

Behavioral versus genetic measures of fitness in bison bulls (*Bison bison*)

MICHAEL S. MOORING* AND M. CECILIA T. PENEDO

Department of Biology, Point Loma Nazarene University, 3900 Lomaland Drive, San Diego, CA 92106, USA (MSM)
Veterinary Genetics Laboratory, University of California, Davis, 1 Shields Avenue, Davis, CA 95617, USA (MCTP)

* Correspondent: mmooring@pointloma.edu

Accurate measures of fitness are important for both basic research on sexual selection and applied conservation actions to promote genetic diversity. For polygynous mammals, good estimates of male reproductive success are often critically important, but especially difficult to obtain. Because the genetic contribution of males is impossible to directly measure in the field, investigators have developed surrogate measures of fitness based on behavioral observations. Such measures are founded on the assumption that observed mating success can reliably predict reproductive success, yet only a few studies have been in a position to validate the accuracy of this assumption. We studied the bison herd at Fort Niobrara National Wildlife Refuge for 8 years, conducting intensive behavioral observations on breeding behavior during the rut (2003–2009) and collecting tissue samples of calves born the following year (2004–2010) for genetic paternity analysis. Our results reveal 2 major trends also observed in other studies: Estimates of mating success were positively correlated with reproductive success when we pooled the entire herd across age classes or years. However, copulatory success did a poor job of predicting the actual number of offspring sired by individual males. For example, 44% of observed matings did not result in the birth of offspring, and 60% of the copulations that did produce a calf did not accurately predict the sire bull. Generalized linear mixed model analysis revealed that observation of mating by a given bull in itself had no predictive power regarding likelihood of paternity, whereas total copulations per season, dominance status, and age of bull or dam significantly influenced the probability of siring offspring. Although use of behavioral data was unable to predict the sire for particular cows, it did give insights into patterns of reproductive success that use of genetic data alone could not provide, such as the role of alternate mating strategies and sperm competition for male reproductive success. We conclude that both behavioral and genetic measures of fitness are needed to understand sexual selection and meet the challenges faced by species of conservation concern.

Key words: behavior, bison, copulations, fitness, genetic parentage, mating success, offspring, paternity, reproductive success, sexual selection

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INTRODUCTION

Reliable measures of individual fitness are needed for understanding sexual selection (Darwin 1871; Andersson 1994) and to better manage threatened species for genetic diversity. Fitness, the contribution made to the gene pool of the next generation, is often operationally defined as the number of surviving offspring (viz., reproductive success), but this is particularly difficult to measure for males of free-ranging, polygynous mammals. Although estimation of female reproductive success can be obtained with relative ease by recording offspring, only genetic paternity data provide incontestable measures of male reproductive success. Yet genetic parentage studies of large, free-ranging polygynous mammals have been limited because of technical and logistical constraints. To study

sexual selection, investigators have long used behavioral measures of reproductive effort as surrogates of fitness (e.g., Maher and Byers 1987; Komers et al. 1992), including ability to hold a harem (Pemberton et al. 1992) or copulation success (e.g., Lott 1979, 1981; Berger and Cunningham 1994; Wolff 1998). These studies often assumed that behavioral measures of mating success were reasonable estimates of reproductive success (e.g., offspring sired).

Estimates of male reproductive success also are needed to address a number of conservation issues, including those facing



American bison (*Bison bison*—Freese et al. 2007; Gates and Aune 2008). For example, the development of specific management recommendations for retaining genetic diversity in bison herds is constrained by the absence of solid data on variation in male lifetime reproductive success (Gross et al. 2006; Dratch and Gogan 2010). The establishment of effective population sizes of bison also is critically limited by a lack of data on the distribution of reproductive contribution among males (Harris and Allendorf 1989; Dratch and Gogan 2010). Also, confident estimates of inbreeding along with recommendations for maintaining genetic variation and inbreeding avoidance in bison require a detailed pedigree of at least several generations along with reliable monitoring of fitness-related traits, such as mortality, natality, and mating success (Halbert et al. 2004; Hedrick 2009). Although bison are more intensively managed than most species, information on the fitness of bison bulls can inform the research programs of other polygynous species of conservation concern.

Review of bison reproductive biology.—Bison exhibit male-dominance polygyny during the breeding season (or rut). Most breeding takes place in July and August (Lott 1981; Meagher 1986), but some breeding continues into the fall and has been observed as late as December. During the rut, bulls move through the herds seeking cows that are approaching estrus by sniffing the anogenital region and performing flehmen. When a cow interests a bull, he tends (guards) her by staying close alongside until she comes into estrus and then mates with her (Lott 2002). During tending, bulls display by bellowing, scent-urination, pawing, rubbing, and wallowing (Wyman et al. 2008, 2012). Tending bulls have high dominance status and elevated levels of androgens and glucocorticoids (Mooring et al. 2004, 2006a); they are frequently challenged by rival bulls surrounding the tending pair (“attending” or “satellite” bulls), and head-to-head fights are common. Most aggressive interactions occur within 15–30 m of the tending pair, with a succession of tending bulls often being displaced by more-dominant bulls before copulation occurs. Tending bulls guard a cow until they breed, are displaced by a more-dominant bull, or lose interest (Wolff 1998). Copulations are quite brief, usually less than 10 s from mount to dismount (Lott 1981). In this study, multiple copulations by the same cow on the same day were rare. During 8 seasons of observations, we only observed 1 cow mate multiple times with different bulls during the same estrous period (in 2004, cow 9456 copulated 3 times with 2 different bulls in a 2.5-h period). In all other cases, multiple mating by cows occurred during different estrous periods separated by an average 21-day interval. Wolff (1998), working at the same study site, observed 3 instances of multiple matings within the same estrous period out of 166 matings over 3 years (1.8%).

Bison are seasonally polyestrous; although cows were once thought to breed only once in a season (Lott 1981; Berger and Cunningham 1991; Wolff 1998), examination of more recent data shows that females can experience up to 2 or 3 estrous cycles per season and thus may mate multiple times (Vervaecke and Schwarzenberger 2006). Immediately follow-

ing a successful copulation, the cow arches her back, expels a small volume of clear or milky secretion from the vulva (presumably vaginal fluids and semen), and erects her tail (Lott 1981; Berger 1989; Berger and Cunningham 1991; Komers et al. 1992; Wolff 1998; Vervaecke and Schwarzenberger 2006). The “tail-up” response is distinctive from tail elevation performed by bison in other contexts (e.g., defecation, urination, or agonistic interactions) in that the angle of the raised tail may be 135° or higher (if 180° is pointing straight up), and the duration of the tail-up can be several days. Previous investigators have reported that the tail-up display is so reliable an indicator of copulation that it can be used to infer copulations not directly observed (Berger 1989; Berger and Cunningham 1991; Wolff 1998; Vervaecke and Schwarzenberger 2006). After copulation, the male usually continues to tend the female for a variable length of time (from 1 h to several days) before searching out new mates (Lott 1981; Wolff 1998).

