

**Remove Threats to Irreplaceable Bison Herd
at
Wind Cave National Park**

FY 2006 Challenge Cost Share Program
Final Project Report
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Abstract

The Wind Cave National Park bison herd represents a culturally, historically, and genetically valuable and irreplaceable natural resource. To most effectively manage this critical resource and prevent mixing of trespass bison from the nearby bison herd in Custer State Park, it is necessary to understand the breeding structure of this herd. In this study, we employed a suite of 26 nuclear microsatellite markers to assign parentage for 761 bison born from 1999-2006. The 4 Custer State Park trespass bison evaluated were not assigned to any of the calves in the study, indicating that these bison did not successfully breed and were likely detected shortly after entering the Wind Cave National Park herd. Preliminary parentage analysis reveals differences between males and females in breeding age classes, and among age classes for multiple sire events in a given year. Uneven culling among sex cohorts across years was noted, resulting in large differences in retained allelic diversity across age classes. This study provides critical information regarding the breeding structure bison in Wind Cave National Park, which will be used to model the effects of various management scenarios.

Background

Although more than 500,000 bison (*Bison bison*) exist today, long-term species conservation is not assured (Freese et al. 2007). For example, fewer than 5% of all bison are maintained in conservation herds (Boyd 2003), while the remaining 95% exist in private herds subjected to various levels of artificial selection. Furthermore, DNA from domestic cattle (*Bos taurus*) has been detected in nearly all bison herds examined to date, reflecting the efforts of cattle ranchers to create more robust breeds by hybridizing the two species (Coder 1975). The bison maintained in Canadian and U.S. federal herds are a critical germplasm (DNA) resource for species conservation, since the majority of extant bison are derived from these herds (Coder 1975; Boyd 2003). Of the 11 U.S. federal bison herds, however, only two have neither historical nor genetic evidence of domestic cattle introgression: Yellowstone National Park and Wind Cave National Park (WC; Halbert and Derr 2007). While the Yellowstone National Park herd currently suffers from widespread brucellosis, the WC bison herd has been free of brucellosis for over 20 years and harbors no known diseases at this time. The WC herd has a particularly high value for bison conservation due to the combination of high levels of genetic diversity (Halbert 2003), lack of detectable domestic cattle introgression (Ward et al. 1999; Halbert and Derr 2007), and disease-free status of the herd.

The maintenance of genetic variation is critical to the long-term survival of closed populations (Couvot 2002; Franklin 1980; Lande 1994). Careful management of WC bison as a closed population, including historical, ecological, and genetic considerations, is necessary to maintain the currently high levels of genetic variation (Halbert 2003) in this valuable genetic resource. Unfortunately in the past 5 years there have been several trespass bison detected within the WC bison herd from the neighboring Custer State Park (CSP) herd, a known source of bison/cattle hybrids (Ward et al. 1999; Halbert et al. 2005). It is uncertain how long these animals had been co-mingling with the WC bison herd.

The limited biological resources at Wind Cave National Park result in an estimated bison carrying capacity of 350-400 individuals, and an annual cull is necessary to maintain the herd at this level. Current management of WC bison includes culling of the yearling (approximately 1.5 year-olds) age class each fall with a goal of keeping back 10 males and 10 females of this age class. Other age classes are culled only in special circumstances; for instance, two conservation satellite herds from WC stock have been recently created using both younger (calf) and older age classes. The overall age and sex structure of the herd, however, should approximate a 1:1 sex ratio and equal class sizes for the age cohorts 2.5 years and older. The genetic effects of this management strategy compared with other potential strategies are currently unknown.

