Spanish mustangs that survived offshore shipwrecks in the 1500's. We found a close genetic resemblance between the Assateague Island horses and the Paso Fino breed (Table 4, Fig. 1), which descended from animals brought to the New World by the Spanish. The Assateague Island herd also shows a close resemblance to the "cold blooded" horses (draft horses and ponies), probably reflecting Shetland pony introductions made early in the 1900's to increase the number of animals with the tobiano pattern (Keiper 1985). The Ocracoke Island horses, in contrast, have a closer genetic resemblance to Standardbred horses (trotters and pacers), a breed that developed in the United States in the late 17th and early 18th centuries from Thoroughbreds and other strains. The Shackleford Banks horses show genetic resemblance to the Ocracoke Island population, from which breeding stock reportedly was derived, as well as to several work horse breeds.

The Cumberland Island horses are genetically similar to several breeds, including the Tennessee Walking horse, Quarter Horse, Arabian, and Paso Fino. Free-roaming horses were reported on Cumberland Island in 1788 (letter on file at Ga. Dep. Hist. and Arch., Misc. File 470, AC 75-559); however, we do not know if the horses present on the island shortly after the Revolutionary War contributed significantly to the current gene pool or if the population is of more recent origin.

DISCUSSION

Heterozygosity detected in the feral horse populations indicates that the limited herd size possible in small island habitats has not greatly affected levels of genetic diversity relative to those of larger mainland populations. Kaminski (1978), in a study of genic variability in 18 horse breeds, found mean heterozygosity at 6 polymorphic loci varied from 0.223 in the Arabian to 0.468 in the Normandy Cob. Average heterozygosity for 11 pony breeds at the same 6 loci ranged from 0.276 in Shetland ponies to 0.382 in Fjord ponies (Kaminski and Urbanska-Nicholas 1979). The levels of mean heterozygosity we detected in feral horse populations were similar to these values ($P > 0.05$), with the exception of the value for the Assateague population when compared to those of the Arabian ($H = 0.223$, $t = 2.02$), Anglo-Arab ($H = 0.245$, $t = 2.39$), and Trotteur Francais ($H = 0.25$, $t = 2.32$) reported by Kaminski (1978). In these earlier studies, however, heterozygosity may have been underestimated, because the highly variable *PI* locus was not examined and because the methods used could not detect some alleles now known to occur, such as at the *TF* locus. Our values for mean heterozygosity were slightly lower, but not significantly different ($P > 0.05$), than those Bowling and Clark (1985) reported for 7 horse breeds sampled at 14 known polymorphic loci (range from 0.378 in Thoroughbreds to 0.481 in Peruvian Pasos).

Heterozygosity is a measure of allele distribution at a locus and is relatively insensitive to the variety of alleles that may be present. A more efficient measure of allele richness is the mean number of alleles per locus. The actual number of alleles per protein locus that we counted in the Assateague Island population (Table 1) fell within the 3.17–4.60 range of alleles reported for 36 horse breeds (Kaminski 1978, Kaminski and Urbanska-Nicholas 1979, Bowling and Clark 1985). We found fewer alleles per protein locus in the Cumberland Island, Shackleford Banks, and Ocracoke Island populations. Reduced numbers of alleles without a corresponding decline in mean heterozygosity may be caused by a population bottleneck of short duration (Allendorf 1986). However, it is more likely that our measures of allelic variation were affected by the small number of samples collected from horses on these islands. Some alleles present at low frequencies probably were not detected, particularly in the Shackleford Banks population. Comparison of allelic presence and frequency at the 16 loci studied in the 4 populations supports this conclusion. In general, the most common alleles in the Assateague Island herd, where sample size was adequate, also were detected at relatively high frequencies in the other herds. The greater allele diversity estimated for the Assateague Island population resulted primarily from the presence of 23 alleles detected at frequencies <0.05, including 13 alleles not found in the other populations.

Small populations, such as those found on islands, frequently are subject to random genetic drift or chance fluctuations in allele frequency.

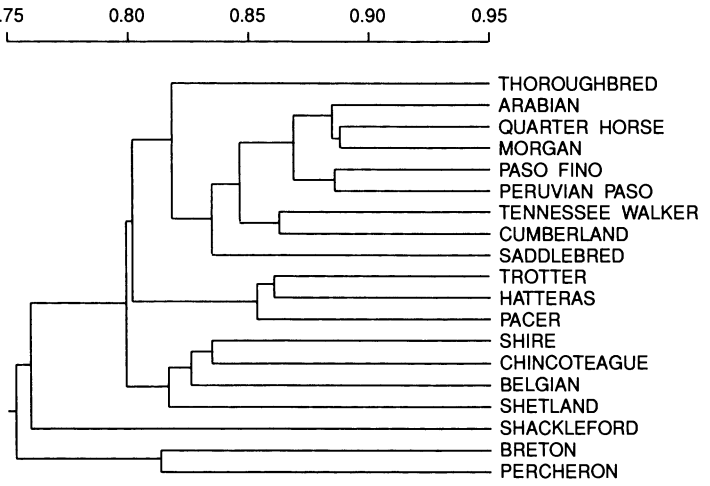

Fig. 1. Dendrogram from cluster analysis of Rogers' similarity coefficients (using unweighted pair group method with arithmetic averages) for 4 eastern United States barrier island feral horse herds and 15 recognized breeds.

