Mating Tactics and Reproductive Success in Male Columbian Ground Squirrels
(Urocitellus columbianus)

by

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Abstract

The widespread discovery of multiple mating tactics, particularly among males, highlights the diverse ways sexual selection may operate within a population. In polygynandrous populations, multiple paternity may arise from females who can produce multiple offspring at once. Alternate mating tactics can arise if there is an increase in competition among males or in the presence of sperm competition. Questions have been raised on how fitness is maximized in mating systems in which there is multiple paternity in a single litter. To understand sexual selection in polygynandrous mating systems, I used Columbian ground squirrels (*Urocitellus columbianus*) as my study species. Male Columbian ground squirrels have two main tactics, territorial and non-territorial. Futhermore, their reproductive success is influenced by the male's mating order in the female's queue. If the first male mate sires the majority of the offspring, he is said to have the "first male advantage." Variation in the number of female mates that the male is able to obtain in a season may also influence his reproductive success (Bateman's 3rd principle).

The purpose of my first study was to examine the relative importance of number of mates (Bateman's 3rd Principle) and average rank for male Columbian ground squirrels. Using a standardized partial regression, I quantified the relative contributions the number of female mates and average rank to the reproductive success for Columbian ground squirrel males. I found that the number of mates influenced male reproductive

success three times more than average rank. I also examined if territoriality affected reproductive success and found that territorial males sired more offspring, had more mates, had lower average rank, and were older than non-territorial males.

In my second study, I examined whether being a subordinate male was an equivalent alternative mating tactic to being a territorial male or whether subordinate males are simply making the "best of a bad job." I found that territorial males received a higher proportion of matings, had a higher proportion offspring, and had higher odds at receiving a mating for the first four matings. Territorial males were not more efficient than non-territorial males at inseminating females at any copulation rank.

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Chapter One

Literature Review on Male Reproductive Tactics in Columbian Ground Squirrels
(*Urocitellus columbianus*)

In The *Origin of Species,* Darwin (1859) first proposed the theory of sexual

selection to explain the evolution of sexually dimorphic characteristics. Darwin (1871)

further developed this idea in *The Descent of Man and Selection in Relation to Sex,* where

undertook the task of making a distinction between the two modes of sexual selection,

later termed intersexual (or epigamic) and intrasexual selection (Huxley, 1938). One of

the keys to better understanding evolutionary biology is to enhance understanding both

intersexual selection and intrasexual selection, how mating systems have influenced

them, and how they have shaped behavior and morphology.

During the past three decades, numerous studies have been conducted in order to

better understand inter- and intraspecific variation in mating behaviors (e.g. Andersson,

1994). In situations where there is a female mate preference, intersexual interactions

influence a male's mating success. Intrasexual competition between the males can

additionally increase their potential for mating opportunities. Species across a broad

range of taxa including mammals (Clutton-Brock, 1989), birds (Hirth, 1997), fishes

(Taborsky, 2001) and arthropods (Alcock, 1997) have been found to have intraspecific

variation in mating behaviors. Intrasexual selection increases when a sex, usually the

male, invests fewer resources in their offspring (Trivers, 1972). In mammals, females

usually provide all of the parental care and compete for access to resources, while males

produce little if any parental care and compete over access to females (Trivers, 1972).

Differences in parental investment, which includes investment in the zygote and feeding

of offspring, lead to competition, and ultimately favor the evolution of behavioral and

morphological differences between the sexes via one or both forms of sexual selection.

To acquire mates, males of different species adopt a variety of different strategies

(Cade, 1979; Andersson, 1994). In turn, the reproductive strategies employed by both

males and females determine the mating system of a species (Clutton-Brock, 1989). It is

not a surprise to find a myriad of research on mating systems in which the males mate

with multiple females (e.g., Orians, 1969). However, more recently there has been

substantial evidence that suggests that mating systems with females who have multiple

mates may be just as common (e.g., Gibson & Jewell, 1982; Lane et al., 2008).   The

potential benefit of having multiple mates has been debated by many scientists

(Andersson, 1994), however, numerous studies have revealed a strong advantage for

females that mate with multiple males in a single reproductive cycle, including improving

the female's fitness (Fisher et al., 2006).