Since 1999, WC park personnel have collected blood, hair, and tissue samples from bison during annual roundups and from a handful of individuals in the field following death. It is believed that nearly all individuals from the herd have been sampled. To better understand the breeding structure of the WC bison herd, we use genotypic information from multiple nuclear markers (microsatellites) in this study for reverse pedigree analysis. Analysis of data from multiple calving years (cohorts) will allow for several important issues to be addressed. First, this analysis will identify any calves produced by CSP trespass bison, which is fundamental to maintaining the integrity of the WC bison herd. Second, basic breeding parameters will be accurately measured, such as the number and ages of breeding males and females over time, which will in turn facilitate management of WC bison. The basic data collected from this study will be used in a currently funded project through the National Park Service Biological Resource Management Division to evaluate the long-term genetic effects of various management scenarios and assist the management of this critical bison herd.

Materials and methods

Blood, hair, and/or tissue samples were collected during annual round-ups from 1999-2006 by park personnel. Upon receipt, all samples were given unique laboratory identifiers. Demographic and phenotypic data from WC park personnel was merged into an ACCESS database maintained in our laboratory to allow cross-referencing between datasets. Appendix A lists the samples analyzed in this study and includes both laboratory and field identifiers. DNA was extracted following previously published protocols (Halbert et al. 2004; Halbert and Derr 2007).

All samples were examined for the presence of domestic cattle introgression in the mitochondrial (Ward et al. 1999) and nuclear (Halbert et al. 2005; Halbert and Derr 2007) genomes following published protocols. The mitochondrial assay is a single-tube PCR with a binary categorical outcome (domestic cattle or bison). The 14 microsatellites used to detect nuclear introgression have non-overlapping allele size ranges defining domestic cattle and bison. These nuclear markers are amplified in 3 PCR assays and detected using the methods described below.

The selection of bovine microsatellite markers and description of multiplexed PCR assays with these markers has been previously detailed (Halbert *et al.* 2004). From the original group of 54 markers (Halbert *et al.* 2004), a total of 26 nuclear autosomal microsatellites (different than the introgression detection panel above) were utilized in this study (Table 1). Two additional markers were used to confirm the sex of each individual, but were not otherwise utilized in this analysis: BMS911 on the X-

chromosome and INRA189 on the Y-chromosome. Amplification was performed in 5- μ L volumes in 96- or 384-well plates, and PCR products were separated on an ABI 377, 310, 3100, or 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California). A Rhodamine-X (ROX)-labeled internal size standard (Mapmarker LOW, Bioventures, Inc., Murfreesboro, Tennessee) was utilized for inter-assay standardization. A selected set of reference samples were analyzed on all four systems to determine allelic range differences and standardize allele calling. The reverse primers for BMS410, and BMS527 were 5'-tailed with a viral DNA sequence (GTGTCTT; Brownstein *et al.* 1996) to either facilitate allelic identification or prevent problematic overlapping with other multiplexed markers. The fragment analysis programs Genotyper 3.6 and GeneMapper 3.7 (Applied Biosystems, Foster City, California) were used for allele identification and comparison.

The Microsoft Excel Microsatellite Toolkit (Park 2001) was used to identify duplicates and calculate basic genetic parameters such as heterozygosity and allelic frequencies. A total of 762 bison born from 1999 through 2006 were parentage tested using the program CERVUS 2.0 (Marshall *et al.* 1998). The following simulation parameters were utilized: 50,000 cycles, 10 candidate parents, 97% sampling proportion, 97% proportion loci typed, 1% genotyping error rate, 80% relaxed confidence level, and 95% strict confidence level. For each of the 8 birth cohorts (1999-2006), only individuals which were at least 1 year old during the rut were kept as potential parents (e.g. for calves born in 2006, potential parents were born in 2004 or earlier). Each birth cohort was analyzed separately for maternal, paternal, and biparental assignments with at least 15 genotyped markers necessary for analysis as follows: 1) all calves were compared to all potential dams; 2) all calves were compared to all potential sires; 3) the two most likely dams for each calf were included as known parents and compared to all potential sires, with at least 15 genotyped markers necessary for analysis. Biparental assignments were accepted under the following criteria: LOD score >10 regardless of the number of genotype mismatches; or, LOD score >6 and <5 mismatches; or, LOD score >5 and <4 mismatches; or, LOD score >4 and <3 mismatches; or, LOD score >3 and <2 mismatches. Assignments with LOD scores of <3 were not accepted. If a biparental assignment was not accepted for a calf, the maternal-only and paternal-only runs were checked to see if a dam or sire match was found using the same LOD-mismatch cutoff criteria as above. The stringent criteria used to assign parentage were chosen to minimize type I error (false positives).

