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**Examination of precopulatory and postcopulatory sexual  
selection mechanisms in the American black bear, *Ursus  
americanus***

By Mark S. Teshera

Thesis

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The Department of Biological Sciences

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## **Abstract**

Most extant bear species are threatened or endangered. One of the species not endangered, the American black bear (*Ursus americanus*), provides a practical reproductive model that can be applied to the conservation of other ursid species, due to their high abundance and similar reproductive biology.

This thesis contains detailed information regarding precopulatory and postcopulatory sexual selection in this species. Specifically, female mate choice, sperm competition, and the possibility of cryptic female choice are examined. This is the first study able to thoroughly examine these reproductive strategies in bears, through the use of an experimental setup. Chapter 1 is comprised of an introduction, experimental aims, and hypotheses. In chapter 2, we demonstrate that females do not select mates based upon morphological traits or social status, and provide evidence indicating that females may use cryptic female choice. Chapter 3 discusses the implications of the results for management of captive and wild bears.

We quantified successful mounts (those that resulted in ejaculation), unsuccessful mounts, quivering durations (a proxy for ejaculation quantity), paternity assessment of embryos, and breeding order of males. Our results indicate that large, prime-age, dominant males were not as strongly selected for as mates as would be expected in the wild. Additionally, males that sired embryos did not ejaculate more than non-sires, which is the opposite trend that

would be expected in the presence of sperm competition. In fact, the prenatal reproductive success of large, prime, dominant males was significantly less than the postnatal reproductive success of these same types of males in the wild, further calling into question the hypothesis that black bears solely use sperm competition. Our results suggest the need for reevaluation of pre- and postcopulatory sexual selection in black bears.

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## Abbreviations and Terms

**Anogenital glands**: located near the female's anal and genital regions, these secrete chemical signals that may signal a female's estrous state

**Average vulva score**: a categorical rank, 0-3, assigned to a female based on the color, visibility and degree of swelling exhibited by her vulva; an average vulva score of  $\geq 2.5$  corresponds with peak estrus

**Cryptic female choice (CFC)**: a hidden, physiological process that serves as a postcopulatory sexual selection strategy. CFC occurs after copulation but before birth, increasing the female's control over her reproductive fitness by allowing her to selectively favor one male's sperm or sired embryo over his competitors within her reproductive tract. CFC can exaggerate or counter male sexual selection strategies such as sperm competition and infanticide

**Delayed implantation (embryonic diapause)**: a physiological process that exists in some carnivores, including bears, in which the blastocyst develops to a certain stage, then enters a period of "suspended animation" during which time it does not implant, but instead remains free-floating in the uterus; throughout diapause, the corpus luteum (CL) secretes only low levels of progesterone; in response to some external cue, hours of daylight for example, the CL reactivates,

the blastocyst implants, and the pregnancy progresses

**Estrus**: behaviorally, that time in the female's ovarian cycle when she is most receptive to mating; physiologically, the point at which the female's vulvar region is at maximum swelling, bright red and highly visible from the rear

**LPD**: large, prime-age, dominant. Large body size in our study was equivalent to a bear with 135-170 kg body mass. Prime age was considered from 7-10 years old. Dominance status was assessed by generating a dominance hierarchy from behavioral observations.

**Microsatellites**: non-coding regions within DNA in which alleles contain variable numbers of base pair repeats; useful in determining paternity

**Multipaternity**: a sexual selection strategy in which offspring from the same litter are sired by different males

**Polyestrus**: when a female comes into estrus more than once in a single mating season

**Quivers**: the rapid, convulsive movements of a male's hindquarters during mating; corresponds with ejaculation

**Semi-free ranging**: descriptive of a captive population housed in relatively large open air enclosures, and with minimal human contact. This type of setting allows for more numerous and broader expression of natural behaviors, while at the same time, less development of stereotypical behaviors associated with stress or other pathologies (i.e. pacing). Semi-free ranging bears are superior to zoo bears for most behavioral studies.

**Sexual size dimorphism**: a distinct difference in body size based upon gender; among mammals, males are usually larger than females, as is the case in American black bears

**Sperm competition**: the sexual selection strategy whereby sperm from different males simultaneously present in the reproductive tract of a female will compete to fertilize the ova. During sperm competition, a sire outcompetes other males by having the highest quality (motility, morphology)/most viable sperm, highest quantity sperm volume, and/or the greatest concentration of sperm.

**Superfetation**: the capability of a female to conceive again while currently pregnant. This results in multiple conceptuses within the womb, which will be born at the same time as part of the same litter, but that differ in total gestational age. This is a very rare phenomena in animals. Black bears are likely capable of this feat due to their unique (among most vertebrates) combination of

sequentially fertile polyestrous and delayed implantation.

## **Chapter 1: Introduction**

Many aspects regarding the breeding behavior and reproductive ecology of the American black bear (*Ursus americanus*) are poorly understood relative to other aspects of the species' biology. The basic mechanisms of their reproductive behavior are known, but they are a difficult animal to study, due to their solitary nature and large home ranges (Powell *et al.* 1997, Lariviere 2001). Body size, age, and dominance are important contributors to reproductive success of free ranging bears (Kovach and Powell 2003, Costello *et al.* 2009), and the dogma is that females select mates based upon these traits. However, females may not need to be selective when considering mates, as they might be capable of employing cryptic female choice (CFC), which is a postcopulatory ability that allows females to select desirable sperm or embryos. Additional research in preferential characteristics for reproductive success is vital to our continued understanding of the behavioral ecology and evolution of sexual selection in this species.

### **Life history of black bears**

There are eight species of bears in the world, with six of these listed as vulnerable or endangered (IUCN, 2016). Bears reproduce slowly, making it difficult to manage their population numbers. The black bear has one of the slowest reproductive rates of any terrestrial mammal in North America, due to its

slow sexual maturity (2-8 years of age), lengthy interbirth interval (1-4 years), and small litter sizes (1-4 cubs) (Pelton 1982, Eiler *et al.* 1989, Lariviere 2001).

Among free ranging populations, these solitary carnivores range in weight from 40-140 kg as adults, with a body length of 1.5-2 m, and they can live over 20 years in the wild (Powell *et al.* 1997). Male black bears are generally solitary, except when seeking out females during the breeding season; sometimes female black bears with cubs form groups with other females possessing cubs (Pelton 1982).

Black bears have large home ranges, with males typically inhabiting a larger area than females (Powell *et al.* 1997). Large home ranges, combined with their solitary nature, means that there will be greater distances between conspecifics. Thus, finding mates may sometimes be difficult. Therefore, a male's larger body size, maintained by a high energetic intake, will enable him to search greater distances in search of mates and so large body size in males should serve as an accurate indicator of fitness.

### **The role of age, body size, and dominance in mate choice**

Each breeding season, male bears will roam in search of females, altering or expanding their home ranges based on female density and dispersal (Powell *et al.* 1997). Prior research has shown that males that are older, larger, and have good fighting ability tend to be more successful in acquiring access to

mates. Costello *et al.* (2009) showed that age was a strong indicator of male reproductive success, and also suggested that body size and fighting ability may also be important for mating. Additionally, Kovach and Powell (2003) conducted a study indicating that large males encountered twice as many receptive females than medium or small males did. In this same study, they determined that mate access was largely determined by fighting ability. They concluded that large, dominant males sired 91% of the cubs produced during that 3 year study. In a black bear population in Long Island, WA, Barber and Lindzey (1986) were able to observe large, dominant males sequester, or mate guard, females against smaller males, thereby limiting mating opportunities for these subordinate bears.

These studies were beneficial for the foundation of my aims and hypotheses, but none of them were able to examine in detail the aspect of female choice, simply because they did not have the facilities and the continuous observation capabilities that this study does. Larger, older, dominant males appear to receive the most breeding opportunities because they out-compete subordinate males. But, if given a choice, does the female actually *prefer* these types of bears, or are they limited to copulating with these larger, older, dominant males when encountering them in the wild during the mating season?

Additionally, these prior studies could not account for unobserved matings, making their studies unable to determine the properties of CFC (although assessing CFC was not a goal of any of these studies). It is possible that nocturnal breedings occurred, which would have been unobserved in our study,

but DNA testing of embryos and cubs would inform us which individual bear was the sire, if any successful unobserved breedings did occur. Moreover, no previous study has examined reproductive success prior to birth and so it remains unknown if CFC mechanisms contribute to postmating sexual selection in bears.

A male bear gains a larger body size and proficient fighting skills as a consequence of aging, so all three factors are interrelated. Females may choose to breed with large, prime, dominant (LPD) bears because these types of males may signify “good genes” (discussed in more detail under the “female sexual selection” sub-heading). Another possible advantage of body size from the female’s perspective is the potentially larger amount of sperm produced by a larger bear. Dewsbury (1982) stated that male ejaculates are energetically costly, so male bears could project a higher degree of “fitness”, because they are able to manufacture more ejaculate. Additionally, larger, older males with good genes that obtain practiced fighting skills can defeat rivals with lower-quality genes in combat, thus proving to the female that he is the most genetically superior male (Andersson 1994). Moreover, Steyaert *et al.* (2012) determined that, in brown bears (*Ursus arctos*), female choice for these traits may be partly due to their indication of genetic quality.

## **Polyestrous female bears and asynchronization of estruses as an adaptive reproductive strategy**

Female black bears are polyestrous (Barber and Lindzey 1986, Gonzales *et al.* 2013), meaning that they come into estrus more than once in a single mating season (estrus is when a female is most receptive to breeding). This strategy provides an adaptive advantage for females, because it provides them maximal opportunities to get pregnant. This can be especially advantageous since males can be dispersed over great distances. Home range overlap for females appears to be highly variable, with minimal overlap (Jonkel and Cowan 1971), low-to-moderate overlap (Schenk *et al.* 1998) and substantial overlap (Reynolds and Beecham 1980, Powell 1987, Horner and Powell 1990) occurring in different populations. When not in close proximity to each other (*i.e.* minimal overlap), females display asynchronous estruses (Jonkel and Cowan 1971). This is also adaptive, because it means that few females are in physiological estrus at any given time, which results in several males competing for breeding rights with each one (Bunnell and Tait 1981). This makes the female a limiting resource, which is why males will travel great distances for the opportunity to breed with them. Furthermore, the result is a male-biased sex ratio, another advantage for the female (Emlen and Oring 1977).

However, when females are housed in close proximity to each other for at least 40 days, their estrous cycles can become synchronous (Gonzales *et al.* 2013). Although most likely to manifest in the dense housing conditions of

captivity, synchronous estrus could happen under certain free-ranging conditions. For example, bears are known to congregate and coexist with minimal aggression where food density is exceptionally high (garbage dumps, salmon runs), including during the mating season (Glenn *et al.* 1976, Lariviere 2001). The ability of females to synchronize estruses when living in close proximity may be a way for them to maximize breeding opportunities while minimizing energetic output. Similarly, plentiful resources could also dictate female congregation to a particular area. Estrus synchronization based on a resource-rich area would be more likely in a wild bear population, as our study bears are not limited by food abundance. Therefore, female density and home range overlap, and/or food availability, may explain differences in whether individual females synchronize estruses within an area.