The diversity of mating tactics may become greater in systems with multiple

mating; especially with promiscuous females, since neither sex limits themselves to one

"best" mate. Females in polygynandrous mating systems have been shown to mate with

males possessing a number of different characteristics including age, dominance, or

courtship style during a single breeding season (Kodrie-Brown, 1993; McLintock &

Uetz, 1996; Kempenaers et al., 1997). In systems in which they have multiple mates,

females should exercise mate preference if her reproductive success is affected by her

choice.   In resource biased systems, a female may choose a male who is able to offer her

superior resources, therefore increasing her reproductive success. The resources a male can offer can include access to his territory (Hixon, 1987), nutrition either indirectly or directly (e.g. nuptial gifts Karlsson, 1998), parental care (e.g. Møller & Thornhill, 1998), or any other direct benefit. In non-resource based systems, females tend to choose males who have attractive, heritable traits (Fisher, 1958; Hedrick, 1988; Broussard et al., 2005). Males who have attractive heritable traits have attractive sons, who are able to mate with other "choosy" females (Weatherhead & Robertson, 1979). The heritable trait the female found attractive would then be passed on to her son. If a trait is considered attractive to the females in that species, the sons who have the trait should have a better chance of mating. Mating experience may also be a favoured male trait, since it may predict future breeding success.

Males in a strong polygynous species tend to develop a more intense competition among each other than males in monogamous species (Clutton-Brock & Harvey, 1978). They can encounter different constraints that affect their reproductive success. These constraints can be intrinsic (physiological) and include such characteristics as body size, age and growth rate (e.g. Leary et al., 2005). For example, large male broad-headed skinks are able to prevent smaller males from to gaining mating access to females (Cooper & Vitt, 1997). The constraint could also be external (environmental), including distance between mates and other males. In order to maximize their reproductive opportunities, they adopt a tactic depending on their current status, including size, age, or energy reserves (Waltz & Wolf, 1984).

To increase their number of offspring, males in polygynandrous systems may have to use a tactic that not only attracts a female mate, but a tactic that will attract the

highest number of mates. Bateman (1948) conducted a laboratory experiment on *Drosophila melanogaster* that showed that the total number of females a male copulated with was directly proportional to the number of offspring he produced. Three principles were formed from Bateman's experiment; the first two principles are seen as intra-masculine sexual selection. They are a) that there is a greater variance of male reproductive success than female reproductive success; and b) the sex that should be more sexually selected should be the sex with the greater variance in reproductive success, which is typically the male. Bateman's third principle is identified as the cause of sexual selection on males. This third principle provides a correlation between the quantity of mates and the number of offspring. If a male is able to mate with an increased number of females, he should also have an increase in reproductive success as well. Even though numerous studies have supported Bateman's principles, a few studies have conclusions which are inconsistent with them (see Snyder & Gowaty, 2007). These studies have demonstrated that although Bateman's principles are a well-tested theory, there are exceptions and the principles should not be assumed for every system.

In several multi-male mating systems, with the use of microsatellite DNA analysis, females in have been found to give birth to multiply sired litters (Hanken & Sherman, 1981; Pearse et al., 2002; Waser & De Woody, 2006; DeYoung et al., 2009). Both Hamilton (1964) and Trivers (1972) suggested that mixed paternity might affect how social behavior evolves in such species. This evolution may be even more accelerated if the order in which the male mates with the female affects his chance of paternity.

Among some species (e.g., Simmons et al., 2003; Lane et al., 2008), the mating

order does not affect a male's chance of paternity. Nevertheless, in numerous other species, the order of matings has a significant influence on the percentage of the clutch or litter sired by each male (e.g., Kraaijeveld-Smit et al., 2002). In mating systems where the last male sires the most offspring, he is said to have the last male advantage (e.g., Pitcher et al., 2003). When the first male mate has the potential to sire a higher percent of offspring, he gains a "first male advantage" (e.g., Christenson & Cohn, 1988). Males have to adjust their mating tactics in order to give them the highest fitness, which may depend on order.

When there is interspecifc variation in male mating behaviours, males who use the most successful tactic should out-compete other males, giving them the potential to sire a higher number of offspring (Trivers, 1972). This male mating tactic may be considered a conventional tactic, with the most competitive individuals in the population employing that behavior (Wolfe, 2008). The mating tactic a male chooses to adopt should depend on his age, size or energy reserves, and how these factors affect his ability to successfully accomplish the necessary defense of a female or resources (Emlen & Oring, 1977b). If a male is unable to successfully defend the resources or the female in order to attract a mate, he may opt for a subordinate alternative-mating tactic.

Environmental factors such as temporal availability of mates and spatial dispersion pattern play a large role in determining which tactic a male chooses (Emlen & Oring 1977). A defense mating system may arise when females are clumped or when the female's oestrus is not synchronous since the dominant males can better defend access to individual females (Emlen & Oring 1977). In many systems, territorial defense by a male can provide him with more opportunities to mate (Wilson, 1971). If a sexually

mature male is subordinate and he exploits a territorial male's resources, including mating opportunities, he is known as a satellite male (Wirtz, 1981).