In prior studies of bison fitness, 3 investigators used behavioral observations to estimate mating success as a surrogate of reproductive success (Lott 1979, 1981; Berger and Cunningham 1994; Wolff 1998), whereas 3 other studies used molecular techniques to assign parentage without behavioral observations (Wilson et al. 2002; Roden et al. 2003; Halbert and Derr 2007). These studies were conducted on conservation herds (bison managed principally for conservation purposes) in national parks or national wildlife refuges with herds of 172–775 bison that contained 22–62 mature bulls (5–6 years old or older). A variety of techniques were used to identify individual bulls: photographs or line drawings of natural features (horns and fur), ear tags, and brands. Using ad libitum or focal sampling (Altmann 1974), the investigators engaged in intensive observations during daylight hours to record copulations directly (mounting) and indirectly (tail-up behavior). The identity of sires was inferred on the basis of copulations and the assumption that most cows only bred once during the breeding season.

Objectives and hypotheses.—The “gold standard” of male behavioral fitness measures is the number of females with which a male copulates (e.g., Say et al. 2003). The measurement of mating success based on copulations must assume that all or most matings are recorded. However, observed copulations can still fail to accurately estimate reproductive success if females mate multiple times with different males (Jennions and Petrie 2000), if some females fail to conceive following copulation (Wilmut et al. 1986), if some males use an alternative mating strategy (e.g., “sneaky” matings) that is less likely to be observed than the dominant strategy (Hogg 1988; Gross 1996), if sperm competition or sperm precedence is practiced (Ginsberg and Huck 1989; Birkhead and Møller 1998), or if spontaneous abortion occurs in some females during pregnancy (Wilmut et al. 1986). Our working hypothesis is that if one or more of the above conditions are present, measures of mating success will be inaccurate estimates of reproductive success. Specifically, any of these conditions will either over- or underestimate measures

of offspring produced as determined by genetic parentage analysis. The purpose of this study was to compare fitness measures of bison males gathered from both behavioral and genetic data in order to test this premise.

MATERIALS AND METHODS

Study site.—The Fort Niobrara National Wildlife Refuge (77 km²) is located along the Niobrara River in the Sandhills of north-central Nebraska (42°53′39″N, 100°28′28″W) and is the site of previous studies of bison reproductive behavior (Maher and Byers 1987; Wolff 1998). The topography of the refuge and surrounding region is flat or rolling hills of native grassland (mixed and sandhill prairie). Established in 1912 as a sanctuary for bison, elk, and native birds, the refuge supports a population of plains bison (*Bison bison bison*) that was managed to maintain 350 individuals (before calving) from 2003 to 2005, and 325 individuals from 2006 to 2011. During the calving season, the herd size increased to around 400–475 individuals. During the spring and summer (April–September), bison grazed over two-thirds of the refuge and were rotated among different fenced units to avoid overgrazing. Unique among public bison herds, every yearling in the Fort Niobrara herd is individually marked with a brand indicating year of birth plus a unique identifier number, allowing individual identification in the field. During the study period (2003–2010), age classes ranged up to 20 years for bulls and up to 23 years for cows. Because 2-year-old bulls occasionally breed and produce offspring, all males 2 years old or older were considered potential breeders. Based on this definition, the herd contained 101–125 potentially breeding males in any given year, along with 112–150 calving-age females (3 years or older), for a sex ratio varying between 0.78 and 0.91 (\bar{X} = 0.84). However, most bulls did not start breeding until age 6. There were 61–84 mature males 6 years old or older in the herd in any given year. For further information on the study site see Mooring et al. (2004, 2006a, 2006b) and Wyman et al. (2008, 2012).

Behavioral observations.—We conducted breeding observations during July and August of each year, which bracketed peak rut for this species (Meagher 1986). We considered the rut to have begun on the day of the 1st observed copulation. The flat or gently rolling hills of prairie vegetation with very few trees provided good visibility from most locations. Observations were conducted within the herd using 4-wheel-drive vehicles to which the bison were well habituated. During the breeding season, 2–4 observers took shifts to maintain continuous surveillance of the herd during daylight hours (0600–2000 h). With the exception of a small exhibition herd near the Visitor Center, the entire herd was maintained in the same grazing unit, and herd members aggregated in 1 or several large groups that could be monitored by 4-wheel-drive vehicle. Observers drove around all parts of the herd so as to account for all tending pairs every 1–2 h. We identified the brand number of tending bulls and cows, recorded tail-ups by cows, documented copulations, and

monitored bull dominance interactions. Noncontinuous observations conducted at night using night-vision equipment (as part of a related study on acoustical communication) confirmed that breeding behavior continued throughout the night, although regular herd observations were not conducted at this time because of the difficulty of reliably identifying individuals.

A tending pair (or “consortship”) was recorded when a bull stood parallel to a cow and followed her movements closely, attempting to exclude competitors from the cow (Lott 1974, 1981). Observations of mating behavior involved 3 different measures: observed, inferred, and ambiguous copulations. Copulations were recorded as “observed” when visually observed from mount to dismount, or as “inferred” when postcopulatory evidence was observed in females that had been tended by the same male before and after this evidence was noted (Berger and Cunningham 1994; Wolff 1998). Postcopulatory evidence included the female displaying an elevated tail (tail-up) accompanied by additional indications of copulation (e.g., swollen vulva, presence of vaginal secretions, frequent squatting, and urination by the cow). Whenever a cow was observed with tail-up, this was noted. When a female was observed with tail-up but the tending bull was different from the bull observed tending in the previous scan, we recorded the copulation as “ambiguous.” Ambiguous copulations were not incorporated into our measure of mating success because, without genetic data, we had no way of knowing which bull was the copulating male.

Agonistic interactions among bulls were opportunistically observed and recorded. Aggressive behavior patterns included supplanting, threats, and overt fighting (Lott 1979). Aggressive interactions between males were recorded whenever one male approached another to within 2 body lengths (< 10 m) and either male turned away using at least 2 steps (Komers et al. 1992). We assumed that the male turning away was displaced by the other one, and the male that supplanted was considered the winner (Komers et al. 1992). Dominance rank was calculated as the proportion of agonistic interactions won, in which dominance rank of a given male is equal to the number of interactions won divided by the total number of interactions won and lost (Berger and Cunningham 1994:149; Mooring et al. 2006a). By this measure, a dominance rank of 1 indicates all wins, a dominance rank of 0 indicates all losses, and a dominance rank of 0.5 indicates an even win–loss record. Dominance rank of males was computed separately for each year and compared with mating and reproductive success.