Results

A total of 982 samples were initially analyzed. Despite repeated attempts at extracting adequate DNA, sufficient ($>75\%$) genotypes were not obtained from four samples; these poor-quality samples were excluded from analysis. A number of duplicates were identified from the original dataset and were also discarded such that 940 individual bison were analyzed for this study.

Nine bison from this dataset were originally classified as immigrants from CSP. Of these, 5 were found to be most likely misclassified (i.e. of exclusively WC origin): WC1854, WC1856, WC1905, WC1739, and WC1740. The 5 misclassified bison were identified on the basis of duplicate genotypic information from samples collected in previous years, overlooked but matching tag numbers, and/or parentage analysis.

Specific details of these analyses have been provided to park personnel. However, 4 bison were confirmed from CSP: WC1306, WC1736, WC1737, WC1738. Three of these bison harbored alleles not previously identified in WC bison. Neither parents nor offspring for any of these bison were identified within the dataset. Only the remaining 936 bison of exclusively WC origin were included in further analysis.

As in previous reports (Ward et al. 1999; Halbert et al. 2005; Halbert and Derr 2007), domestic cattle introgression was not detected among the WC samples in this study. Each of the WC samples contained bison mitochondrial DNA and no domestic cattle alleles were detected at the 14 microsatellites examined for nuclear introgression.

The current WC herd structure is depicted in Figure 1. Sex ratios, heterozygosity, and allelic diversity (% of total alleles present in cohort) was calculated for each calf cohort from 1999-2006 (Table 2). This analysis reveals two age classes in which one of the sexes was completely removed from the herd: only three males remain from the 2004 cohort, while only nine females remain from the 2002 cohort. Additionally, a ratio of less than 1:2 was noted in the 2005 cohort. These 3 cohorts also contain the smallest numbers of bison and substantially fewer alleles than the other cohorts analyzed (Table 2).

Parentage analysis was performed on 761 bison born from 1999 to 2006. Assignment success varied by year (Table 3), with a larger proportion of biparental assignments in the most recent cohorts (2004-2006). Overall, 13 bison (1.7%) were not assigned to any parents. Dam ages at conception ranged from 1.5 to 23.5 years (Figure 2). Three different dams produced calves at 23.5 years (WC1100, WC1039, and WC1070). The majority of dams were 2.5 to 5.5 years of age at breeding (Figure 2). Sire ages at conception ranged from 1.5 years to 18.5 years, with only a single incident of a bull producing a calf at 18.5 years. The majority of sires were either 1.5 or 5.5-8.5 years of age at breeding (Figure 2).

While most sires produced only a single calf during the study period, a few bulls were very successful at producing multiple calves (Figure 3). In fact, two bulls produced a total of 15 calves each during the study period. Within a given year, bulls produced between 1 and 5 calves each, with an average of 1.5 calves/sire/year. The oldest sires were most likely to produce more than one calf in any given year (Figure 4).

Several cases were noted of a single dam apparently producing 2 calves in a given year. Genotype matches and high LOD scores (>10) indicate these assignments are real. These data indicate possible errors in aging estimates, since in each case one of the calves was aged in the year of birth (probably few errors since calves are easily identifiable) while the other was aged the year following birth (possibly misclassified as 1.5 years of age, when actually 2.5 years of age). Systematic errors in aging estimates further confounds the definition of potential parent pools, especially for the oldest cohorts, and likely contributed to assignment failures in the dataset (Table 3).