Waltz and Wolf (1984) described two classes of tactics. The first class of tactics suggests that there are two equally competitive alternative mating tactics. The second class depends on the size or the age of the male. Younger or smaller males are unable to compete successfully against stronger or older males, and they are forced to adopt a subordinate alternate mating tactic, or increase their fitness by "making the best of a bad job" (Koprowski, 1993; Alcock, 1997). There should be unequal fitness between the alternative mating tactics when the male's ability to defend the resource changes with age.

Columbian ground squirrels (*Urocitellus columbianus*) exhibit a polygynandrous mating system. They hibernate for up to nine months out of a year, leaving them a short reproductive period. A female will enter oestrus approximately four days after emerging from hibernation (Murie & Harris, 1982), and mate with up to eight males (Raveh et al., 2010a) in only a few hours. A short two to three week mating period as individuals emerge from a long (nearly 9-month) hibernation is a time of intense competition among males. Thus, multiple mating tactics by males seem likely.

It has been suggested that male Columbian ground squirrels use different mating tactics within a local population. Two of these mating tactics include territorial and non-territorial behaviours (Murie & Harris, 1978; Dobson, 1984),. Territorial males have been observed to not only defend a specific area that includes both females and resources, but also defend females directly (Manno & Dobson, 2008). Manno and Dobson (2008) found that being a territorial male did not guarantee mating; however, it did provide the

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male a high chance of obtaining the first copulation with estrous females from his territory. Non-territorial males were younger males that were behaviorally subordinate but were still able to achieve copulations (Manno & Dobson, 2008).

Females who reside on a male's territory become familiar with that male, which may give him a better opportunity to mate with her. Some females have been observed to move their sleeping burrows to the territories of neighboring males before they enter oestrus. Females also feed on different territories during the days leading up to oestrus, particularly the day before, increasing interactions with other males than the territory holder whose territory she sleeps in. This suggests that female choice is an important factor in determining the mating system, though the details of this are unknown.

Manno & Dobson (2008) found that females often sleep in the same burrow as the dominate territorial male the evening before she enters into oestrus, which lead to a copulation before they emerge in the morning (Murie, 1995a). If there was no territorial male claiming the area around a female's nest burrow, she mated with the oldest neighboring male. The dominant males were continually challenged in their territory by other males in the colony, especially on the days the females in their territory are in oestrus. A dominant male aggressively defended the ovulating females on his territory, sometimes with great effort. The females, however, were able to escape and search for additional male mates. Mating order plays a large roll in male reproductive success, allowing the fist mates to receive a higher chance of paternity (Raveh et al., 2010a). Larger males, and males of intermediate age were to found to be the most reproductively successful.

As with other rodents such as house mice (*Mus domesticus*; Firman et al., 2009)

or guinea pigs (*Cavia porcellus*; Martan & Shepherd, 1976), and even with closer related species such as the Artic ground squirrel (*Spermophilus parryii plesius;* Lacey et al., 1992), Belding's ground squirrel (*Spermophilus beldingi*; Hanken & Sherman, 1981), and the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus;* Schwagmeyer & Foltz, 1990), the Columbian ground squirrel has been found to have a first male advantage (Raveh et al., 2010a). Males who mate first receive 57.5% off the offspring, but this advantage weakens as the female increases her number of mating partners (Raveh et al., 2010a). The males, however, do not appear to adjust their strategy with respect to females who mate with more males. Furthermore, as a male moves down in the mating order (increase in rank), his percentage of offspring greatly decreased, with the second male only receiving 23.1% of the offspring, and the third through fifth males received 14.1%, 3.9% and 1.5% respectively, of the offspring. This, nevertheless, did not deter males from mating with previously mated females, since Raveh et al. (2010a) also suggested that a male still gains substantial fitness by mating with previously mated females. The question is then, what provides a male with greater gains in reproductive success, a higher rank or more mates (Bateman's principle)?

In the second chapter the aim was to compare the reproductive success of males from a decrease in rank with an increase in matings. My first hypothesis is that variance in reproductive success of male Columbian ground squirrels is due to differences of the number of matings. I predicted that the number of total offspring for each male would increase with the number of females he mated with (Bateman's principles). Alternatively, variance in reproductive male success should also be influenced by differences of the female's rank of copulations. I predicted that the number of offspring

would be greater the higher the male is, on average, in a female's overall mating order. Since these alternatives are not mutually exclusive, I conducted a standardized partial regression analysis to examine the relative importance of the two processes to male reproductive success.