All behavioral observations were noninvasive, followed guidelines of the American Society of Mammalogists (Sikes et al. 2011), and were approved by the Institutional Animal Care and Use Committee of Point Loma Nazarene University and the University of California, Davis. Behavioral observations were conducted by the 1st author (MSM) and 2–4 student research assistants per year. Every year, the 1st week of fieldwork was devoted to training in behavioral observations. Interindividual reliability tests of 20-min focal animal observations were conducted to ensure that behaviors were

consistently observed and recorded. The correlation coefficient between MSM and students was generally > 0.90 for activity scans, indicating a high level of reliability among observers. Students also were trained in proper reading of brands until they were proficient in accurate identification of individual bison. Although observations during the rut involved breeding behaviors not observed earlier in the season, the initial training sessions and rotation of observational teams during the rut ensured a high level of accuracy in the recording of behavioral data and identification of bison bulls and cows.

Genetic parentage analysis.—Genetic parentage assignments were carried out on the calves born in the year following breeding observations to determine the reproductive success of each bull. Throughout the summers of 2004–2010, tissue biopsies were collected from calves in the herd using biopsy darts (Pneudart Inc., Williamsport, Pennsylvania). Usually, the identity of the dam was recorded based on behavioral observations of nursing behavior in order to save time in the laboratory identifying the dam. Nursing behavior was highly indicative of the mother–offspring relationship, and in only 1 case that we know of did a calf nurse from a female not its dam. In a few cases, nursing behavior was not observed and continuous proximity to the adult female was used to establish the dam–offspring relationship. In either case, subsequent genetic analysis confirmed whether the assignment was accurate, which it generally was. Additional genetic samples (blood or tail hair, or both) from 344 calves were collected during the annual roundup of the herd in late September. Genetic samples of all potential parents (needed for matching parental and calf genotypes) were collected during the roundup or from biopsy darting in the field. Blood samples collected in 2002 for another study ($n = 294$) also were used to establish dams and sires.

Genetic parentage assignments procedure.—Biological material provided for genetic analyses consisted of skin biopsies, hair-root samples, and whole blood in FTA cards (Whatman, Inc., Piscataway, New Jersey). DNA was extracted from biopsies and hair roots by an alkaline lysis procedure (Sancristobal-Gaudy et al. 2000). FTA card punches (1.2 mm in diameter) were processed according to manufacturer's protocols. Forty-four microsatellite markers were amplified in a total of 9 multiplexed polymerase chain reactions containing 1 FTA punch or 3 μ l of extracted DNA at the Veterinary Genetics Laboratory, University of California, Davis. Twelve of these markers (BM1225, BM1706, BM17132, BM4440, BM720, BMS1117, BMS1172, BMS1862, BMS2639, BMS410, BMS527, and RM372) were from the panel developed and validated by Schnabel et al. (2000) for bison parentage analysis. The remaining markers are used at the Veterinary Genetics Laboratory for parentage testing in cattle and bison. Multiplexed polymerase chain reaction products were combined into 5 pools for separation by capillary electrophoresis in ABI 3730 DNA Analyzers (Applied Biosystems, Carlsbad, California). Fragment-size analysis and genotyping were performed with

software STRand (Toonen and Hughes 2001). Details of polymerase chain reaction multiplexes, pooling scheme, marker location on bovine genome, allele size ranges, polymorphism information content (Botstein et al. 1980), and probability of paternity exclusion given a known dam are provided in Supporting Information S1 (DOI: 10.1644/13-MAMM-A-209.S1). Probability of exclusion for each marker and for the whole panel was estimated according to Jamieson and Taylor (1997).

Genepop 4.2.1 software (Rousset 2008) was used with default settings to obtain allele frequencies and to test for Hardy–Weinberg equilibrium for each marker in a set of 205 adults (cows and bulls) from the pool of candidate parents. Bonferroni correction was applied to account for multiple Hardy–Weinberg equilibrium testing. Genotype comparisons were done with computer software developed in house at the Veterinary Genetics Laboratory for parentage analysis of livestock and companion animal species. This software identifies matches and mismatches between parent–offspring pairs or trios but does not estimate paternity probabilities. Parental assignments were made through an exclusion process that required a minimum of 2 loci to exclude individuals as possible sires or dams. Exclusion uses incompatibilities between parents and offspring to reject all but the true parent–offspring pair and is considered the paragon of parentage analysis (Jones and Ardren 2003). Given the large number of markers used, this condition was met for all but 1 case, which we suspect involved bulls related as father and son. Paternity assignments were carried out in a blind manner, that is, without information on possible sire(s) from field observations. Calves were first compared to the given dam to confirm maternity and then potential sires were searched among the pool of candidate males. Parent–offspring mismatches for incompatible homozygous genotypes in either or both BMS3024 and ILSTS5 were not counted as exclusions because null alleles (nonamplifying sequences) were detected in these markers.

Data analysis.—We measured mating success (Copulations) as the number of observed or inferred copulations by a given bull during the rut season. Reproductive success (Offspring) was measured as the total number of offspring sired according to genetic paternity analysis during the breeding season. Mating success and reproductive success were measured for each male for each year of the study. We matched every parentage assignment to our herd behavioral observations for each of the 7 years (2003–2009). Every dam and sire was tracked using detailed tending observations sorted by dam or sire, date, and time, and by the master copulation list assembled throughout each rutting season. Data were first analyzed using the PASW Statistics 18 package for Windows (SPSS, Inc. 2009). Exploratory analysis using Kolmogorov–Smirnov and Shapiro–Wilk tests, plus normal Q–Q plots and normal detrended Q–Q plots indicated that most of our behavioral data were nonnormally distributed. We therefore used nonparametric ranked statistical tests that do not require the assumption of a normal distribution (Siegel and Castellan