### **The mating system of black bears**

The mating system of the black bear can be classified as promiscuous (Schenk and Kovacs 1995), meaning that multiple females will mate with multiple males. Typically, the mating season for black bears will be from late spring to early summer (Spady *et al.* 2007). During this time, usually solitary males will seek out female mates. After the breeding season is over, the male will have no further investment in the offspring, with the female providing sole parental care

(Brown 1993). Females do not immediately gestate the embryo, instead using a reproductive tactic known as delayed implantation (Wimsatt 1963).

Delayed implantation is a reproductive phenomenon that occurs when the blastocyst does not immediately implant in the uterus, instead remaining in a state of diapause for an extended period of time before ultimately implanting into the uterine wall (Wimsatt 1963). A blastocyst refers to a ball of cells that has formed after a period of embryonic development, following conception (Schatten and Constantinescu 2007). Embryonic diapause refers to the blastocyst entering a stage of suspended animation; the blastocyst will continue to develop, but at an extremely slow rate (Renfree and Shaw 2000). Therefore, although black bears conceive during the spring/summer months, they do not begin gestating at this time. Gestation only occurs after the blastocyst implants in the uterus, which typically occurs in November or early December (Wimsatt 1963). Upon implantation, a gestation time of approximately two months begins (Foresman and Daniel 1983). Cubs are usually born in January or February (Lariviere 2001, Spady *et al.* 2007).

### **Sperm competition**

Sperm competition can be defined as a male postcopulatory sexual selection strategy, where the male with the “best” sperm will outcompete other males’ sperm (Birkhead and Pizzari 2002). Typically, the male with the highest

quantity, highest quality sperm will be more successful in fertilizing a female's egg (Birkhead and Moller 1998). Individuals from species that use sperm competition can often be identified by visibly large testes (Birkhead and Pizzari 2002, Dixson and Anderson 2004). Spady *et al.* (2007) noted that male black bears' testes grow to a large size during, and then regress after, the breeding season.

In addition to large testes, sperm competition is often associated with species that have obvious sexual dimorphism (males larger than females) (Andersson 1994). The evolution of a larger body size is important to males, due to the advantage gained in intrasexual competition for breeding rights, which can lead to sustained access to females (Andersson 1994). Barber and Lindzey (1986) observed that dominant male black bears can mate guard, thereby limiting mating access of rivals. Therefore, since black bears are a species that exhibits large testes (Spady *et al.* 2007), sexual dimorphism (Kovach and Powell 2003), and mate guarding behavior for prolonged mating (Barber and Lindzey 1986), they are assumed to use sperm competition.

### **Sexually selected infanticide**

While sperm competition is a precopulatory sexual selection strategy used by males, they can also exercise a postcopulatory, and postnatal, sexual selection strategy known as sexually selected infanticide (SSI). SSI occurs when

a male kills a female's offspring, of which he is likely not the father, in order to breed with her himself, ultimately siring his own offspring and passing on his own genes (Hrdy 1979). For male bears, SSI is an advantageous strategy, because it brings a female into estrus sooner than she would otherwise be receptive (McLellan 2005).

Infanticide occurs in black bear populations (Czetwertynski *et al.* 2007), and females may have developed a counterstrategy against it. Steyaert *et al.* (2012) noted that infanticide is common in brown bears, and that female promiscuity may be a way to confuse paternity among potentially infanticidal males. Furthermore, Bellemain *et al.* (2006) investigated a mate choice dilemma in brown bears: should females mate with the fittest male in a population, or mate with many males in close vicinity to her, *i.e.*, males she is likely to encounter in the future? Their results yielded two important findings relevant to this thesis: they found that females preferentially selected for the geographically closest males, and these males more often sired her cubs. But, these sires were also more often the oldest, largest, most heterozygous males. Therefore, Bellemain *et al.* (2006) suggested that, as a counterstrategy to SSI, females mated promiscuously with nearby males likely to be encountered in the future, and then exercised CFC to select the genes from the fittest male(s).

## **Cryptic female choice (CFC)**

CFC is a postcopulatory sexual selection strategy used by females, in which they can choose higher quality sperm to fertilize their egg(s) (pre-conception), and/or higher quality embryos for gestation (post-conception) (Birkhead 1998). Using cryptic female choice, the female can guard against inbreeding, lower-quality genes, and conceptions resulting from situations where the female has no control over which males she breeds with (Eberhard 1996). Sperm competition and cryptic female choice are physiological mechanisms that have evolved as a result of sexual conflict in animals, and are adaptive for each sex as they try to maximize their reproductive fitness (Birkhead and Pizzari 2002).

Relevant to the idea of CFC in black bears is the fact that, unlike birds, reptiles, insects, and arachnids, bears do not possess sperm storage glands (Erickson et al. 1964). Consequently, the reproductive tract of female bears is unable to keep sperm alive, or select for sperm using these glands. In fact, most mammalian sperm cannot survive longer than 7 days (Hafez and Hafez 2000). Therefore, we can rule out delayed fertilization as a method of sperm selection in black bears. Conversely, black bears may be able to use CFC to select for the most fit genes among their mates, and perhaps delayed implantation assists in this process, allowing additional time for pre-implantation offspring assessment.

## Hypotheses

This thesis empirically tested three experimental hypotheses in order to identify the sexual selection strategies employed by American black bears, and to further elucidate the male (sire) characteristics most associated with paternity success.

Hypothesis 1 is that female mate choice is achieved primarily or solely through CFC.

Prediction 1 is that females will not preferentially mate with large, prime-age, dominant males.

Females should not need to be selective with respect to phenotypic mate quality in the presence of CFC. If black bears were largely monogamous, they would not need to use CFC. However, since they mate with multiple partners, including copulation with many subordinate, or “lesser” males observed at our study site, this multiple mating affords the female numerous opportunities to conceive and acquire quality genes, with CFC acting as the final selection mechanism.

In wild grizzly bears, Craighead *et al.* (1995) determined that males are more likely to mate guard when females are more widely dispersed. However, when female densities are greater, males will instead breed with multiple females. Even if males do mate guard, they may not be able to entirely exclude conspecifics. Since females are polyestrous, they may mate with additional

males after the guarding male has departed. Even if a male does successfully guard a female throughout the entire breeding season, which encompasses multiple estruses, then the female still may choose to reject his genes via CFC. Additionally, at our study site, we found that if males did choose to mate guard, it was impossible to entirely exclude rivals. Lesser males still achieved breeding opportunities while the guarding male slept.

Hypothesis 2 is that CFC counters or negates sperm competition.

Prediction 2 is that ejaculate quantity will be similar among males; LPD males will not ejaculate more than other males.

Black bears are assumed to use sperm competition, however, no prior study has been able to quantitatively measure this in wild bear populations. Since our bears display the same mating behaviors seen in wild bears, they provide an accurate representation of actual breeding conditions. Therefore, given our up-close mating observations with respect to ejaculation quantities, we are able to quantitatively assess the properties of sperm competition, and in the presence of CFC, these properties should not be apparent.

The extent of control that females have with respect to CFC is unknown. They may have complete, or partial, control over sperm competition. Due to the unknown extent of control exerted by CFC, it is still logical for males to engage in all forms of intrasexual competition for females. Returning to mate guarding, guards will still be posed with the threat of “sneaky” breeders attempting covert

copulations, and so both guards and sneaks can expect to be faced with sperm competition (Wedell *et al.* 2002). Therefore, we may assume a link between mate guarding and sperm competition. Aspects of both sperm competition and CFC may be present in black bears, but we are suggesting that CFC is at least partially present.

Hypothesis 3 is that CFC enhances male-male competition.

Prediction 3 is that prenatal paternity success will be similar among all males, but postnatal paternity success will be greater for LPD males.

If black bears use sperm competition, we would expect that only males of superior genetic quality would be able to achieve fertilization. But, if females use CFC, we can instead expect that males of lesser genetic quality can also conceive. These embryos will likely be selected against by the female *in utero*, and this is why the majority of postnatal cubs are expected to be sired by LPD males.

LPD males in the wild are known to achieve high postnatal reproductive success rates (Kovach and Powell 2003, Costello *et al.* 2009), and we can logically expect that LPD males from our study site will achieve similar postnatal rates. However, strictly speaking in terms of prenatal reproductive success rates, LPD traits should not matter. We can still expect male-male competition to occur, however, as sperm competition and CFC may not be mutually exclusive.

## **Chapter 2: Analysis of pre- and postcopulatory sexual selection**

*This chapter is a stand-alone manuscript in preparation for publication to the peer-reviewed journal, Behavioral Ecology.*

### **Abstract**

Female American black bears (*Ursus americanus*) are thought to prefer large, prime-age, dominant males when selecting mates. However, if given a free choice of mates between these, as well as smaller, younger, subordinate males, this may not be true. Furthermore, females may exhibit a postcopulatory cryptic female choice, nullifying the importance of precopulatory female mate choice. Controlled mating experiments on semi-free ranging black bears were conducted, wherein females were given a free choice of mates from different cohorts, comprised of males exhibiting variable morphological traits and social status. Genetic paternity of embryos was assessed and compared with the morphological traits of sires. Large, prime, dominant males achieved significantly less successful mounts than our expected value of 91%. Non-sires had a longer mean  $\pm$  SD ejaculation duration than sires ( $t=-2.18$ ,  $df=13$ ,  $p=0.048$ ). Large, prime, dominant males achieved significantly less paternity success when evaluated prenatally, compared to 91% postnatal paternity success. Our results indicate that female black bears do not prefer large, prime, dominant males to

breed with. Furthermore, we suggest that sperm competition may not be a postcopulatory sexual selection strategy used by this species, as females may instead use cryptic female choice to select embryos *in utero*. These findings provide new, key insights into the reproductive physiology of sexual selection in ursids, advancing knowledge in this field and affording better management practices.

## **Introduction**

Precopulatory and postcopulatory sexual selection in the American black bear (*Ursus americanus*) require closer examination, in order to further enhance our understanding of this species' breeding behavior. The basic mechanisms of their reproductive behavior are known, but they remain a difficult animal to study, due to their solitary nature and large home ranges (Powell *et al.* 1997, Lariviere 2001). Nevertheless, the black bear provides a practical model that can be applied to the conservation of other ursid species, due to their high abundance (Pelton 1982) and similar reproductive biology (Spady *et al.* 2007).