Over a long period, genetic drift fixes alleles at previously polymorphic loci, producing a deficiency of heterozygotes (Hartl 1981). Small, isolated populations also may be subject to inbreeding or reduced allelic diversity due to founder effects. The 4 feral horse herds, however, have maintained relatively high levels of heterozygosity, despite small population size. This was particularly evident in the Assateague Island population, where 74 of 95 alleles known to occur at the loci we analyzed were present (Table 1). These high levels of heterozygosity possibly are the result of repeated introductions of horses to the islands from a variety of sources.

MANAGEMENT IMPLICATIONS

Genetic Uniqueness and Diversity

The close genetic resemblance and wide range of alleles shared between the barrier island horses and recognized breeds on the mainland indicate that the Cumberland, Ocracoke, and Assateague (Va.) horse populations are not genetically unique with respect to the blood markers we studied. We reserve judgment regarding the uniqueness of the Shackleford Banks population until more individuals are examined. The herds also have been augmented by introduced stock during the relatively short period they have occupied island habitats. Consequently, we do not believe that the horses meet genetic criterion established by Bratton (1988) for retention of feral animals on public lands.

Nor do the horses meet the second, environmentally based, criterion for retention; i.e., they should pose no threat to endemic or endangered species. Excessive grazing of dune-stabilizing vegetation can adversely affect nesting habitat of the loggerhead sea turtle (*Caretta caretta*) and deplete inland habitat used by alligator (*Alligator mississippiensis*), wood stork (*Mycteria americana*), and other wetland species (Hillestad et al. 1975). Intensive grazing by horses in the salt marsh reduces aboveground biomass of saltwater cordgrass (*Spartina alterniflora*), which may adversely affect estuarine ecosystems (Turner 1988). Based on ecological and genetic considerations, we recommend that feral horses be removed from, or reduced in number on, Cumberland and Assateague Islands to minimize grazing-related damage of habitat. The corralled Hatteras Island population does not represent a source of ecological danger at its currently managed size of about 20 individuals. The Carrot Island population might require control measures in the future. Managers reduced population size from 52 to 19 after 19 animals died during the severe winter in 1986–87, but the population grew to 26 horses by summer 1990 (J. Taggert, N.C. Div. Coastal Manage., pers. commun.).

Our recommendation to remove the island feral horses is based on ecological considerations; however, other concerns and values of the animals must be considered. Some feral horse populations may contribute to local historical and

cultural elements for which a park was established. Other herds, such as on the southern portion of Assateague Island, provide economic benefits to their private owners. Maintenance of viable, but reduced breeding populations, therefore, may be justified to meet these public and private needs.

Minimum Population Size

General Calculations.—Limiting horse population size on these islands, in combination with the natural force of random genetic drift, could lead to fixation of deleterious alleles, loss of rare alleles, and declines in mean heterozygosity (Fuerst and Maruyama 1986). Such reductions in genetic diversity might impair vigor, fertility, and disease resistance and could limit ability to respond to environmental variation (Beardmore 1983). Population size, particularly the number of breeding adults, is a major factor controlling the rate at which genetic diversity is lost. Effective population size (N_e), which represents the number of individuals that contribute genetically to the next or future generations, is approximated by

$$F = \frac{1}{2N_e},$$

where F represents the acceptable level of genetic loss per generation. Animal breeders have determined that inbreeding depression can be avoided if the rate of genetic loss is <2% per generation, but, for most species, a more conservative rate of 1% may be needed. At this rate of genetic loss, the effective population size is 50 animals (Soule 1980). Inbreeding at the 1% level in Standardbred horses had little impact on reproductive performance (Cothran et al. 1984).

Effective population size as calculated above is applicable only for genetically ideal populations. A number of formulas have been published to adjust estimates of N_e for populations with disparate sex ratios or overlapping generations. One of the more accurate (Harris and Allendorf 1989) is the modified formula of Reed et al. (1986),

$$\frac{1}{N_e} = \frac{1}{(4)(L_M)(M_{BR})(\bar{k}_M)(\bar{l}_M)} + \frac{1}{(4)(L_F)(F_{BR})(\bar{k}_F)(\bar{l}_F)},$$

where L_M and L_F are the mean ages of males and females that reproduce, M_{BR} and F_{BR} are the numbers of breeding males and females, $\bar{k}_M$ and $\bar{k}_F$ are the numbers of young sired by a male or born to a female per year, and $\bar{l}_M$ and $\bar{l}_F$ are the probabilities that a newborn male or female survives and breeds. Accuracy of the formula may be increased if the product is multiplied by the following to account for non-Poisson reproductive success (Harris and Allendorf 1989):

$$\frac{\bar{K}}{(\bar{K} + 1) + (V_K/\bar{K})},$$

where $\bar{K}$ and V_K are the mean and variance, respectively, of the total number of offspring produced by an individual female over her lifetime that also survive to reproductive age.