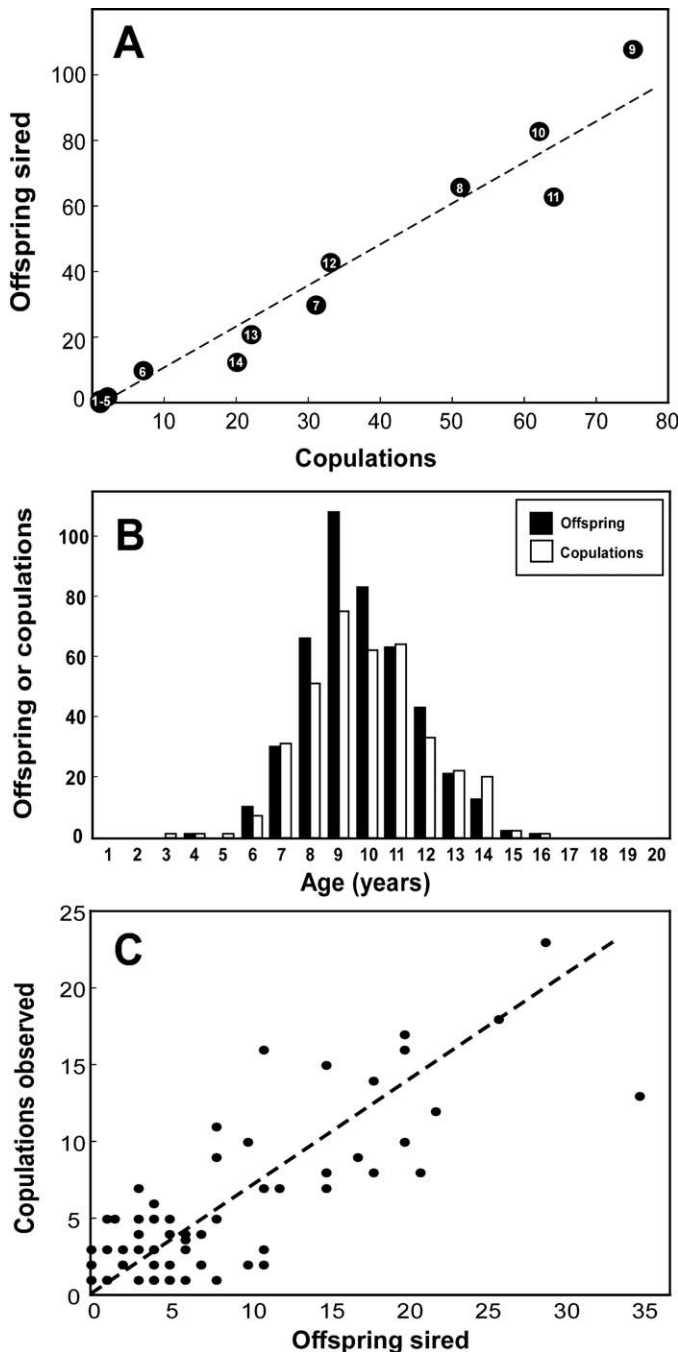


FIG. 1.—A) Positive correlation ($r = 0.98$, $P = 0.0001$) for all copulations versus offspring sired for 14 age classes of bison (*Bison bison*) males pooled across 8 years (2003–2010) at Fort Niobrara National Wildlife Refuge, Nebraska. The age class in years is indicated on each symbol. Note that prime-aged bulls (7–12 years) had the greatest number of copulations and offspring sired. B) Number of all offspring sired from genetic paternity (solid bars) and copulations (open bars) for 70 bison bulls pooled across 8 years (2003–2009). C) Positive correlation ($r = 0.82$, $P = 0.0001$) for all copulations versus offspring sired for 70 bison bulls pooled across 8 years (2003–2009).

1988); Spearman correlation, Mann–Whitney test, Kruskal–Wallis test, and the related samples Wilcoxon signed-ranks test. The level of significance was set at 0.05, and all tests were 2-tailed unless otherwise stated.

We used generalized linear mixed models to determine if observed mating behavior was a good predictor of paternity. The generalized linear mixed model controlled for repeated measures of mating behavior and paternity in different seasons by fitting Bull Identity, Dam Identity, and Season as random effects. Using the R statistical environment (R Development Core Team 2013), we fitted the generalized linear mixed model with a logistic regression and the glmer function of the lme4 library (Bates et al. 2011). The response variable “Paternity” measured whether a given bull sired a calf with a given dam in that year (yes, no). To identify good predictors of Paternity, we considered the fixed effects of Bull Age, Dam Age, Dominance, Copulations, Rivals, and Observed Mating Behavior for every bull–cow interaction observed. Specifically, “Mating” indicated whether the bull associated with a particular cow through an ambiguous, inferred, or observed copulation (yes, no); “Bull Age” was the age of bull in that season; “Dam Age” was the age of cow in that season; “Dominance” was the dominance rank of bull in that season; “Copulations” was the total inferred and observed copulations by the bull in that season; and “Rivals” was the number of prime-aged bulls (7–12 years old) in the herd that season.

We employed 2 approaches in selecting the best model: a chi-square goodness of fit test and an information theoretic approach using Akaike’s information criterion (AIC). These tests were run in R using the drop1 tool. Residual plots showed that there were no systematic errors in the residuals. As with previous analyses, the level of significance was set at 0.05. To determine the best predictors of paternity, we began with a full model without transformations or interaction effects (the “simple model”) and systematically removed single fixed factors in an effort to identify better models. After identifying the best simple model, we added quadratic terms to the age variables and systematically added pairwise interactions among fixed effects until we arrived at a “complex model” that was a slight improvement over the simple model previously found. Because some would argue that the simpler model is more appropriate, we present both models.

RESULTS

Comparison of herd-wide mating success versus reproductive success.—Mating versus reproductive success was analyzed by age class (year) and by bull identification. When we compared the total number of copulations (mating success) and offspring (reproductive success) contributed by each pooled age class for all years (Figs. 1A and 1B), there was a highly significant positive correlation (Spearman rank correlation: $r_s = 0.98$, $n = 14$ age classes, $P = 0.0001$) and no significant difference (related samples Wilcoxon signed-ranks test: $Z = 0.99$, $n = 14$ age classes, $P = 0.32$) between copulations and offspring. When we compared the number of

copulations versus offspring sired by all bulls for all years pooled, there was still a positive correlation (Spearman: $r_s = 0.72$, $n = 70$ bulls, $P = 0.0001$; Fig. 1C), but on average the number of offspring per bull ($\bar{X} \pm SE = 7.8 \pm 0.92$ offspring) exceeded the number of copulations (5.3 ± 0.60 copulations; related-samples Wilcoxon: $Z = 4.24$, $n = 70$ bulls, $P = 0.0001$). However, this herd-wide relationship broke down when examining each individual bull. Averaged across all 8 years, 8.2% (range 0–19% per year) of bulls observed to copulate never sired offspring, whereas 16.5% (range 11–23% per year) of bulls that sired offspring were never observed to copulate. Thus, number of copulations did not reliably predict number of offspring sired for a given bull.