The goal of the third chapter was to determine if satellite (non-territory holding, resident males) males are competitive in terms of reproductive success, in the Columbian ground squirrel mating system. I first want to know why some males defend territories while others (satellites) do not. My first hypothesis follows Waltz and Wolf (1984) class one tactic, which is that the males have two distinct and equal mating tactics, satellite and territorial, as suggested by Murie & Harris (1978) and Dobson (1984). The alternative hypothesis goes along with Waltz and Wolf (1984) class two tactic, the satellite males are forced to use an alternative mating and they are "making the best of a bad job." I predict that if there are two equal tactics, each tactic will have equal mating success. If the alternative hypothesis were true, I would expect the satellites to have lower mating success, therefore, employing that behavioral tactic if the only alternative is to not mate at all. The next question I wanted to address was whether there was difference between satellite and off-territory territorial males with respect to their efficiency of getting offspring from matings other than the first. Raveh et al. (2010a) suggested that males did not differ in patterns of success, but from an examination of the overall pattern of parentage for males in different position. Nonetheless, a small sample of younger males appeared to have a different pattern of success than older males, but the result was not statistically significant. I took a modeling approach that examined the odds of success at siring offspring. The hypothesis is that there will be a difference in mating success

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between the satellite males and the off-territory territorial males, perhaps due to the lack of experience and physiological readiness of younger males, as Broussard et al. (2008) found for females. I predicted that the off-territory territorial males would have better odds at obtaining a higher rank than the subordinate males, compared to what would be expected if the female's order of mates is random.

Chapter Two

Affect of Number of Mates and Mating Order on Male Columbian Ground Squirrels (*Urocitellus columbianus*)

Abstract. Questions have been raised about how fitness is maximized in mating systems in which there is multiple paternity in a single litter. To better understand sexual selection in polygynandrous mating systems, I studied Columbian ground squirrels (*Urocitellus columbianus*). Mating order is known to influence the male Columbian ground squirrel's reproductive success, giving the first male mate the "first male advantage" of siring the most offspring. The number of female mates the male obtains in a single mating season has also been thought to influence the male's reproductive success (Bateman's 3rd principle) in Columbian ground squirrels, but has never been tested. By running a standardized partial regression, I discovered that both number of mates and average rank in the mating order are important to the male's reproductive success. Furthermore, the number of females he is able to mate with is more than three times more influential to the number of offspring he sires than is the male's average rank. This study also looked at the effects of territoriality on male reproductive success. I found that territorial males obtained more matings, sired more offspring, had a lower average rank, and were older than non-territorial males.

INTRODUCTION

To explain sexually dimorphic characteristics, Darwin (1859; 1871) extended his concept of natural selection and proposed the theory of sexual selection. He suggested that sexual selection is a process that acts on traits that increase both competitive ability and attractiveness to mates. Darwin made a distinction between modes of sexual selection and competition, later termed intersexual (mate choice) and intrasexual selection (male-male competition) by Huxley (1938). Intersexual competition was suggested as the main cause for the development of "secondary" sexual characteristics, which are physical or behavioral characteristics that are different between the sexes, but doesn't have a direct reproductive function. Even though they are unlikely to increase survival, Darwin argued that these characteristics increased their opportunities to either breed with or attract a mate. Secondary sexual characteristics lead to individual variation in reproductive success, which is required for natural selection (Darwin, 1859; Fisher, 1930). Differential reproductive success has become accepted as a measure of sexual selection (e.g., Wade, 1979; Arnold, 1994).

Bateman's (1948) experiment on *Drosphila melanogaster,* was one of the most influential papers for modern research on sexual selection. This empirical study looked at the variance in reproductive success using visible genetic makers to identify paternity in an entire population of fruit flies. Bateman came to three conclusions, which were later termed "Bateman's principals." Two of these principals are considered intra-masculine sexual selection: (1) males display a greater variance of reproductive success (number of offspring produced) than females; and (2) there is a greater variance between the number of mates for males than there is for females. Bateman saw the third principle

as being the factor for sexual selection on males (Bateman, 1948). It states that the variance in reproductive success in males is primarily caused by the variation in the number of mates they are able to acquire. In other words, the total number of females a male copulated with is directly proportional to the number of offspring he produced.

Although his ideas are still seen as a breakthrough in sexual selection, Bateman's methods have recently been criticized (see Dewsbury, 2005; Tang-Martinez & Ryder, 2005; Snyder & Gowaty, 2007). Furthermore, an over-reliance on Bateman's predictions may have stifled research in sexual selection, especially with regard to female choice (Tang-Martinez & Ryder, 2005). Even though Bateman's principles are an important to many mating systems, it is imperative not to overlook other factors that influence variance in mating success.