While we understand that large, prime-age, dominant (LPD) males experience the most reproductive success (Kovach and Powell 2003, Costello *et al.* 2009), the extent of female preference for these morphological traits remains relatively unknown. However, this study was able to examine the aspect of female choice at a level of detail previously not possible in other studies. LPD males appear to

receive the most breeding opportunities because they out-compete subordinate males. But if given a choice, does the female actually *prefer* these males, or are they simply breeding with them when encountering them in the wild during the mating season, since most subordinate males have been driven off?

In addition to this precopulatory sexual selection, postcopulatory sexual selection may also occur. Female choice may occur at a physiological level post-mating, a postcopulatory sexual selection strategy known as cryptic female choice (CFC). CFC refers to the female's physiological ability, post copulation, to influence the outcome of sperm competition and paternity success, so that her egg(s) cannot be fertilized by inferior males (pre-conception), and/or *in utero* selection against embryos sired by inferior males (post-conception) (Eberhard 1996, Birkhead 1998). This reproductive tactic evolved in response to a male postcopulatory sexual selection strategy known as sperm competition. Sperm competition posits that the males which produce the highest-volume, highest-quality sperm are more likely to out-compete their rivals, and thus achieve fertilization (Birkhead and Moller 1998).

More specifically, the basis of female cryptic choice is that the female can internally eliminate unwanted sperm or embryos from her reproductive tract in order to exert more control over her reproductive success (Birkhead 1998).

Theorized reasons for natural selection favoring cryptic female choice include avoidance of inbreeding, prevention of genetically inferior offspring, and mating systems where females have limited or no control over which males they

copulate with (Eberhard 1996). No prior study has examined reproductive success prior to birth, or been able to account for unobserved matings in bears, and so it remains unknown if CFC mechanisms contribute to postmating sexual selection in bears. This is the first study to empirically test for the presence of CFC in ursids.

This study was comprised of two aims. Aim 1 was to examine what male attributes female black bears prefer when selecting mates. Aim 2 was to determine if CFC exists in black bears. We proposed three hypotheses. First, we hypothesized that female mate choice was achieved primarily or solely through CFC. Second, we hypothesized that CFC would counter or negate sperm competition. Third, we hypothesized that CFC would enhance male-male competition.

## **Methods**

### **Experimental Design**

All research, animal handling, and care activities were conducted in accordance with approved Institutional Animal Care and Use Committee of California State University San Marcos (CSUSM) protocol #'s 09-003 and 13-002. Field work was conducted at a 300 acre private wildlife reserve (44°N), with a semi-free ranging black bear population, from which study animals were selected and shifted to a 305 m x 488 m breeding pen during the mating seasons. Behavioral

observation of bears in the breeding pen was conducted from 5/27/09 to 7/6/09 (0900 – 1700 MT), 5/31/11 to 7/7/11 (0900 – 1730 MT), 6/3/13 to 7/23/13 (0730 – 1930 MT), and 6/1/15 to 7/14/15 (0800 – 1800 MT).

## **Experimental Methods**

The onset of behavioral estrus was determined using behavioral sampling methods (Martin and Bateson 2007), as well as daily visual examination of vulvar swelling, as measured using a subjective ranking system (vulva scores) described in Gonzales et al. (2013). For each year, 6 females were housed in the breeding pen (since April, prior to start of the mating season). Once at least one of these females was determined to be in physiological estrus (an average vulva score of  $\geq 2.5$ ), 2-3 males (one from each age/size category, as best as possible) were admitted to the breeding pen. Males were then observed using focal sampling (1 observer per male) and continuous recording for 8-12 contiguous hours per day. In each of the 2 cohorts of males per season, we admitted 2-3 males. To test hypothesis 1, we recorded the traits of the males that successfully bred, those that did not successfully breed, and male-male social interactions to identify dominant/submissive behavior. For these data, our sample size totaled 21 males from breeding seasons in 2009 ( $n = 4$ ), 2011 ( $n = 5$ ), 2013 ( $n = 6$ ), and 2015 ( $n = 6$ ). This total is not including 2 males, which were removed early (1-2 days), after entering the breeding pen. Behavioral actions

such as bluff charging, charging, jaw popping, guttural vocalization, and fighting were considered examples of aggressive behavior. A dominance interaction was counted when one male clearly displaced another, based on the criteria from our ethogram (Figure 1). During copulation, a successful mount was defined as one that results in ejaculation, as indicated by the quivering of the male's hind legs (Gonzales *et al.* 2013). Behavioral observation of quivering durations was also used as a proxy for ejaculation. To test Aim 2, we analyzed paternity data from 5 males that received successful breeding opportunities from 2009 and 2011. Male cohorts were kept inside the breeding pen for sufficient amounts of time as to ensure that all estrous females had the opportunity to be bred. After the first cohort of males finished breeding with the females (not all males were allowed by females to breed them) they were removed from the breeding pen. After a minimum waiting period of 10 days, a new cohort of males was introduced into the breeding pen with the females. Ten days were given in between male cohorts because mammalian sperm can survive for up to seven days in the oviduct (Hafez and Hafez 2000). In addition, unlike birds and reptiles, black bears do not possess sperm storage glands (Erickson *et al.* 1964) and so are not anticipated to be able to keep sperm alive and dormant for prolonged periods. Accordingly, this 10-day time period allowed us to be absolutely certain that no sperm from the first cohort of males could have survived by the time the second cohort of males were introduced and thereby complicate paternity assessments.

## **Dominance hierarchies**

Dominance hierarchies were created for each male cohort, indicating where each male ranked in order of dominance. For each male, age and body size were estimated, based on 14 years experience by T. Spady with this population. The categories for age were young (4-6 years of age), prime (7-10 years of age), and old (11+ years of age). Our age categorizations were similar to those of Costello *et al.* (2009). These authors classified their males as young (<7 years old) and mature ( $\geq 7$  years old), but gave no description of which age ranges differentiate intermediate from old males. We visually estimated body size, categorizing males as small (35-90 kg), medium (90-135 kg), and large (135-170 kg). These categories for adult black bears were as defined by Kovach and Powell (2003). After totaling the number of male-male dominance displacements, dominance hierarchies were constructed for each cohort of each season (Figure 2). However, for the 2009, 2011, and 2013 field seasons, it should be noted that male dominance interactions were not experimental aspects that were being closely observed during these studies, and so data are sparse for these years.

## **Vulva scores**

Vulva scores, as defined by Gonzales *et al.* (2013), were used to determine when females were in estrus. Each day, observers would independently

determine each bear's vulva score. If a female's average vulva score was  $\geq 2.5$ , then she was considered to be in physiological estrus.

### **DNA Sample Collection**

Before removing males from the breeding pen, skin biopsies were collected from them using DNA darts (Pneu-Dart, Williamsport, PA) and a Telinject rifle (Telinject, Agua Dulce, CA). In the 2009 and 2011 seasons, diapaused embryos were collected for pre-implantation paternity assessment. In addition, whole blood and serum was collected from females during each season (2009, 2011, 2013, and 2015) for genetic testing and other experiments. Diapaused embryos and blood samples were collected from anesthetized females; these embryos were flushed from the uteri after the uterus and ovaries were removed using a laparoscopic-assisted hysterectomy technique described in Himelright et al. (2014). Extracted uteri were rinsed repeatedly and examined under a dissecting microscope; blastocysts were then collected from the uterine horns. Embryo size was measured using an ocular micrometer. Measurements of the zona pellucida, trophoblast, and inner cell mass were recorded to determine what developmental stage the embryos reached. Skin, blood, and embryos were stored in preservative at  $-20^{\circ}\text{C}$  (DNA All Protect; QIAGEN Sciences, Germantown, MD) and after transport to the lab at California State University San

Marcos, the samples were processed and analyzed for paternity assessments as described below.

### **Paternity Analysis**

A QIAGEN DNeasy Blood and Tissue Kit (QIAGEN Sciences, Germantown, MD) was used to extract DNA from the blood of the females, and from the skin biopsies from the adult males. Homogenization and mechanical lysis was achieved, using a mortar and pestle for the skin samples, and a 20-gauge blunt needle syringe for the blood, followed by 22 hour protease K digestion at 37°C. Tetranucleotide primers specific to American black bears were used to magnify polymorphic microsatellites (Meredith *et al.* 2009), using Platinum Blue PCR Supermix (Invitrogen, Grand Island, NY). PCR products were then separated on 2.5% high sieve agarose gels (Fisher Scientific, Fair Lawn, NJ), which were ran at 80 volts for 3-5 hours; an Experion capillary electrophoresis system (Bio-Rad, Hercules, CA) was also used. Male mating partners were already known, so paternity was assigned once the same paternity results were confirmed for each of 2-3 different polymorphic microsatellite primers per cub.

## Statistical Methods

Our independent variables tested included age, body size, and dominance status of males. As we have defined them in our study, age and body size each had 3 levels (age = young, prime, old; size = small, medium, large), and social dominance had 2 levels (subordinate, dominant). Our dependent variables include the specific measures of mate selection (allowed to successfully mount female), prenatal (pre-implantation) paternity success, and postnatal (from published literature) paternity success. To calculate differences between males regarding successful mounts, unsuccessful mounts, embryo sires, breeding order, and quivering durations, we used either one-way ANOVA with Tukey pairwise comparisons or chi-square tests, with a significance level of  $\alpha < 0.05$ . Two sets of chi square analyses were conducted for the measure of % successful mounts, % non-breeding males, and % offspring sired. The first set compared our data to an expected 0.91 proportion, derived from Kovach and Powell (2003) or expected 0.92 proportion (Lacey *et al.* 1997). The second set compared to a null difference expected proportion. Only the expected null difference was used for the % of time males were first in the breeding order, since we had no other sources for comparison in this category. Minitab v16.0 (Minitab, Inc., State College, PA) was used for all statistical analyses. Our ethogram and dominance hierarchies were developed as described in Martin and Bateson (2007).

## Results

### Mate Choice

We conducted two separate sets of chi square analyses of the percentage of total successful mounts of the season(s) obtained by males. In the current study, dominant males successfully mounted females significantly less (Figure 3a;  $X^2=23.86$ ,  $df=1$ ,  $p<0.001$ ) than the 91% expected value intuited from a previous study of paternity success in free-ranging black bears by Kovach and Powell (2003). Similarly, prime males (Figure 3b;  $X^2=15.59$ ,  $df=2$ ,  $p<0.001$ ) and large males (Figure 3c;  $X^2=33.47$ ,  $df=2$ ,  $p<0.001$ ) also had significantly less successful mounts than expected. When further examined within each male category, there was no difference in the percentage of successful mounts between dominant or subordinate males ( $X^2=0.12$ ,  $df=1$ ,  $p>0.7$ ); young, prime, or old males ( $X^2=1.72$ ,  $df=2$ ,  $p>0.3$ ); and small, medium, or large males ( $X^2=1.39$ ,  $df=2$ ,  $p=0.5$ ).