Parentage assignments in relation to observed matings.—Details of the 44-marker panel are summarized in Supporting Information S1. The 44 markers were distributed in 22 chromosomes of which 14 contained 2 or more markers. Among the 44 markers tested, 3 were fixed (BM1818, BM4208, and CSSM36) and did not contribute information for parentage analysis. The remaining 41 markers showed varying number of alleles (2–8) and polymorphism information content values ranging from 0.05 to 0.79. Significant Hardy–Weinberg equilibrium departure was found for BM4107 and ILSTS5 ($P < 0.001$), both explained by heterozygote deficit. The result for ILSTS5 was not unexpected because evidence of null-alleles, that is, single-locus mismatch of homozygous types in parent–offspring pairs, for this and BMS3024 was observed during parentage analysis comparisons, and further confirmed through multiple events of null allele transmission. Evidence for null alleles in BM4107 was not observed. Based on a subset of 28 unlinked markers, that is, located in different chromosomes or separated by ~ 50 centimorgans if syntenic, the combined power of the panel to exclude nonsires given a known dam was estimated to be at least 0.99999 (see Supporting Information S1).

A total of 607 parentage assignments were made for calves conceived during 2003–2009 (born 2004–2010), ranging from a high of 106 (2005) to a low of 58 (2009) per year. Because all possible sires were genotyped, exclusion of all but 1 bull based on the criteria of at least 2 excluding markers, except for the noted exceptions of ILSTS5 and BMS3024, provided strong support of sire assignment to each calf. The number of calves birthed per year (Supporting Information S2, DOI: 10.1644/13-MAMM-A-209.S2) tended to decline during this period due to a reduction in the herd size and declining calving rate for reasons not fully understood (an aging cow population and nutritional deficiencies are under consideration). About 60% of parentage assignments did not conform to the behavioral observations, meaning that the sire bull would have been misidentified in 60% of cases had we relied only upon behavior (Supporting Information S3, DOI: 10.1644/13-MAMM-A-209.S3). Of the nonconforming parentage assignments ($n = 365$), the sire male was never observed tending the dam in 51% of cases, and the cow was never observed with tail-up (indicating copulation) in association with the sire male in another 46% of cases; a small percentage (3%) were unobserved because the

dam and sire were in the small exhibition herd near the Visitor Center, which we did not regularly observe. In contrast, only 40% of parentage assignments ($n = 242$) conformed to the behavioral observations (Supporting Information S3), meaning that we observed the sire male tend the cow, with the cow in turn performing tail-up and other postcopulatory signs in association with the sire bull (either while the sire was tending or with the next tending bull in the case of ambiguous copulations). When the dam was last seen being tended by the sire bull (or another) at the end of the day, and the following morning the cow exhibited tail-up in the presence of another bull (or the sire), we presumed that the copulation had occurred overnight. Approximately half of observed copulations leading to offspring occurred overnight (52% [Supporting Information S2]), indicating that bison performed mating behavior at the same rate day and night.

Observations of mating behavior took 3 different forms: observed, inferred, and ambiguous copulations (Fig. 2A), although only observed and inferred copulations were used to assign mating success to males. These observation types varied in the accuracy with which they predicted the sire male (Fig. 2B; Supporting Information S4, DOI: 10.1644/13-MAMM-A-209.S4). Observed and inferred copulations were the most accurate estimator, successfully predicting the sire male 62% and 64% of the time, respectively. Ambiguous copulations were the least accurate method; in only 43% of cases was the sire male 1 of the 2 bulls indicated as responsible for the copulation.

In addition to examining copulations in which the female gave birth to a calf in the following year, we examined copulations that did not result in offspring. A total of 227 matings were observed over the 7 years that did not result in the birth of offspring (Supporting Information S5, DOI: 10.1644/13-MAMM-A-209.S5). Of these cases, 11% involved observed copulations, 47% were inferred, and 42% were ambiguous. This ratio (11%–47%–42%) was similar to the ratio of observed, inferred, and ambiguous copulations that produced offspring, which was 13%, 46%, and 41%, respectively. When we examined all the females that were observed to mate (either by observing copulations or tail-ups), only 56% actually resulted in the birth of offspring (Fig. 3A). Thus, any time that we observed mating behavior, there was a 44% chance that no calf would be born the following year to that cow.

Generalized linear mixed model analysis: does mating behavior predict paternity?—The information theoretic approach identified the full model as the best simple model ($AIC = 750.55$), although the chi-square test suggested that we could remove the Rivals variable without a significant change in goodness of fit (Supporting Information S6, DOI: 10.1644/13-MAMM-A-209.S6). Because every other model yielded a higher AIC (indicating a worse fit), we retained the full model. The best model fit was then used to assess the relationship between each of the fixed factors and Paternity (Supporting Information S7, DOI: 10.1644/13-MAMM-A-209.S7). For this simple model (and indeed all the models we ran), “Mating”

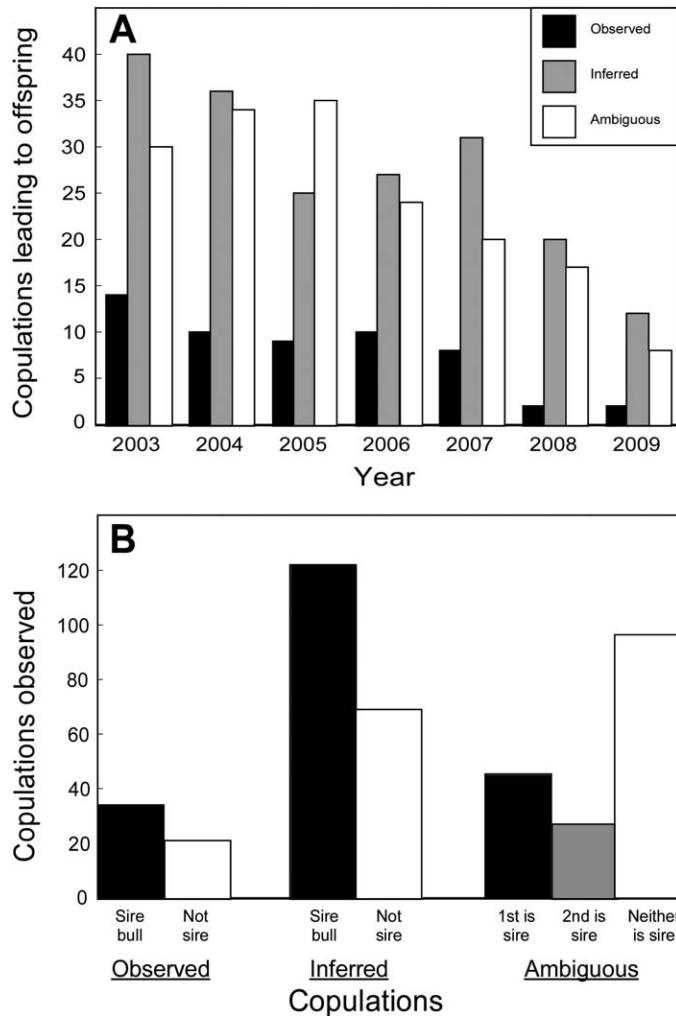


FIG. 2.—A) The number of recorded matings leading to offspring that involved observed (black bars), inferred (gray bars), and ambiguous (open bars) copulations for bison (*Bison bison*) observed at Fort Niobrara National Wildlife Refuge, Nebraska, from 2003 to 2009. B) The number of observed, inferred, and ambiguous copulations that predicted or did not predict the sire male.