Discussion

Parentage analysis based on genotypic information is the gold-standard in evaluating breeding success. This technology has been utilized in other wildlife species, such as deer (DeYoung et al. 2002), elk (Talbot et al. 1996), and bighorn sheep (Coltman et al. 2002). A panel of 15 microsatellites was previously developed to evaluate parentage in bison (Schnabel et al. 2000) and assessed on bison from commercial

breeding operations. This panel of markers was found to be insufficient in parentage determination for the WC herd when neither parent was known *a priori* and especially in cases where only one parent was sampled. In total, 26 markers were used for parentage assessment in this study, including 11 from the Schnabel et al. (2000) panel. Parentage analysis has also been used to evaluate reproductive success in wood bison (*Bison bison athabascae*), a bison subspecies of northern latitudes (WC bison are the plains subspecies, *Bison bison bison*). Wilson et al. (2002) used 21 microsatellite markers to assign parentage for 317 wood bison calves born over a 4-year study period in Elk Island National Park. To date, the present study is the largest known study of wildlife breeding success using genotypic data.

The current demographic structure of the WC herd reveals several age classes with highly skewed sex ratios (Figure 1). The sex ratio at birth for each cohort approximates 1:1, but biased culling has resulted in uneven numbers of males and females for several age classes. Two recent age classes (2004, 2002) contain less than 10 individuals of a single sex and substantially less allelic diversity than other age classes (Table 2). In the short-term, it is clear that these age cohorts will be underrepresented in the breeding pool and do not have the same potential to pass genetic diversity on to other generations as other cohorts. The long-term effects of the age and sex structure of the WC herd are currently unknown, but will be investigated using the data collected in this study.

This study revealed critical information about trespass CSP bison. First, detailed genetic analysis indicated 5 misclassified trespass bison were actually of WC origin. These bison were originally classified as trespass based on missing or misread individual identifiers (tags or microchips). Second, no calves were produced by the 4 confirmed CSP trespass bison, indicating that the WC bison genetic pool has likely not been recently mixed with the CSP lineage.

Differences in breeding age trends were noted between sires and dams (Figure 2), with a more limited reproductive age range noted in males than females. Several females over the age of 20 successfully produced calves, while only two incidences of males producing calves over the age of 13.5 were noted (one 14.5 year-old and one 18.5 year-old). Furthermore, the incidence of multiple sire events was not evenly distributed across age classes (Figure 4), indicating that the older males are more successful at competing and mating multiple females per rut season. Interestingly, both male and female 1.5 year-olds were successfully mated, although age misclassifications may have inflated the actual proportion of 1.5 year-old sires and dams (see below). These breeding trends have important implications for management and provide critical data for defining breeding ages and success rates in modeling management effects.

Future directions

Additional sampling and data analysis from bison collected during the 2007 and 2008 round-ups will further extend this dataset and our understanding of multi-generational breeding structure. As more breeding-age bison are sampled (those that were not sampled as calves), a more complete dataset will be compiled and parentage assignment rates should increase. However, bison in the dataset with misclassified ages will continue to cause “noise” in the dataset and attempts should be made to carefully check age assignments and correct age misclassifications in future round-ups if possible.

Breeding ages and success rates from this study will be used in a currently funded project through the National Park Service Biological Resource Management Division to evaluate the long-term genetic effects of various management scenarios and assist the management of this critical bison herd. Taken together, these studies will provide a solid basis for genetic management of WC bison and will make this herd among the most thoroughly studied wildlife populations in the world.

Following additional sampling and analysis in 2007-2008, at least one manuscript will be prepared for publication in a peer-reviewed scientific journal based on these findings.

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Table 1. Microsatellite marker summary information.