Of the males that did not successfully breed any females (no ejaculation), we found that dominant males were just as likely to fail, and terminate their mount prior to ejaculation, as subordinate males (Figure 4a;  $X^2=0.5$ ,  $df=1$ ,  $p>0.3$ ). Similarly, age (Figure 4b;  $X^2=1.00$ ,  $df=2$ ,  $p>0.5$ ); and body size (Figure 4c;  $X^2=0.25$ ,  $df=2$ ,  $p>0.7$ ) did not appear to affect males' likelihood of failed mount attempts.

## **Sperm Competition**

Of the males permitted by females to achieve successful mounts, non-sires had a longer mean  $\pm$  SD quivering duration (1,253 s) than sires did (967 s) (Figure 5;  $t=-2.18$ ,  $df=13$ ,  $p=0.048$ ).

Of all males that received successful mounts, there was no difference in mean  $\pm$  SD quivering duration between dominant and subordinate males ( $t=-0.22$ ,  $df=28$ ,  $p=0.825$ ); nor between young, prime, and old males ( $F_{2,29}<0.001$ ,  $p=0.998$ ). A modest effect of body size on quivering duration was observed ( $F_{2,29}=4.29$ ,  $p=0.023$ ); small males had longer mean quivering duration than medium males ( $p=0.027$ ) (Figure 6).

## **Cryptic Female Choice**

In the current study, dominant males were not as strongly selected for paternity success when assessed prenatally compared to the 91% expected value for postnatal paternity success (Figure 7a;  $X^2=6.51$ ,  $df=1$ ,  $p<0.05$ ). Similarly, prime males (Figure 7b;  $X^2=25.05$ ,  $df=2$ ,  $p<0.001$ ) and large males (Figure 7c;  $X^2=34.93$ ,  $df=2$ ,  $p<0.001$ ) achieved significantly less than expected paternity success when assessed prenatally. When examined further, there was no difference between the prenatal paternity success of dominant or subordinate males ( $X^2=1.00$ ,  $df=1$ ,  $p>0.3$ ); young, prime, or old males ( $X^2=0.67$ ,  $df=2$ ,  $p>0.7$ ); and small, medium, and large males ( $X^2=2.00$ ,  $df=2$ ,  $p>0.3$ ).

In order to test the hypothesis that mating order influences paternity success, we conducted chi-square analyses based on either an expected proportion based on published literature or on a null assumption (see Methods). Five of 6 pregnant females produced embryos that were sired by the male that bred her first in a given estrus, with first-breeding males siring 7 of 9 embryos (Figure 8). This mating order effect on prenatal paternity in the current study was similar ( $X^2=2.47$ ,  $df=1$ ,  $p>0.1$ ) to the 92% postnatal paternity success documented in arctic ground squirrels (*Spermophilus parryii plesius*) (Lacey *et al.* 1997).

When examined within categories, there was no difference between dominant and subordinate males being the first to successfully breed estrous females (Figure 9a;  $X^2=1.00$ ,  $df=1$ ,  $p>0.3$ ); nor between young, prime, and old males (Figure 9b;  $X^2=0.00$ ,  $df=2$ ,  $p>0.99$ ); nor between small, medium, and large males (Figure 9c;  $X^2=1.5$ ,  $df=2$ ,  $p>0.3$ ).

## **Discussion**

Our results yielded three important findings. First, when females are given a free choice of mates, LPD males are not as strongly selected for as would be expected in a wild setting. Barber and Lindzey (1986) observed that dominant males were able to control access to females via mate guarding. However, while some females in our study seemed to show preference for certain males, they did not appear to select them based on dominance, age, or size. This suggests

that there is considerable plasticity in how females choose mates, and the reason for this is unknown. Females may simply be opportunistic breeders.

Alternatively, female preference for certain males could be the effect of pheromones, which are key olfactory indicators important to reproductive assessment in mammals (Dehnhard *et al.* 2006). By evaluating male pheromones, females may be able to determine behavioral and physiological condition of potential mates (Dehnhard *et al.* 2006, Swaisgood *et al.* 2000). This chemical communication has been suggested as a factor which may govern mate selection in the giant panda (*Ailuropoda melanoleuca*) (Swaisgood *et al.* 2000). More recently, both behavioral and anatomical evidence of intra and intersexual chemical communication in black bears and related ursids is mounting (Rosell *et al.* 2011, Gonzales *et al.* 2013).

Second, males that sired embryos did not ejaculate more than non-sires. On the contrary, non-sires actually ejaculated slightly longer than sires, with dominance, age, and body size having no effect on quivering duration. This indicates that sperm competition may not be an important factor in determining which males achieve reproductive success. Although not conclusively proven, black bears are thought to use sperm competition (Schenk and Kovacs 1995, Kovach and Powell 2003). Sperm competition is a postcopulatory sexual selection strategy in which sperm from different males compete inside the uterus or oviducts for the rights to fertilize an egg (Stockley and Purvis 1993). During sperm competition, males that produce the highest-volume, highest-quality sperm are more likely to out-

compete their rivals, and thus achieve fertilization (Birkhead and Moller 1998). Since black bears are assumed to use sperm competition, then we would expect the largest (and likely older and dominant) males to produce the largest volume of quality sperm, simply because they have larger testes. However, using quivering duration as a proxy for ejaculation quantity, we did not see this expected trend in reproductive success in relation to quantity of ejaculation. Additionally, because males in each category were sires, reproductive success was also not likely due to sperm quality differences based on male category. Females may have evolved CFC to counter sperm competition, nullifying its effects. There may also be elements of both postcopulatory sexual selection strategies present, and more research is needed to make a better determination.

Third, the *in utero* reproductive success of LPD males was much less than that of postnatal reproductive success of LPD males. Interestingly, only mate order appeared to have strong influence determining which bear conceived. This suggests that mate order, rather than male characteristics, is the most important factor determining reproductive success. Additionally, CFC could be the ultimate mechanism influencing final reproductive success, because there was a much greater preferential paternity success for LPD males when assessed postnatal but not when assessed prenatal. If sperm competition exists in black bears, we would have expected to see a prenatal reproductive success percentage similar to that reported for postnatal reproductive success by Kovach and Powell (2003), since the best sperm would theoretically outcompete less fit sperm. To the

contrary, DNA analysis confirmed that a male from 2011, classified as young, small, and subordinate, achieved prenatal reproductive success by fertilizing one of the females' eggs. Therefore, we might conclude that if females use CFC, they may select against genetically inferior embryos, rather than inferior sperm.

In addition to these results, certain aspects of the black bear's reproductive physiology would seem to complement cryptic female choice. First, there is the fact that this species is polyestrous. This could be a way for a female to have a "do-over" during the breeding season. For example, if a female breeds with a low quality male during her first estrus, she may receive the opportunity to breed with higher quality males during subsequent estruses. Therefore, if she has a lower quality embryo and a higher quality embryo to choose from for implantation, she has the option to select the higher quality embryo using CFC. In further support of this idea, Himelright *et al.* (2014) determined that, in American black bears, each estrus is equally and independently fertile. Their study also suggested that black bears may be capable of superfetation.

Superfetation is when an animal becomes pregnant, and then becomes pregnant again during a subsequent estrus (Roellig *et al.* 2011). Superfetation has been proven to exist in the American mink (*Mustela vison*), which is another polyestrous, litter-bearing carnivore that exhibits delayed implantation (Shackelford 1952).

Another physiological process that bears possess which may facilitate CFC is delayed implantation. The delay in uterine implantation may be affording the

female additional time for embryo assessment, while they remain in a state of diapause. Perhaps while the female is preparing her body for hibernation, she is also assessing the quality of her embryos during this time. All of these reproductive aspects could help facilitate cryptic female choice, by giving the female multiple opportunities to acquire quality male genes. For example, if the female initially mates with a subordinate male, and then obtains better male genes during subsequent breedings, she will still have the choice regarding whether to gestate a subordinate male's embryo, or abort it. If the female does not gain better male genes (*i.e.* no subsequent breedings or no subsequent conceptions), then she can still gestate the initial embryo(s) that she has, if she chooses to do so.

If CFC exists in bears, it may help explain certain unresolved mysteries about bear reproductive ecology. Miller and Waits (2003) determined that in a small, isolated population of grizzly bears, where genetic variability was hypothesized to be extremely low, genetic testing showed a much higher genetic diversity than previously feared. Could this be explained by CFC actively rejecting some or most of the genetically incompatible (*i.e.* related) embryos, and instead choosing more heterozygous embryos? Another aspect to consider in relation to CFC is sexually selected infanticide (SSI). SSI occurs when males kill other males' offspring, in an effort to breed with the mother and pass on their own genes (Hrdy 1979). Bellemain *et al.* (2006) concluded that female brown bears may employ a strategy of multiple mating, in order to confuse paternity among potentially

infanticidal males, and ultimately use CFC to select embryos.

Our results indicate that American black bears may employ CFC, especially when considered in conjunction with this species' reproductive physiology.

Further research will be necessary in order to definitively prove that CFC exists in black bears. If proven to be true, this invaluable knowledge would advance the management, conservation, and captive breeding of ursids, as there still remains much we do not know about bear reproductive ecology and the evolution of sexual selection.

## Black bear dominance behavior ethogram

The following criteria were used to score dominance in males:

1. An interaction began when one male bear interacted with another male bear (aggressive popping, guttural grunting, huffing, bluff charging, charging, stiff-legged walking, stomping/swatting the ground, staring, open-mouthed displays, physical fighting). Any of these aggressive behaviors, when directed from one male to another, that caused the “loser” to retreat (displaced), was counted as a dominance interaction.
2. If a bear charged another and caused him to retreat, but then that bear (the initial loser) initiated his own charge (causing the initial charging bear to retreat) (known as a dominance reversal), then this was considered two interactions. In the first interaction, the charging bear was the victor. In the second interaction, the initial loser became the victor. Both bears would therefore be scored as victors for their respective victorious interactions.
3. Bears clearly could not be communicating with each other anymore for an interaction to be considered over. If charging, fighting, *etc.* was over, but there was still eye contact, noises, *etc.* being directed from one bear to the other, then this encounter was still ongoing. Only when all eye contact/communication ceased between the bears would the encounter be considered over. Following the end of such an encounter, another encounter could begin immediately after, and this was considered a new, separate encounter.
4. A dominance interaction could only be considered to have occurred if there was a clear winner and loser. If the observer thought that the result of the interaction was ambiguous or unclear in any way, then this interaction would not be counted (*i.e.* if a male was sitting near a den and another male walked by, causing the sitting bear to huff at him, and the walking bear did not stop or respond in any way, and passed by without incident, then this was not an interaction (in terms of the dominance hierarchy).
5. Displacement could also occur if one bear was wary of another (*i.e.* if two bears were approaching each other and the subordinate bear altered his path to give the dominant bear a wide berth, the subordinate bear ran away huffing, or even if the subordinate bear was copulating with a female and fled upon approach from a more dominant bear).