had no significant impact on Paternity ($P = 0.99$). “Rivals” also did not appear to influence Paternity ($P = 0.09$), and “Bull Age” was marginally nonsignificant ($P = 0.07$). “Copulations” had the largest single influence on Paternity ($P < 0.0001$), followed by “Dam Age” ($P = 0.0005$) and “Dominance” ($P = 0.02$). Thus, the simple model suggested that breeding-age females that mated with dominant males that had copulated many times over the season were most likely to give birth to offspring, regardless of the age of the sire.

The best complex model utilized quadratic transformations for Bull Age and Dam Age, added an interaction effect between Dominance and Copulations, and removed the Rivals fixed effect (Supporting Information S6). The final model yielded a slightly better fit compared with the best simple model (AIC = 727.74). Interestingly, the influence of the fixed effects on Paternity was very similar to that of the simple model except for age (Supporting Information S7). “Mating”

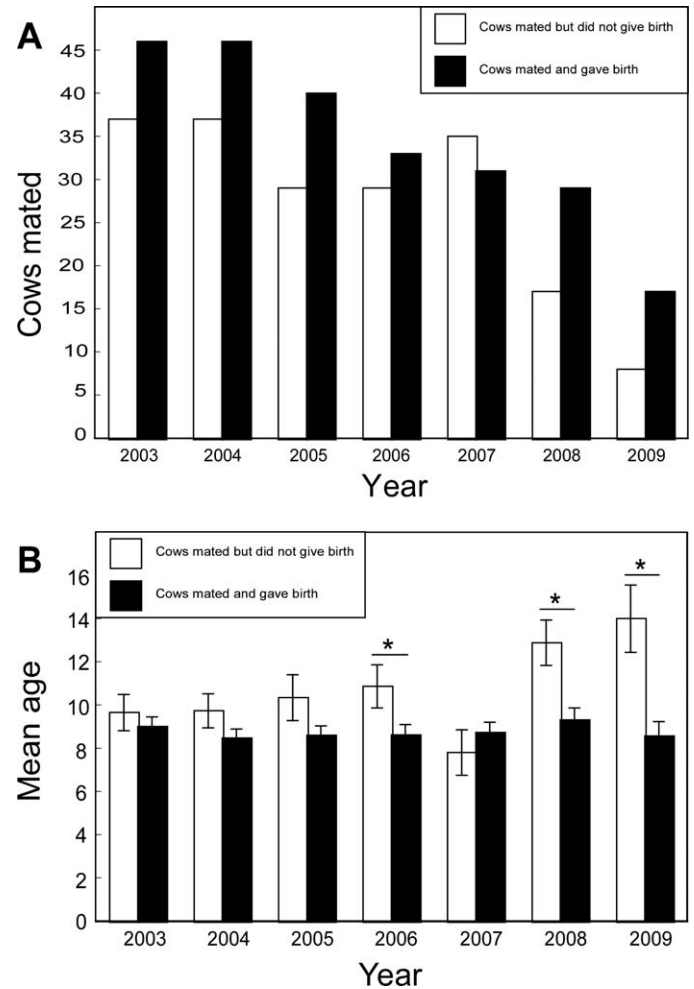


FIG. 3.—A) The number of bison (*Bison bison*) females for which mating was observed that subsequently gave birth (filled bars) or failed to give birth (open bars) at Fort Niobrara National Wildlife Refuge, Nebraska. B) Mean (\pm SEM) age in years of bison females observed mating that did not give birth to a calf (open bars) or did give birth (filled bars). Asterisks (*) indicate a significant difference in age ($P < 0.05$) for the years indicated. Sample sizes: 2003: no calf born = 37, calf born = 100; 2004: 37, 97; 2005: 29, 106; 2006: 29, 87; 2007: 35, 82; 2008: 17, 77; 2009: 8, 58.

again had no significant impact on Paternity ($P = 0.99$), whereas “Copulations” had the largest single influence on Paternity ($P < 0.0001$), followed by “Dominance” ($P = 0.0004$) and “Dominance–Copulations interaction” ($P = 0.0008$). However, in this model Bull Age had a significant influence on Paternity (linear term: $P = 0.001$; quadratic term: $P = 0.002$), whereas Dam Age had no significant influence (linear term: $P = 0.4$; quadratic term: $P = 0.1$), the opposite result from the simple model. There was no significant improvement in the complex model when Dam Age or other fixed effects were individually removed (Supporting Information S8, DOI: 10.1644/13-MAMM-A-209.S8), demonstrating that this was the best model. Thus, the complex model suggested that dominant, prime-aged males that copulated many times over the season were most likely to sire offspring,

regardless of dam age. Although the simple and complex models contradicted each other in regard to the influence of bull and dam age, it makes biological sense that both bull age and dam age should influence the probability of paternity.

Influence of bull age and dam age on paternity.—Despite the disagreement between the 2 best generalized linear mixed models, there is reason to believe that the age of both dam and sire makes a difference in regard to reproductive outcome. For instance, failure of females to conceive following copulation, or to bring a developing fetus to full term, could be due to senescence. This is suggested by the finding that “elderly” cows (≥ 16 years) were less likely to give birth to a calf than “prime”-age (3–5 years) cows (Kruskall–Wallis test: $n = 1,617$, $\chi^2_2 = 387$, $P = 0.0001$; Scheffé multiple comparisons: $P = 0.0001$). Elderly cows had a 36% chance of producing offspring in a given year compared with 69% for prime cows. Thus, one could predict that elderly cows would be less likely to produce offspring following copulation than younger ones. Because this was a directional prediction, we used 1-tailed Mann–Whitney tests to test this hypothesis by comparing the age of cows that mated but did not produce offspring versus cows that mated and produced offspring. Females that did not give birth to a calf in the year following an observed or inferred copulation were significantly older than those that did give birth for years 2006, 2008, and 2009 (Mann–Whitney test: 2006: $Z = 2.05$, $n = 116$ cows, $P = 0.02$; 2008: $Z = 2.71$, $n = 94$ cows, $P = 0.0035$; 2009: $Z = 2.67$, $n = 66$ cows, $P = 0.004$); there was no significant difference in the age of the 2 classes of cows in 2003, 2004, 2005, and 2007 (2003: $P = 0.25$; 2004: $P = 0.10$; 2005: $P = 0.07$; 2007: $P = 0.07$; Fig. 3B). There was a marginally nonsignificant trend for cows that copulated but did not produce offspring to be older than cows that did produce offspring for 6 of the 7 years (binomial test: $n = 7$, $k = 1$, $P = 0.062$).