Locus	Chromosome (Position)^a	Size range	# bison alleles^b	# WC alleles^c
BL1036	14 (78.7)	177-193	5	5
BM1225	20 (8.0)	239-273	10	7
BM1706	16 (80.6)	232-254	6	5
BM17132	19 (58.6)	85-95	5	3
BM1862	17 (86.3)	201-215	6	5
BM1905	23 (64.3)	172-184	4	2
BM2113	2 (106.2)	127-153	9	7
BM4107	20 (52.4)	159-185	8	5
BM4311	6 (89.7)	90-104	6	6
BM4440	2 (55.0)	123-143	7	7
BM47	23 (9.1)	103-111	4	3
BM711	8 (83.6)	161-177	6	4
BM720	13 (38.6)	203-235	9	6
BMS1001	27 (5.1)	107-115	5	5
BMS1074	4 (74.9)	152-160	5	4
BMS1315	5 (31.8)	135-149	5	4
BMS1675	27 (64.1)	85-91	4	4
BMS1716	11 (47.7)	185-197	6	4
BMS1857	29 (0.9)	142-170	9	7
BMS410	12 (0.0)	83-97	6	3
BMS510	28 (22.1)	91-95	4	4
BMS527	1 (55.9)	159-177	8	6
HUJ246	3 (67.9)	256-264	5	4
ILSTS102	25 (6.5)	113-153	6	5
RM372	8 (19.1)	114-138	8	8
TGLA122	21 (67.3)	136-150	6	4
Average			6.23	4.88

^a chromosomal positions (cM) as reported in the USDA cattle gene mapping database (<http://www.marc.usda.gov>, last accessed 03-31-07)

^b number of bison alleles across 11 US federal herds (including WC) from Halbert (2003) and Halbert and Derr (unpublished data)

^c number of alleles in WC herd based on 936 bison from this study, with 2 alleles not previously identified in Halbert (2003): BMS1857 allele 162 and BMS527 allele 183

Table 2. Sex ratios and genetic diversity for each WC calf cohort produced 1999-2006 both before and after culling. The data line indicated by “ALL” includes the entire data set (pre-cull) and the current herd (post-cull 2006). Average and standard deviation (SD) calculations taken across the 8 birth-year cohorts.

		PRE-CULL				POST-CULL			
		N	M/F ratio	Heterozygosity	% alleles	N	M/F ratio	Heterozygosity	% alleles
	ALL	936	0.828	0.643	100.0%	389	0.677	0.644	98.4%
COHORT	2006	77	1.081	0.644	96.1%	NA	NA	NA	NA
	2005	120	1.000	0.641	97.6%	17	0.417	0.632	87.4%
	2004	106	0.893	0.642	97.6%	3	3/0	0.729	66.1%
	2003	83	1.024	0.642	96.1%	42	1.211	0.649	95.3%
	2002	104	0.825	0.648	96.9%	9	0/9	0.660	81.1%
	2001	62	1.067	0.639	96.9%	27	1.250	0.644	92.9%
	2000	97	1.064	0.645	97.6%	26	0.733	0.648	91.3%
	1999	112	0.931	0.643	97.6%	41	0.577	0.641	92.9%
	Average	95.13	0.99	0.64	97.0%	28.88	0.80	0.66	87.8%
	SD	19.56	0.09	0.00	0.7%	20.41	0.52	0.03	9.9%

Table 3. Parentage assignment summary statistics, indicating the percentage of total offspring tested which were assigned 2 parents (biparental), a single parent (dam/sire only), or were unassigned.

Cohort	Samples	biparental	dam only	sire only	unassigned
1999	112	26.8%	67.9%	1.8%	3.6%
2000	97	23.7%	72.2%	1.0%	3.1%
2001	62	33.9%	61.3%	3.2%	1.6%
2002	104	44.2%	52.9%	1.0%	1.9%
2003	83	43.4%	51.8%	2.4%	2.4%
2004	106	55.7%	42.5%	1.9%	0.0%
2005	120	50.0%	44.2%	5.0%	0.8%
2006	77	83.1%	14.3%	2.6%	0.0%
All	761	44.5%	51.4%	2.4%	1.7%

Figure 1. Bison herd demography by age and sex classes following 2006 cull.