**Figure 1:** Ethogram, defining the criteria used to determine when a male-male dominance displacement occurred. The total number of dominance displacements, based upon the requirements from the ethogram, was used to construct dominance hierarchies.

a

		M2-09	M4-09	M3-09	M5-09
# of times individual supplanted another	M2-09	N/A	N/A	12	N/A
	M4-09	N/A	N/A	N/A	1
	M3-09	1	N/A	N/A	N/A
	M5-09	N/A	0	N/A	N/A

# of times individual was  
supplanted by another

b

		M4-11	M1-11	M5-11	M6-11	M3-11
# of times individual supplanted another	M4-11	N/A	N/A	17	28	N/A
	M1-11	N/A	N/A	N/A	N/A	6
	M5-11	9	N/A	N/A	N/A	N/A
	M6-11	2	N/A	N/A	N/A	N/A
	M3-11	N/A	0	N/A	N/A	N/A

# of times individual was  
supplanted by another

c

		M4-13	M1-13	M3-13	M2-13	M5-13	M6-13
# of times individual supplanted another	M4-13	N/A	N/A	N/A	N/A	48	25
	M1-13	N/A	N/A	110	5	N/A	N/A
	M3-13	N/A	6	N/A	0	N/A	N/A
	M2-13	N/A	2	0	N/A	N/A	N/A
	M5-13	0	N/A	N/A	N/A	N/A	30
	M6-13	0	N/A	N/A	N/A	0	N/A

# of times individual was  
supplanted by another

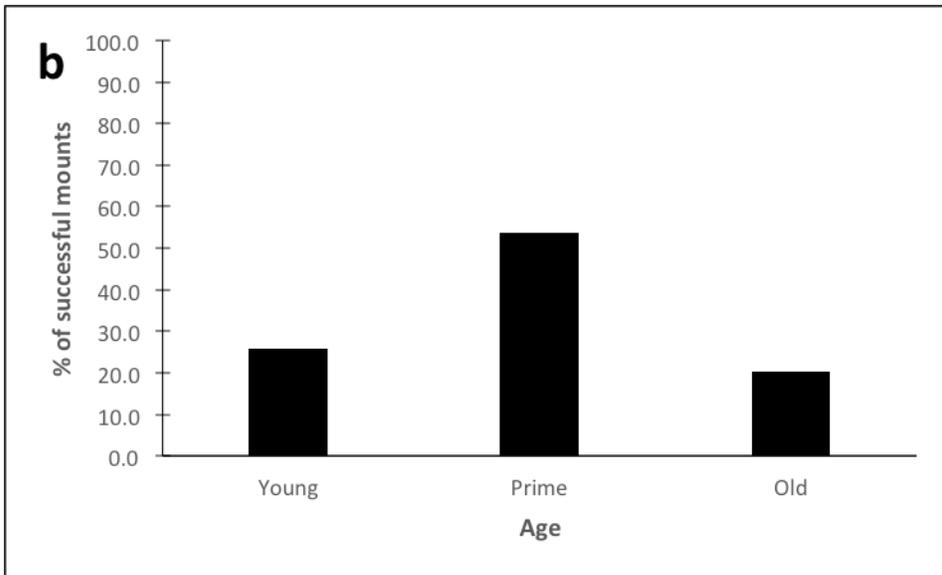
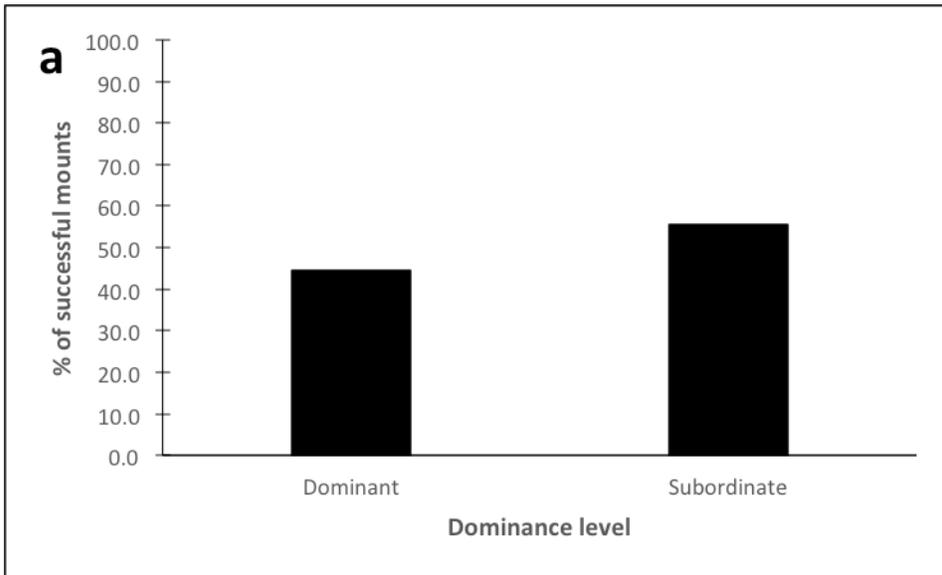
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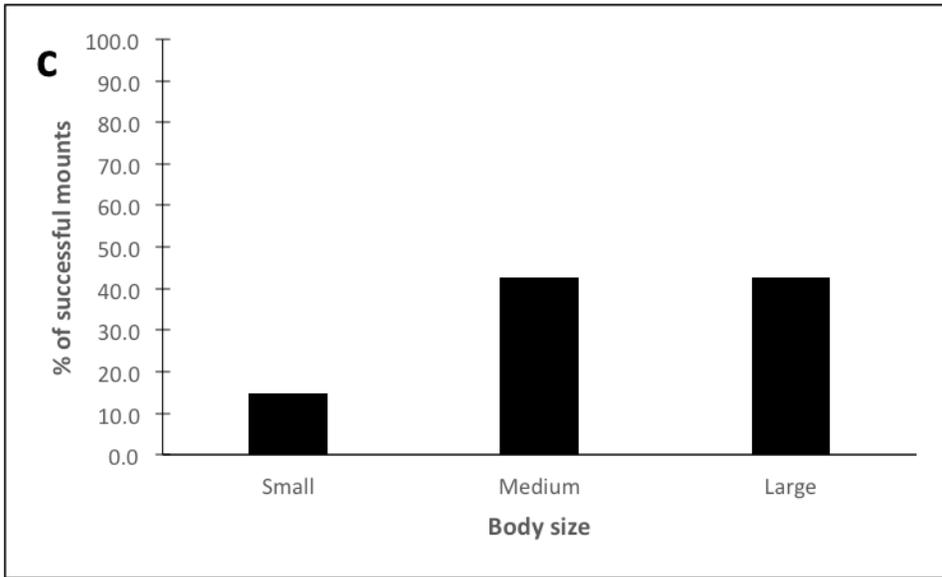
		M3-15	M6-15	M1-15	M4-15	M5-15	M2-15
# of times individual supplanted another	M3-15	N/A	N/A	64	N/A	N/A	37
	M6-15	N/A	N/A	N/A	0	29	N/A
	M1-15	0	N/A	N/A	N/A	N/A	9
	M4-15	N/A	0	N/A	N/A	7	N/A
	M5-15	N/A	0	N/A	4	N/A	N/A
	M2-15	0	N/A	3	N/A	N/A	N/A

# of times individual was  
supplanted by another

**Figure 2:** Dominance hierarchies for male cohorts, indicating the number of observed male-male dominance interactions. The males are listed in order of dominance, from most dominant to least dominant (left to right on top column, top to bottom on side column). The respective cohorts are from 2009 (2a), 2011 (2b), 2013 (2c), and 2015 (2d)

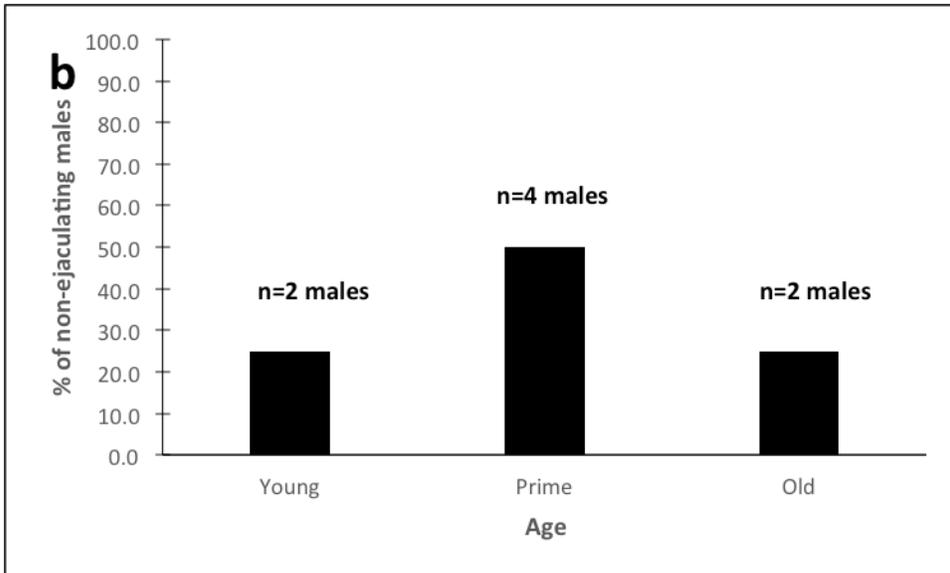
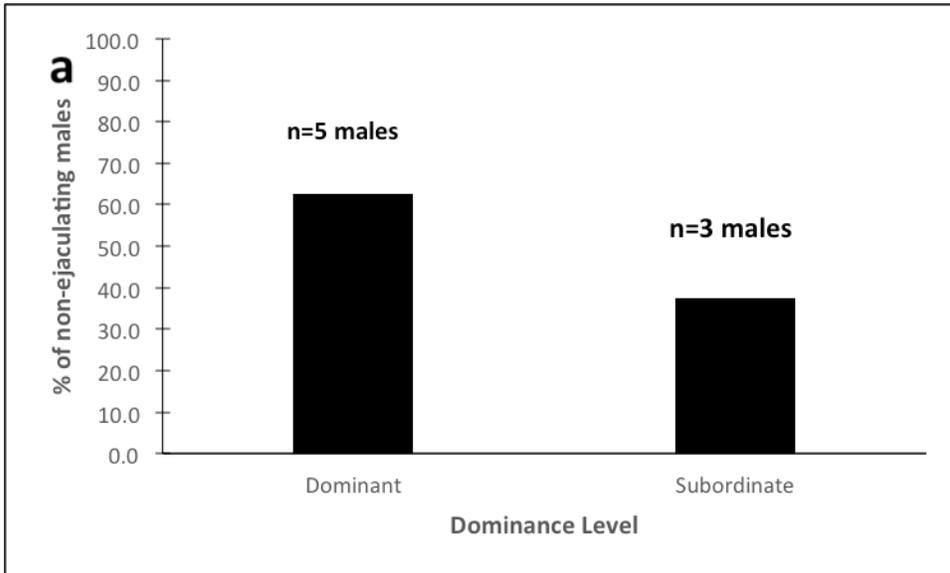
**Figure 3**