It also seemed conceivable that older or less-dominant bulls might be less successful in siring offspring; therefore, we tested for any differences in age or dominance rank of bulls that copulated and did (or did not) sire a calf by that cow. Bulls that did not sire offspring following mating were significantly older than those that did sire offspring only in 2006 (Mann–Whitney: $Z = 2.16$, $n = 46$ bulls, $P = 0.015$). However, there was a trend in all years for mated bulls that did not produce offspring to be older (pooled mean = 10.23 years) than bulls that mated and produced offspring (pooled mean = 9.19 years), which was significant by the binomial test ($n = 7$, $k = 0$, $P = 0.008$). There was no significant difference in dominance rank in any year according to the Mann–Whitney test. Finally, an average of 24% (range = 16–34% per year) of bulls that copulated with 1 or more females that gave birth to a calf also mated with a cow that did not give birth the following year, suggesting that it was not necessarily the bulls that were responsible for failure to produce offspring.

Another approach to trying to disentangle the effects of bull and cow age is to consider the possibility of assortative mating. Assortative mating occurs when individuals with certain traits or phenotypes (e.g., age or body size) mate more often with

each other than is expected by chance. For example, in fallow deer (*Dama dama*), yearling females were more likely to mate with younger, less-dominant males than older females (Farrell et al. 2011). However, we saw no indication that bison mated with individuals of similar age. For example, only 3 females conceived as yearlings and of those only 1 mated with a young male (another yearling); the others mated with bulls ranging from 6 to 10 years. Most females first conceived as 2 year olds.

DISCUSSION

In the past, studies of the reproductive biology of bison assumed that behavioral measures of mating success were reasonable estimates of reproductive success (Lott 1979, 1981; Berger and Cunningham 1994; Wolff 1998). Although these seminal studies provided cutting-edge insights into bison behavioral ecology and sexual selection, they did not possess the technological tools to directly measure reproductive success by genetic assignments and thus could not test their assumptions. More recent genetic parentage studies (Wilson et al. 2002; Roden et al. 2003; Halbert and Derr 2007), although providing accurate measures of offspring sired, did not include behavioral observations to estimate mating success. Thus, this is the 1st study to estimate fitness in bison bulls using both behavioral observations and genetic analysis.

Our comparison of behavioral versus genetic measures of fitness reveals 2 distinct patterns. At the herd-wide level, when pooled across age classes or bulls, number of copulations was strongly correlated with number of offspring sired ($r = 0.72$ – 0.98), although copulations underestimated actual offspring sired by prime-aged bulls. However, at the level of the individual bull, observations of mating behavior were a poor predictor of paternity or even whether offspring would result. Considering the intensive dawn-to-dusk observations conducted on this herd throughout the rut and the nearly ideal conditions for observations (limited area, treeless terrain, and ability to use motorized vehicles within the units), it is surprising that 60% of matings were never observed. Even more unexpected was the finding that observed copulations (in which mounting was visually confirmed) were no more accurate a predictor of reproductive success than inferred copulations based on tail-up and other postcopulatory signs. To summarize, 44% of observed matings did not result in the birth of offspring, and 60% of the copulations that did produce a calf did not accurately predict the sire bull. Thus, what appeared to be a good herd-wide association between behavioral and genetic measures was highly misleading regarding the fitness of individual bison.

The results of the generalized linear mixed model analysis highlight a surprising conclusion of our study. Merely observing a single incident of a bull with a cow in a breeding context (observed, inferred, or ambiguous copulation = “Mating”) had no predictive power regarding the likelihood that the bull would subsequently sire a calf birthed by that female. This is supported by our finding that observed mating behavior only yielded a 56% chance that the dam gave birth to

a calf at all, regardless of whether the observed bull was the sire. However, the sum total of all copulations over a season (observed or inferred copulations = “Copulations”) did have a strong influence on the likelihood of paternity. As a measure of the competitive ability of a bull, “Dominance” status also significantly predicted paternity. Finally, the age of the mating bull and cow appeared to play a role in the likelihood of paternity, with indications that older cows and bulls have a lower probability of siring or giving birth to offspring due to the effects of senescence.

Why do copulations not always predict paternity?—The measurement of fitness based on copulations assumes that most events are observed. Our results indicate that this is not the case. Half of all paternity assignments that could be traced to tending by the sire male and postcopulatory behavior of the dam occurred during the night, when direct observations were difficult even with night-vision equipment. Copulations also could go unobserved after observations were completed in late August. Although it is impossible to know with certainty (because parentage assignments do not come with time stamps to indicate the date of conception), observations during the fall and winter of 2004–2005 revealed that breeding continues at a low rate into December. At the National Bison Range, Borgreen (2010) found using ultrasound examinations that 20% of the cows sampled bred after October. Failure to observe all copulations would underestimate measures of mating success, which might partially explain why herd-wide analysis showed copulations to underestimate reproductive success.

Even if all copulations are observed, behavioral measures may fail to accurately predict offspring sired if copulations do not always lead to conception. This can happen if females mate multiple times but conceive only once per season, in which case copulations will overestimate reproductive success. Bison females are seasonally polyestrous and may experience 2 or 3 estrous cycles, mating with different males during each estrous period (Vervaecke and Schwarzenberger 2006). Previous studies reported that 9–15% of breeding females engaged in multiple copulations during different estrous periods (Berger and Cunningham 1994; Wolff 1998), whereas we observed that around 15% of breeding-age cows at Fort Niobrara (range 7.6–23.5% per year) bred 2 or more times during successive estrous periods. In addition, some cows may not conceive because of failure by the bull to fertilize, which also would overestimate behavioral estimates of reproductive success. This may happen if older males become infertile or produce lower-quality sperm (Curren et al. 2013). Also, if males have access to a large number of females and copulate often, the most prolific males can experience sperm depletion (Preston et al. 2001). However, physiological studies indicate that bison bulls produce adequate sperm for breeding throughout the year (Helbig et al. 2007), and bulls at Fort Niobrara were rarely observed to copulate more than 20 times in a season.