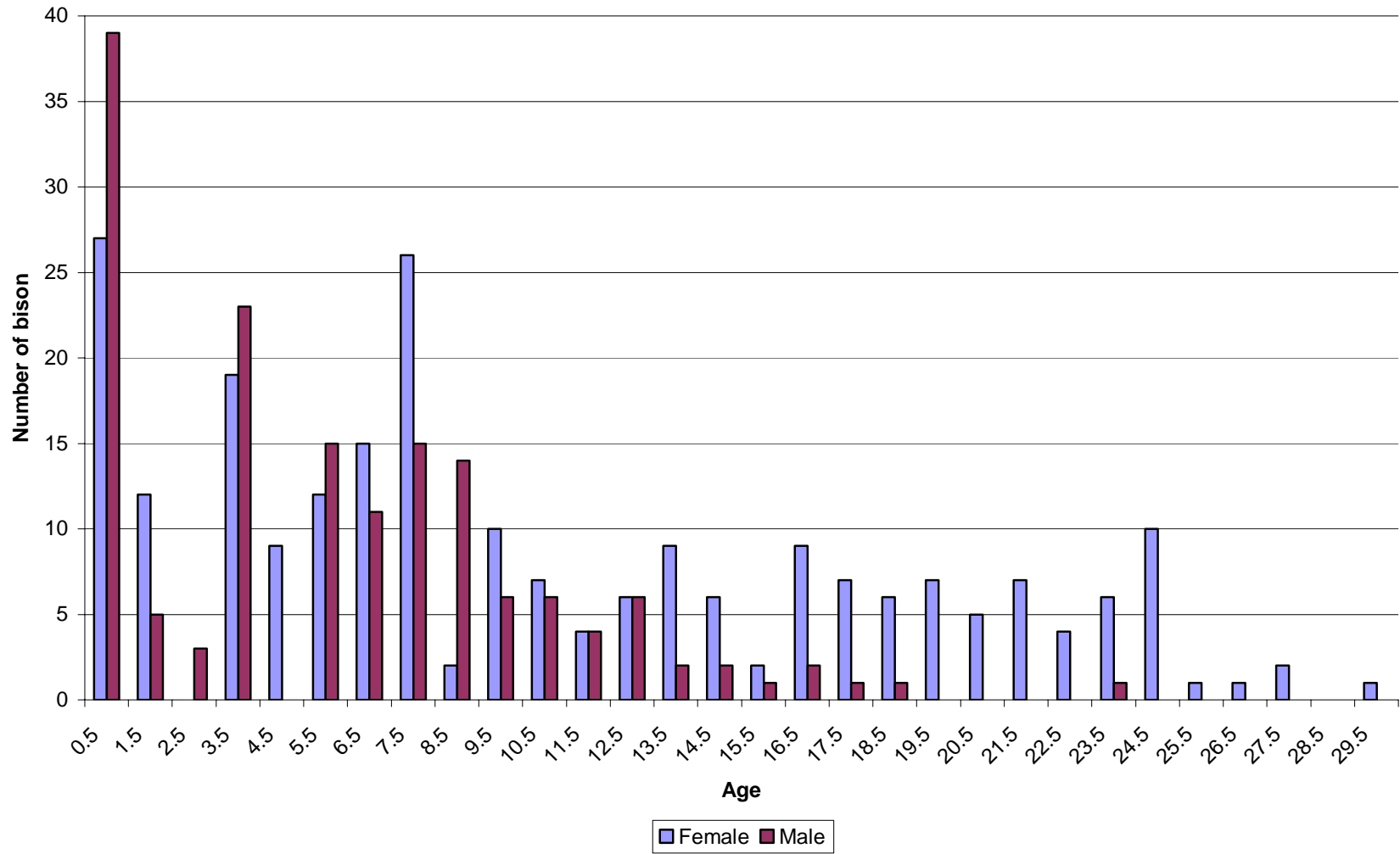


Figure 2. Breeding age of assigned parents, 1999-2006. For each age class, the number of total offspring produced is indicated (e.g., 42 calves were produced by females which were 1.5 years old at breeding.)