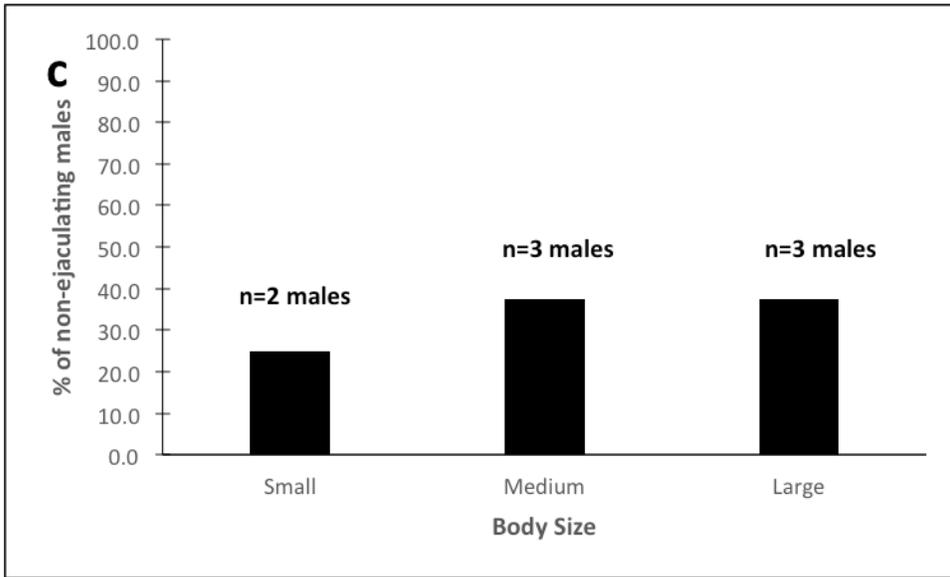




**Figure 3:** Percentage comparison of successful mounts achieved by males, categorized by dominance level (3a), age (3b), and body size (3c).

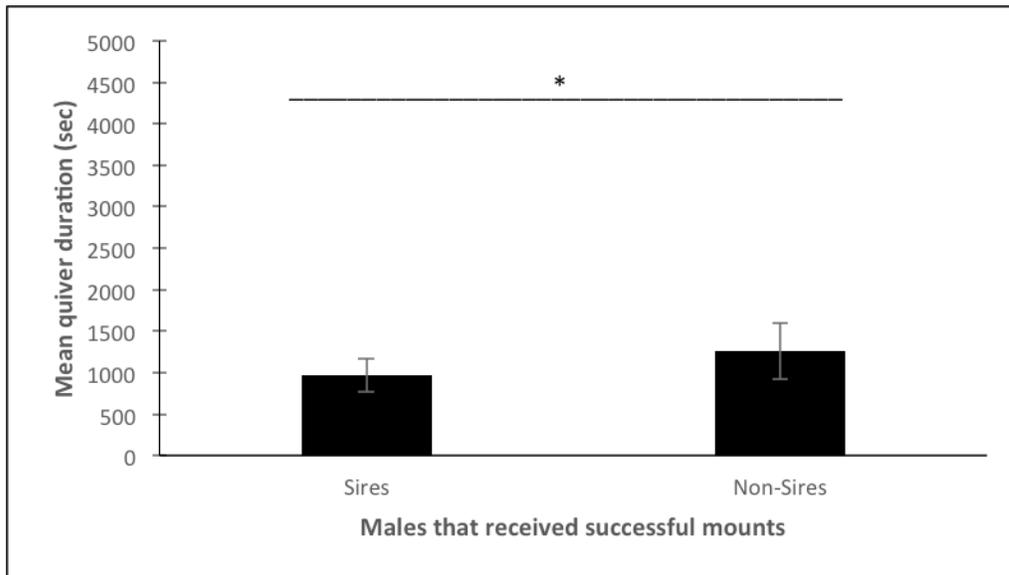
**Figure 4**





**Figure 4:** Percentage comparison of males that did not successfully breed any females (no ejaculation), categorized by dominance level (4a), age (4b), and body size (4c).

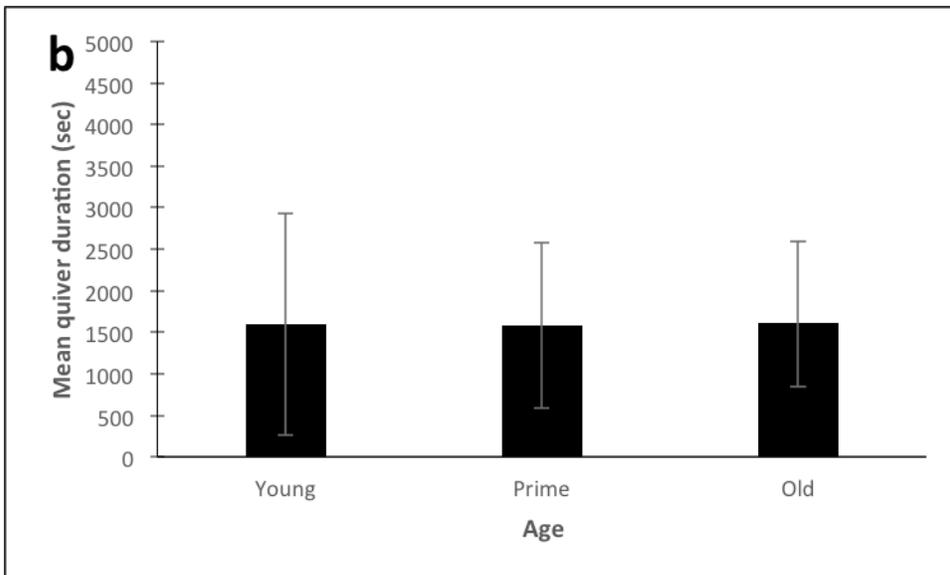
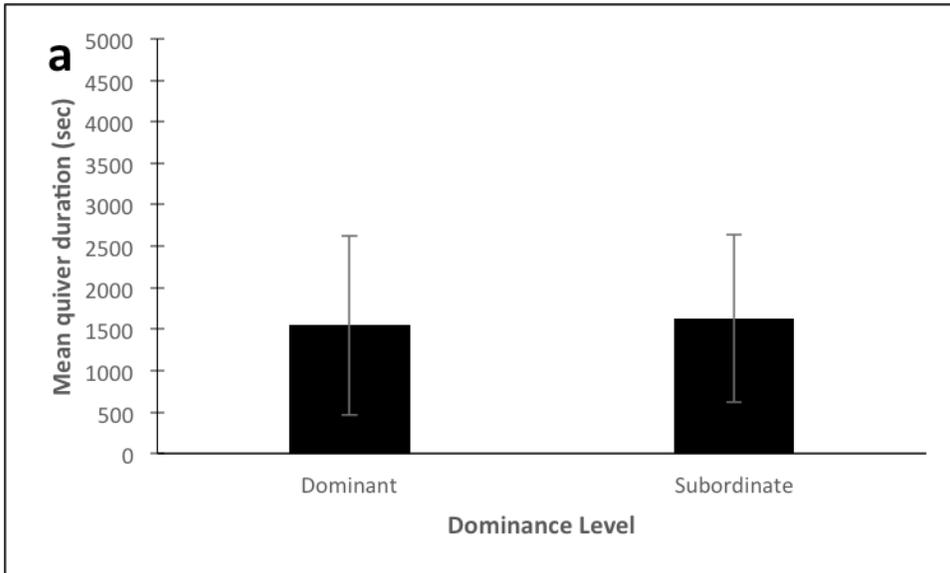
**Figure 5**

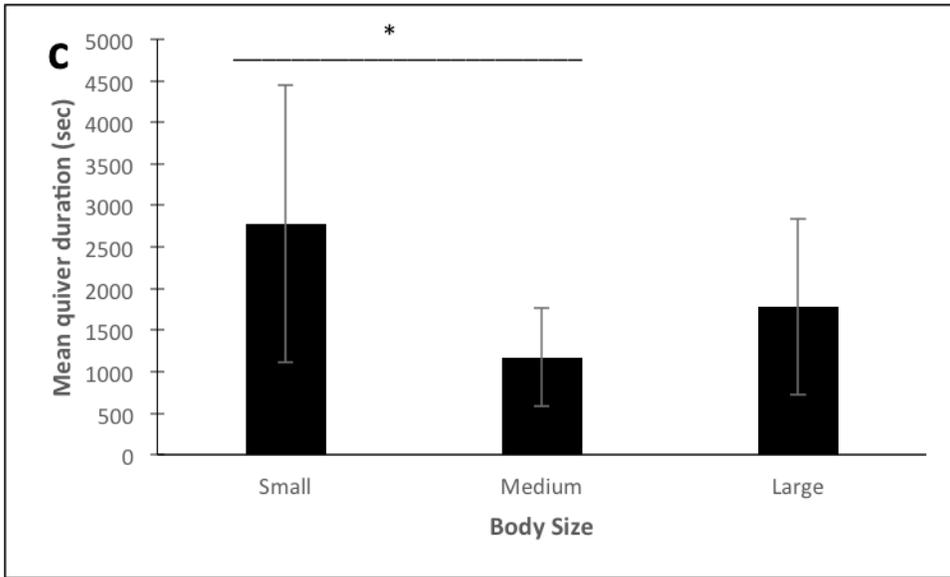


**Figure 5:** Comparison of mean +/- SD quiver duration of both sires and non-sires during successful mounts.

\* = statistically significant ( $p < 0.05$ )