Behavioral measures may not accurately predict paternity for other reasons. For instance, when males practice alternative mating strategies that are less likely to be observed than the

dominant strategy, measures of reproductive success based on copulations could be underestimated. Depending on their competitive ability, bulls use different mating strategies to gain access to mates and maximize their reproductive success (Wolff 1998). High-quality bulls defeat rivals and tend as many females as possible (dominant strategy), whereas less-competitive bulls may become “perpetual challengers” that follow tending pairs and opportunistically mate when the dominant bull is distracted (sneaky challenger strategy). There are indications that cows may practice mate selection for sneaky males (Wolff 1998). Because our observations focused on tending pairs, sneaky challengers could have gone unnoticed. On the other hand, sperm competition would overestimate reproductive success due to the displacement of sperm by rival bulls. It is unlikely that sperm competition plays a role in the reproductive success of male bison as it does for bighorn sheep (*Ovis canadensis*—Hogg 1988), because cows rarely mate with a 2nd male during the same estrous period. Finally, spontaneous abortions would tend to overestimate behavioral measures of fitness based on copulations. A recent study monitored the reproductive status of free-ranging bison cows using fecal hormone assays and ultrasound and found that 12–26% of pregnant cows lost their calf during gestation (Borggreen 2010). Our finding that 44% of copulations failed to produce offspring supports a role for spontaneous abortions.

Support from previous studies.—Although only a few studies have attempted to compare behavioral and genetic measures of fitness in polygynous mammals, those that did came to similar conclusions as we did. Although mating success was strongly associated with reproductive success at the population level, observations of the mating behavior of individual males were a poor predictor of paternity. Pemberton et al. (1992) used genetic paternity to investigate behavioral estimates of male mating effort in red deer (*Cervus elaphus*) and found that the probability that a male fathered a calf was closely related to the number of days he held the female in his harem. Although harem membership accurately identified the relative success of males, it was a poor predictor of their absolute success. Amos et al. (1993) compared the breeding behavior and genetic paternity of gray seals (*Halichoerus grypus*) and found that the sire male was near the mother around her estrous period in 89% of cases where paternity was assigned. However, the most likely sire male based on behavioral data was not the genetic father in 36% of cases. Coltman et al. (1999) compared behavioral and genetic measures of fitness in Soay rams (*Ovis aries*) and found a positive correlation between the number of females that a male consorted with and the number of offspring sired, and that males observed in consort with a female were 18 times more likely to have sired her offspring than other candidate rams. Nonetheless, in 73% of the genetic assignments the offspring were sired by a male that was not observed in consort with the female during her estrous period. Finally, Say et al. (2003) examined copulatory success in fallow deer in relation to genetic paternity assignments. The study confirmed that the number of copulations performed by males was “globally” a

good estimator of their reproductive success insofar as males that performed more matings sired more offspring. However, in 16% of cases in which only 1 male was observed with the female, another male had in fact sired the fawn.

A common observation was that behavioral measures underestimated the offspring sired by successful males and overestimated the success of males that sired few or no offspring (red deer [Pemberton et al. 1992], gray seals [Amos et al. 1993], and fallow deer [Say et al. 2003]), as also observed in bison. As a consequence, the variance in male reproductive success based on genetic assignments was greater than what the behavioral estimates of mating success suggested. These studies reported that behavioral estimates of mating success were imprecise because of mating at night, assortative mating among young individuals, and the use of alternative mating strategies by subordinate males, such as sneaky mating strategies (e.g., Hogg 1988; Røed et al. 2005). The authors concluded that behavioral measures of mating effort or mating success could be used to identify the males most likely to father offspring, but these measures were not good at predicting the true reproductive success of males.

Conclusions.—Whenever it has been possible to compare estimates of fitness from observed mating behavior with reproductive success measured by parentage assignments with DNA markers, several conclusions have emerged. First, estimates of mating success correlate with reproductive success to some extent, especially from a population perspective. This is expected, because mating is a prerequisite for siring offspring, and more matings increase the likelihood of successful reproduction. Second, measures based on observed mating behavior are poor predictors of actual reproductive success for individual males, and can be misleading. The reasons for this lack of concordance between behavioral and genetic fitness measures are many and varied, but these factors can be expected to operate in many species of polygynous mammals. Third, use of behavioral data enables patterns to be detected that would go unnoticed in a purely genetic study. Even though parentage analysis using molecular markers is likely to be the only reliable method of accurately estimating fitness in species of polygynous mammals, this does not imply that genetic assignments alone are sufficient for understanding patterns of reproduction and sexual selection. For example, comparisons of both behavioral and genetic fitness measures have provided new insights into the role of alternative mating strategies, sperm competition, and multiple estrous cycles on sexual selection, and have revealed the importance of nighttime copulations and the frequent failure of females to conceive or carry an embryo to term. The goal of most reproduction studies is not simply to find out who the fathers are, but also to gain insights into why some males sire more offspring than others. We contend that a combination of behavioral and genetic data are best suited to clarify sexual selection and address the conservation challenges faced by threatened species of polygynous mammals.

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SUPPORTING INFORMATION

SUPPORTING INFORMATION S1.—Details of 44 markers used for genotyping and parentage analyses.

Found at DOI: 10.1644/13-MAMM-A-209.S1

SUPPORTING INFORMATION S2.—Parentage assignments in relation to behavioral observations.

Found at DOI: 10.1644/13-MAMM-A-209.S2

SUPPORTING INFORMATION S3.—Comparison of copulations leading to offspring (determined by genetic parentage assignments) that were observed or not observed.

Found at DOI: 10.1644/13-MAMM-A-209.S3

SUPPORTING INFORMATION S4.—Copulations from behavioral observations of bison bulls.

Found at DOI: 10.1644/13-MAMM-A-209.S4

SUPPORTING INFORMATION S5.—Copulations observed in which the cow did not later give birth based on behavioral observations.

Found at DOI: 10.1644/13-MAMM-A-209.S5

SUPPORTING INFORMATION S6.—Generalized linear mixed models run to determine the combination of fixed effects that best predict Paternity.

Found at DOI: 10.1644/13-MAMM-A-209.S6

SUPPORTING INFORMATION S7.—Output for generalized linear mixed models.

Found at DOI: 10.1644/13-MAMM-A-209.S7

SUPPORTING INFORMATION S8.—Tests for dropping individual terms from complex model.

Found at DOI: 10.1644/13-MAMM-A-209.S8

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