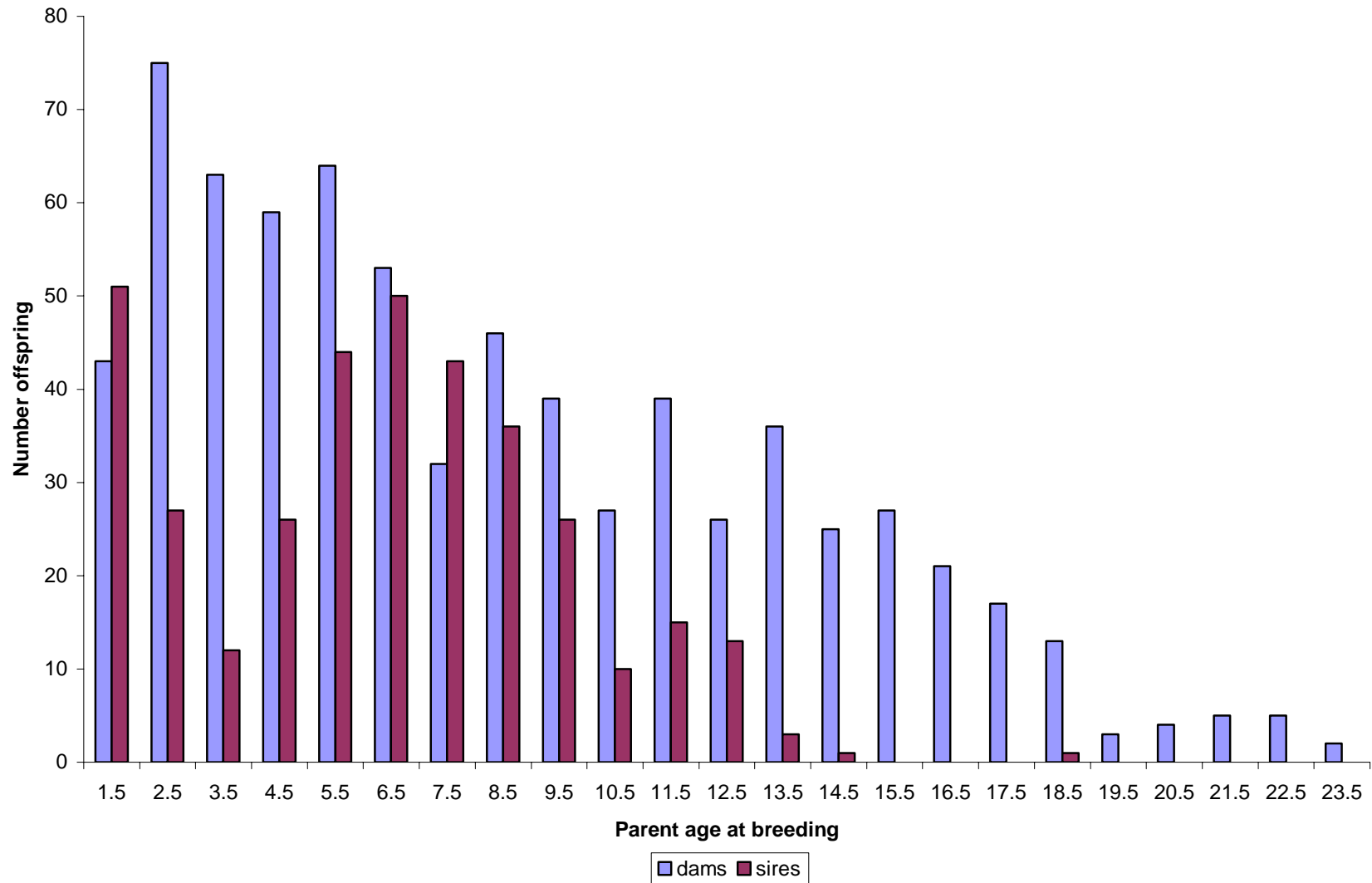


Figure 3. Male breeding success overall 1999-2006 (e.g., 52 males produced a single calf during the study period).