**Figure 6**

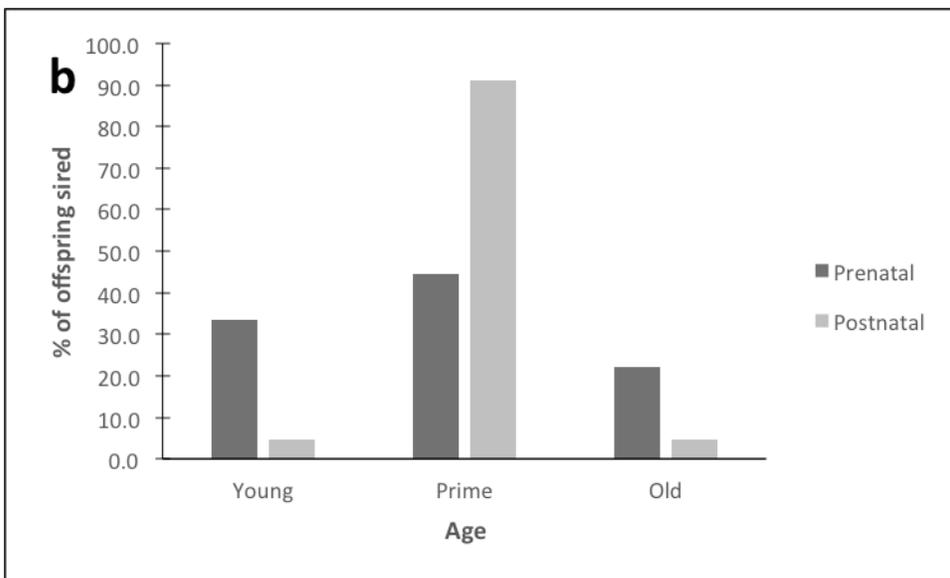
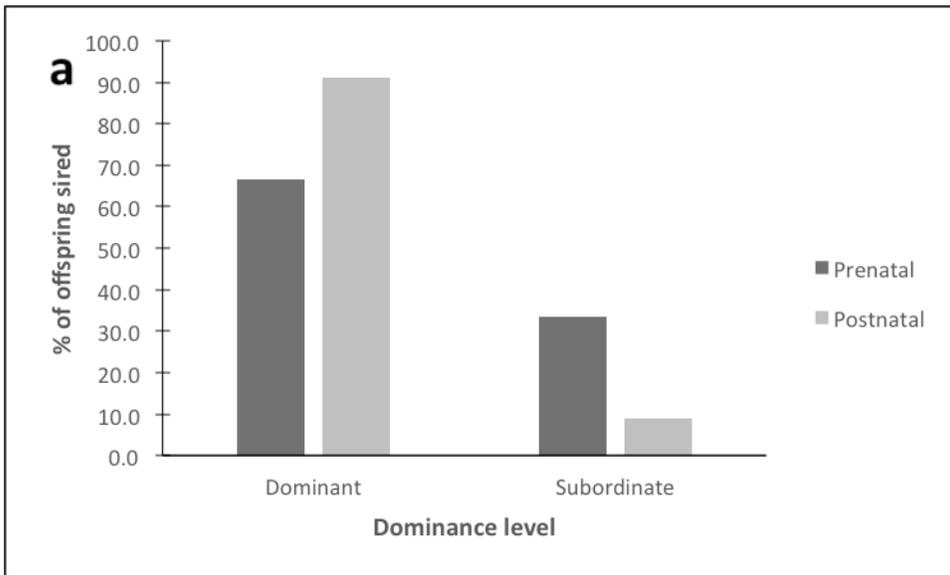


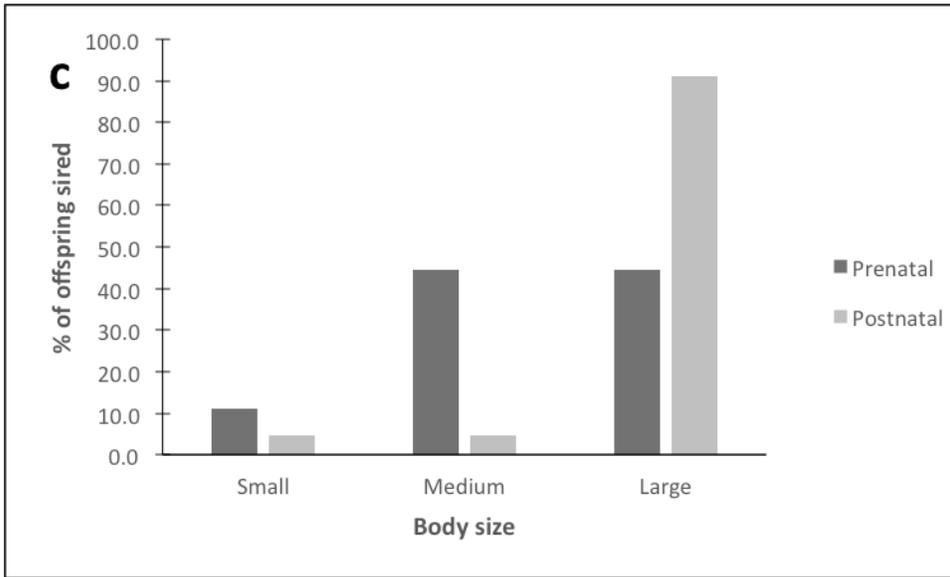


**Figure 6:** Comparison of mean +/- SD quiver duration of all males that achieved successful mounts, categorized by dominance level (6a), age (6b), and body size (6c).

\* = statistically significant ( $p < 0.05$ )

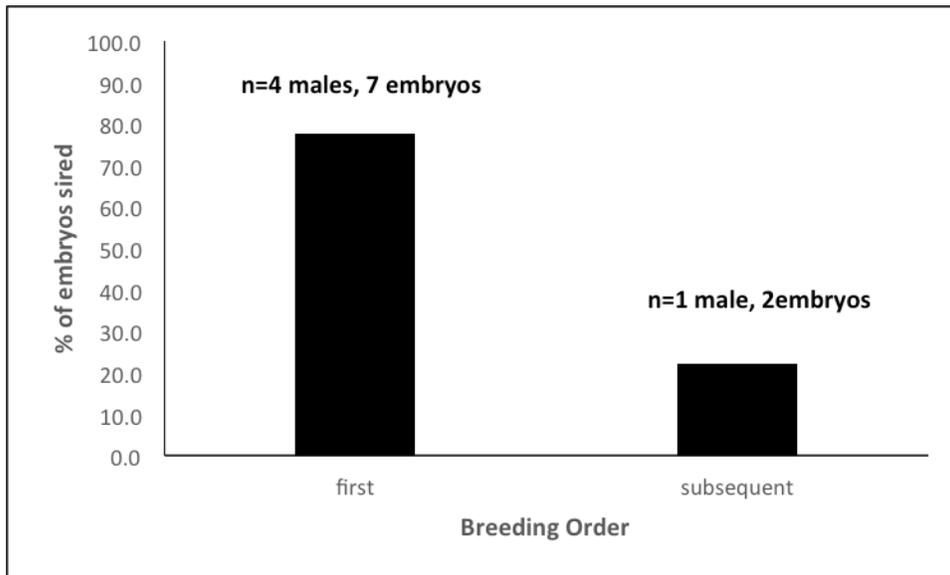
**Figure 7**





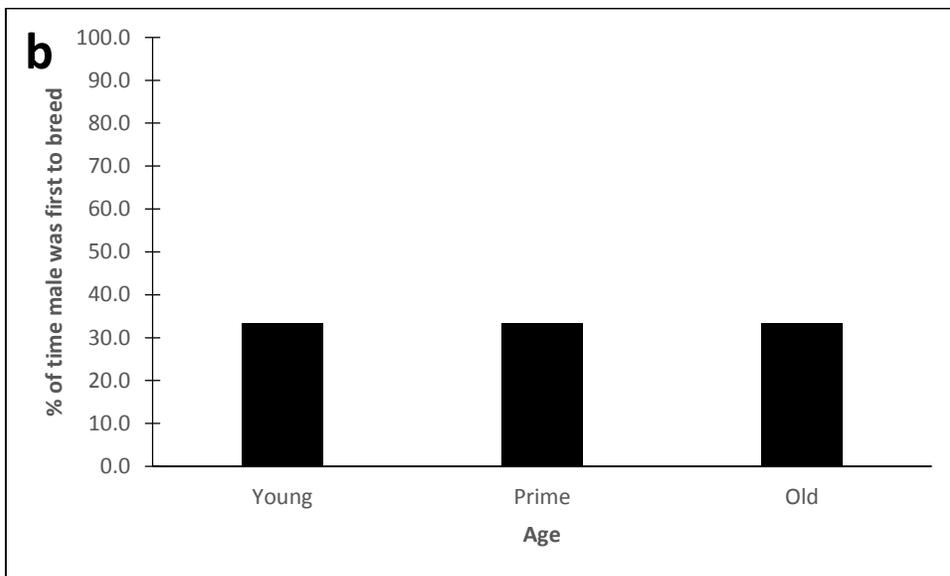
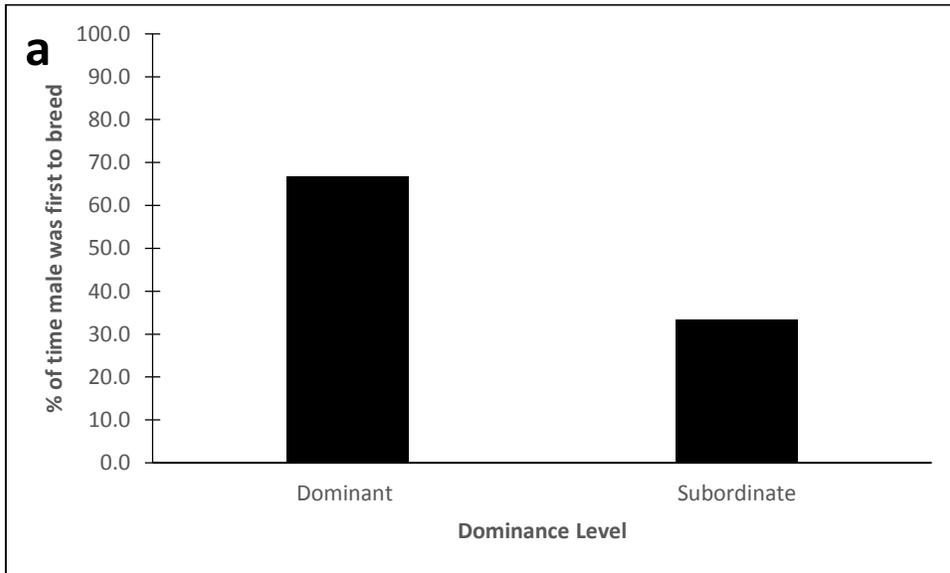
**Figure 7:** Percentage comparison of prenatal (our data) and postnatal (Kovach and Powell 2003) offspring sired by males, categorized by dominance level (7a), age (7b), and body size (7c).

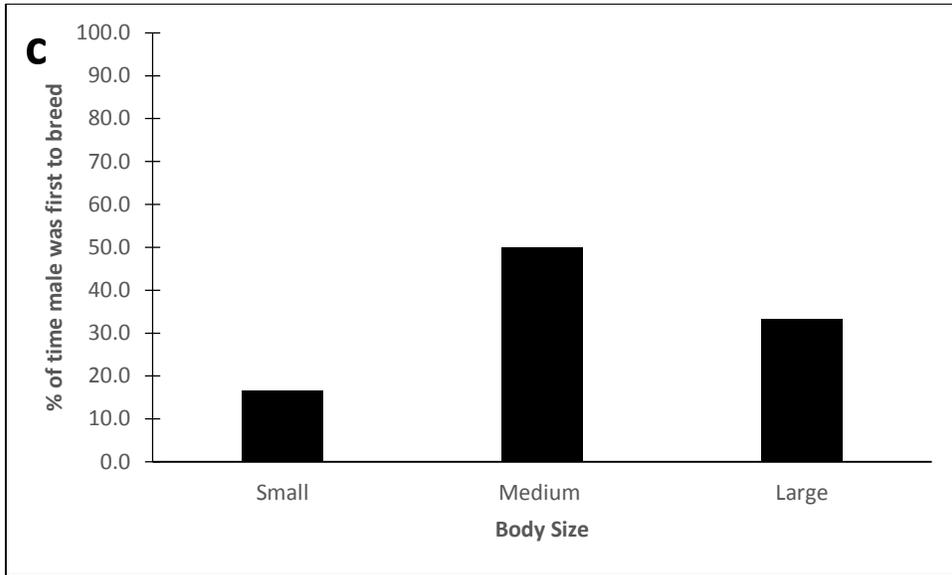
**Figure 8**



**Figure 8:** Percentage comparison of male breeding order in relation to embryos sired. We assumed that males who bred first would sire more embryos.