We applied these models to estimate minimum effective population sizes in 3 feral horse herds with different harem structures, age structures, or management regimes. Our projections should be viewed only as general estimates for population management goals, which might need to be modified if more complete demographic data or effective models become available in the future.

Application to Cumberland Island National Seashore.—The Cumberland Island horses represent an unmanaged population where harem size is relatively small. Between 1986 and 1990, the population grew from about 186 to 220 horses organized into 37 harems, 2 all female herds, and a number of unstable bachelor groups (Goodloe 1991). The mean size of herds that contained at least 1 adult female (≥3 yr old) was 4.6 during this 5-year period, and each adult harem male, on average, supported 1.6 adult females. A large proportion of the adult males on the island were bachelors; only 46.4% of the male population were dominant or codominant stallions of harems that contained females ≥3 years old. In contrast, an average of 60.1% of the adult females produced foals during each breeding season, yielding values of 0.481 and 0.309 for $\bar{k}_M$ and $\bar{k}_F$, respectively. Survival of foals produced by island females was poor; only 59.4% of male foals and 62.4% of female foals survived to 1 year, and approximately 75% of the surviving female yearlings lived to breeding age. Assuming that survival of male yearlings approximates that of females, we calculate that the probability a newborn male or female will survive and breed is 0.205 and 0.291 ($\bar{l}_M$ and $\bar{l}_F$, respectively). We estimated that generation length averages 10 years for males and 9 years

for females. Subadults compose about 32% of the Cumberland Island population (Goodloe 1991). We made no corrections for non-Poisson distribution of progeny because our estimated values for $\bar{K}$ and V_K were 3.63 and 3.40.

If park managers retain a herd composition and age distribution similar to that in the unmanaged Cumberland Island herd, we calculate that 23 males must breed annually to maintain an effective population size of 50 animals where no more than 1% of the genetic diversity is lost per generation. The current population could be reduced by about half, or to 122 individuals, at the end of the breeding season (23 harem M, 26 bachelor M, 35 mares of breeding age, and 38 subad M and F). Simulation models developed by Turner (1988) suggest that a population of 49–73 horses is needed to prevent excessive damage to the salt marshes.

Application to Assateague Island National Seashore.—Mean size of harems with adult females in the unmanaged herd on Assateague Island National Seashore averaged 11 individuals from 1975 to 1984 (calculated from data presented in Keiper and Zervanos [1979], Keiper and Hunter [1982], Keiper [1980, 1983, 1984]), which is much larger than on Cumberland Island. Population size during this 10-year period increased from 45 to 107 horses, despite the removal of 13 animals in 1984 (Keiper 1984). Foaling rates were similar to those on Cumberland Island (53.9% including more productive 3-year-old females [Keiper and Houpt 1984] than on Cumberland Island [Goodloe 1991]), but a much larger percentage of the Assateague Island foals (88.3%) survived to 1 year (Keiper and Houpt 1984). Bachelor males are known to disperse to the southern portion of the island, and a greater proportion of remaining males breed annually than they do on Cumberland Island (calculated at 63.7% of ad M from data presented in Keiper and Zervanos [1979], Keiper and Hunter [1982], Keiper [1980, 1983, 1984]). Assuming that 70% of males and 85% of females remain on the site and survive to a mean breeding age of 10 and 8 years, respectively, we calculate that a managed Assateague Island National Seashore population should contain at least 72 animals (10 ad M, 26 ad F, and 36 subad) after the breeding season to maintain an effective population of 50 ($\bar{k}_M$ and $\bar{k}_F$ = 1.1 and 0.27, $\bar{l}_M$ and $\bar{l}_F$ = 0.39 and 0.41, respectively). The population in late 1990, following an outbreak of eastern equine encephalitis that killed 30 animals, was estimated at 135 horses (J. Kumer, Assateague Island Natl. Seashore, pers. commun.). The National Park Service recommends that the population be limited to 150 ponies (Keiper 1984).

Application to Chincoteague National Wildlife Refuge.—In contrast to the feral horses on the northern portion of Assateague Island, those on the Chincoteague National Wildlife Refuge are managed through annual removal of foals aged 1–3 months. In 1990, 80 foals, or approximately 95% of the foal crop, were sold at the annual auction (H. Thornton, Chincoteague Volunteer Fire Dep., pers. commun.). Nine of the female foals sold were donated to the fire department and returned to the island, raising the total population to 140 horses (8 stallions, >100 ad mares, limited subad, and an estimated 13 foals [H. Thornton, pers. commun.]). If managers continue to remove most (95%) male foals and to retain about 25% of the female foals, we estimate that an effective population size of 155 horses would limit loss of genetic diversity to <1% per generation (9 ad M, 110 ad F, 36 subad). Currently, managers maintain population size at 150 or fewer animals. Our estimate of minimum population size would be reduced if additional foals were retained or if there was significant dispersal of animals to the Refuge from the herd on the Assateague Island National Seashore. Minimum population size would increase to an estimated 305 individuals if 95% of both male and female foals were removed annually.

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