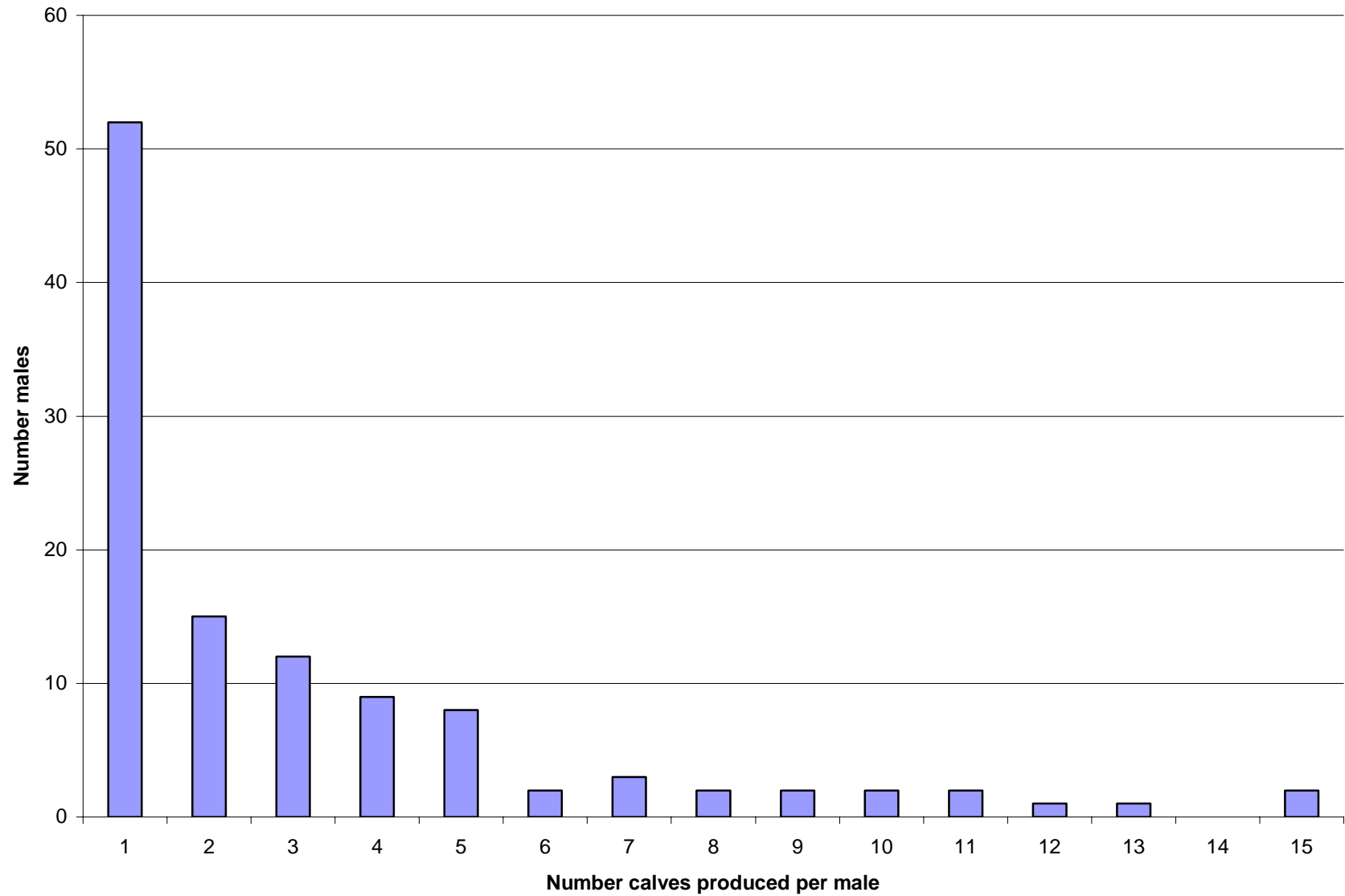


Figure 4. Multiple breeding events per year by sire age, summarized from 1999-2006 (e.g., five 1.5-year-old males produced 2 calves/year).