**Figure 9**





**Figure 9:** Percentage comparison of which males were first to successfully breed estrous females, categorized by dominance level (9a), age (9b), and body size (9c).

## Chapter 3: General Discussion

Given the need for continued research and new ideas regarding pre- and postcopulatory sexual selection in mammals, let alone bears, the results from this study offer valuable insights. All 3 of my hypotheses were supported, leading to different conclusions than what are currently assumed regarding sexual selection in black bears.

Hypothesis 1: female mate choice is achieved primarily or solely through CFC.

We analyzed 601 mounts, with 54 successful and 547 unsuccessful. We determined that LPD males did not achieve as many successful mounts as would be expected based on comparison to wild bears. Furthermore, LPD males were just as likely to experience failed mounts as smaller, younger, subordinate males were, indicating that females did not select mates based upon morphological features and social status. Therefore, my hypothesis was supported. This is a significant result because it is contrary to the currently accepted notion that females *do* prefer these types of mates. The reason for this intriguing result is likely due to the unique experimental setup, which allowed us to observe the aspect of female mate choice at a level of detail previously undocumented.

What makes our experimental setup so unique is that the behavior, mannerisms, and vocalizations of our bears act as a facsimile of wild bear populations. By admitting 2-3 males into the breeding pen, females have enough

space to escape them if uninterested, allowing them to maximize mate choice among these males. These cohorts are similar to the number of males that estrous females might encounter in the wild during any single estrus, with the exception being that dominant males are not able to entirely exclude subordinates, due to them all being fenced in together. Therefore, we were essentially able to observe how wild bears act, but under more controlled conditions.

Females did not select mates based on body size, age, and dominance. This makes sense when considering the properties of CFC and the mechanics of the black bear mating system, both of which allow the female to mate freely among males, and then use this postcopulatory sexual selection mechanism to select the best genes. While not showing preference for physical features or social rank, females did appear to prefer certain individual males. This may be due to pheromones, which serve as olfactory indicators that allow females to assess reproductive fitness of males (Dehnhard *et al.* 2006). Therefore, females may select males based on olfactory cues, but more research is needed to validate this.

#### Hypothesis 2: CFC counters or negates sperm competition.

Upon analysis of the properties of sperm competition, and using quivering duration as a proxy for ejaculation quantity, we determined that sires ejaculated for a total of 967 seconds, while non-sires ejaculated for 1,253 seconds. Thus,

my hypothesis was supported. This is a significant result because it is again contrary to the dogma that black bears use sperm competition. Black bears are assumed to use sperm competition due to their sexual dimorphism (Kovach and Powell 2003), large testes (Spady *et al.* 2007), and prolonged mating bouts (Barber and Lindzey 1986. Additionally, Kovach and Powell (2003) indicated that multi-paternity litters are also indicators of sperm competition. However, prenatal reproductive success was not measured in that study, and multi-paternity litters could also have been the result of CFC, as females could select high-quality embryos from multiple males. Our findings with respect to sperm competition also stem from the fact that this experiment is the first to afford such a large sample size of observed successful mounts for analysis. Due to the solitary nature of wild bears, close-range observations of their copulations, and especially of male quivering durations, are extremely difficult. However, at our study site, we were able to examine these acts up close, while the bears act naturally and are unperturbed by our presence.

### Hypothesis 3: CFC enhances male-male competition.

No previous study has been able to empirically test for the presence of CFC in bears. By comparing prenatal paternity success of LPD males to postnatal paternity success of LPD males, we were able to establish that CFC may be a key sexual selection strategy employed by black bears. Among 5 males, LPD bears achieved significantly less prenatal paternity success when compared to postnatal paternity success of LPD males in the wild.

Consequently, this result supported my third hypothesis. This is an important result, because if males use sperm competition, we would expect LPD males to fertilize a significantly higher number of eggs, thus resulting in a similar pre- to postnatal paternity success percentage.

An important note is that dominant, but not large or prime, males did sire a slight, but not statistically significant, majority (67%) of offspring when assessed *in utero*. Moreover, 60% of sires were dominant, and 83% of litters were sired by the male that bred the female first. Of the sires that bred first, 80% of these were dominant (1 dominant male bred 2 different females, being the first breeder both times). Therefore, it appears mate order, rather than any male characteristic, is the most important indicator of reproductive success. The fact that LPD males did not achieve significantly higher prenatal paternity success than lesser males suggests the possibility of CFC.

### **Implications for management of captive and wild bears**

American black bears are abundant (Pelton 1982) and can provide a reliable research/management model for other bear species, due to their similar reproductive biology (Spady *et al.* 2007). Our results indicate that American black bears may exhibit CFC, especially when considered in conjunction with this species' reproductive physiology. If so, this would mitigate the outcome of male-

male competition and male mate selection. This knowledge could have valuable application to various aspects of bear management and conservation.

Since LPD males sire the most cubs, and are therefore vital to a population's survival, these types of males should be given careful consideration when determining hunting limits to avoid excessive long term loss of genetic fitness and/or depopulation. Moore *et al.* (2015) noted that traits which tend to be associated with reproductive success are also those that are desired by hunters, and overexploitation of LPD males can negatively alter a population's age and sex ratios, density of LPD males, and age at first reproduction. Additionally, with less LPD males in a population, young males are more likely to encounter females and less likely to disperse, due to decreased intraspecific competition; therefore, removing LPD males from a population can have deleterious consequences due to an increased risk of inbreeding, loss of genetic diversity, and acquisition of less fit traits. LPD males would be especially important for a small bear population. In an area where such a population exists, wildlife managers could reduce or stop hunting, in order to ensure that these males remain in the population, and are available to breed with females to help bolster population numbers. Therefore, it is vital to keep LPD males in all bear populations, because if CFC exists, females will need these higher-quality genes to select from, as the only other option would be genes from less fit males, which her body may reject entirely.

Additionally, the results of this study could assist zoos with their captive breeding programs for endangered and threatened bears. Some bear species reproduce more easily in captivity than others (Ball 1994, Kolter 1995, Linke 1999, Rosenthal 1999, Frederick and Shrake 2002, Vineyard 2002, Zhong and Gipps 2004).

Due to the female's ability to select the most fit embryo(s) *in utero* via CFC, zoos should be encouraged to allow mating with multiple males, while reducing concerns about inbreeding, as related embryos will likely be selected against due to genetic incompatibility. Zoos should avoid simply pairing a female with a "genetically superior" male, since the female may not prefer this type of male, and even if copulation does occur, this male's genes could be rejected *in utero* via CFC. Exposing an estrous female to only one "fit" male would be forced mating, and this can have dire consequences for the female and her offspring, as forced mating in captive animals can lead to spontaneous abortion, lower conception rates, miscarriage, and abnormal postbirth offspring (Moller and Legendre 2001). However, if females are given a choice of mates, her body could simply reject such an embryo and choose a more genetically compatible one from different mates. Furthermore, Martin-Wintle *et al.* (2015) demonstrated that free mate choice among captive giant pandas resulted in more copulations, leading to higher birth rates. In addition to providing females multiple males to mate with, it is very important to grant males access to them just prior to peak

estrus, as our research indicates that mate order, not physical traits or social status, is crucial to reproductive success.

Further research will be necessary in order to ultimately prove that cryptic female choice exists in black bears. If proven to be true, this invaluable knowledge would advance the field of reproduction in ursids, as there are many assumptions about their breeding behavior, but also many unknowns.

## **Future Research**

In order to better investigate CFC in black bears, a more direct comparison of prenatal and postnatal paternity success should be made. While prenatal paternity success was assessed using embryos from our study bears, postnatal paternity success was determined by consulting previous studies conducted on wild bears. I suggest that postnatal paternity success should also be assessed using cubs from our study population. Additionally, I also suggest the collection of additional pre-implantation embryos, as our current sample size consists of 6 litters from 2 years. This is a good sample size as is, but analysis of additional litters, regarding what types of males sired them, will only bolster our findings.

If feasible, I think the use of video cameras at the study site would be beneficial for future studies. This would allow additional behavioral analyses of

male-male dominance and mating interactions, while at the same time increasing the sample sizes of both.

An additional aspect of this would be the useful behavioral training. Those of us that spent summers at the study site became very familiar with bear behavior. I think it would be very worthwhile to train others in behavioral observation of bears. The next Master's student could have one or more undergraduate assistants, as I had, and train them on aspects of bear behavior. Once sufficiently familiar with the relevant behaviors, they could review video and record the additional behavioral interactions. This would save the graduate student valuable time, while simultaneously giving undergraduates valuable training.

Also in consideration of being time-efficient, it might be wise to expedite the processing of the paternity analysis by sending out samples to a genetic sequencing company. I understand that training on lab techniques is a vital component to the Spady Lab, and I have gained valuable training and experience in conducting PCR amplification and gel electrophoresis. However, conclusive results were at times slow to come by, and it seemed like there were a number of things that could go wrong with the gels. Nevertheless, I learned about these problems, what they meant, and how to address them going forward, so the experience gained in lab was invaluable, especially for someone like me, who had no lab experience in these areas prior to joining this lab. It might be useful for the next student to become proficient in PCR amplification and gel

electrophoresis through lab training, so that they gain this important experience. Then, in an effort to get results faster, samples could be prepared and sent to a sequencing company. This way, the student will still get the training, and will be capable of doing the lab work, meanwhile results will be obtained in a timely manner. These ideas could also be considered in the context of student interests and goals, time constraints, and future directions of the lab.

In terms of subsequent projects I believe that the current research in this thesis should be continued, in order to analyze cubs from our study bears. I think that using our bears, rather than bears from prior studies of others, will strengthen the results by establishing a direct link to the prenatal and postnatal properties of this specific population of bears.

Gathering larger sample sizes of prenatal and postnatal paternity will better inform our results and further strengthen our conclusions about reproductive strategies used by black bears. Therefore, more field seasons of behavioral observation and additional paternity analysis in the laboratory will be required.

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