With the help of microsatellite DNA analysis, an increasing number of polygynandrous mating systems (both females and males have multiple mates) have been revealed to have multiple paternity in a single litter (Hanken & Sherman, 1981; Pearse et al., 2002; Waser & De Woody, 2006; DeYoung et al., 2009). Hamilton (1964) and Trivers (1972) suggested mixed paternity might affect the evolution of social behavior in a species. The evolution of social behaviors may be affected if the order in which the male mates with the female affects his chance of paternity. In some polygynandrous mating systems with multiple paternity, males are able to maximize the number of offspring by mating either earlier or later in the female's queue of mates, depending upon the species (e.g., Kraaijeveld-Smit et al., 2002; Raveh et al., 2010a). When the first male mate has the potential to sire a higher percent of offspring, he gains the "first male

advantage" (e.g., Christenson & Cohn, 1988). Males may then adjust their behaviors to gain this advantage.

Male reproductive success might depend on both the number of mates (Bateman's principal) acquired and the order in which the male mated with the female. It is unknown which of these two influences has a greater effect on overall reproductive success in different mating systems. If the number of mates were more important to the male's reproductive success, males may be expected to exhibit behaviors that maximize the number of females with which they copulate with (Trivers, 1972), regardless of where in the mating order they are. This tactic might be expected when the order in which males mate makes little difference to their success in siring young (e.g., Lane et al., 2008). Alternatively, if the position in the female's queue were more important to reproductive success, behaviors that allow the male to reposition himself in the female's mating order should enhance his reproductive success, possibly sacrificing number of mates for better access to a few females. In the latter case, males may display behaviors, such as mate guarding, that decrease the number of mates the female has access to during oestrus. Mate guarding may provide reproductive success by securing further matings with the female and preventing other males from contributing sperm to sire her offspring (Sherman, 1989b; Raveh et al., 2010b).

The two strategies that males can purse to increase their reproductive success are 1) to maximize their number of mates or 2) to optimize their position in a female's queue of mates. Males can either purse one strategy completely or try to optimize a combination of the number of mates with the best possible position in a female's queue. The optimal combination of the two may vary across populations or species.

The purpose of my study was to examine the importance of number of mates and order in the queue of multiple-mating females for male Columbian ground squirrels (*Urocitellus columbianus*). This species is ideal for a study of the importance of these two factors on reproductive success of males because both male and female Columbian ground squirrels have multiple mates and litters exhibit multiple paternity (Murie, 1995b; Manno & Dobson, 2008; Raveh et al., 2010a). There is also a first male advantage in Columbian ground squirrels, with over 57% of offspring sired by the first male to mate with the female, with and reproductive success declining for later-copulating males (Raveh et al., 2010a). Despite an average litter size of around three offspring, females mate with up to eight males and the first five males to mate may sire offspring. While males gain fitness advantage by mating with several females, males have higher reproductive success if they mate early in a female's sequence of mates. Columbian ground squirrels hibernate for nine months and have a single mating season each year during a restricted activity season of slightly over three months (Dobson, 1992). Females mate shortly after emergence from hibernation in the spring, during a 2-3 week period (Murie & Harris, 1982). Male mating success is influenced by territorial behavior shortly before and during the female emergence period (Manno & Dobson, 2008; Raveh et al., 2010b).

I quantified the relative contributions of the number of mates that males had during a reproductive season and where these matings were in the queue of individual females to reproductive success for male Columbian ground squirrels. I also tested whether the males that mated with the greatest number of mates during a breeding season were also high in the mating queue of the females that they mated with. Since females

usually mate first with territorial males, the number of females on a male's territory might also have an effect on reproductive success of males. Finally, there is attrition of offspring during the reproductive season (Murie et al., 1980) and reproductive success may differ if defined as number of offspring sired or as number of offspring that survive until weaning. Therefore, I examined whether patterns of reproductive success differed at these two critical points during the reproductive process.

METHODS

Field Methods

I studied a colony of Columbian ground squirrels (*Urocitellus columbianus*) in the Sheep River Provincial Park, Alberta, Canada (50°N, 110°W, elevation 1500m), from April to mid-July over 4 years (2005-2008). Ages and matriarchal lineages were known for the entire population, except for one male that was at least three years of age when first captured. All individuals were trapped within two days of emergence from spring hibernation. Males emerge in mid-April with females emerging a few days to up to three weeks later (Murie & Harris, 1982). Ground squirrels were trapped using live traps baited with peanut butter (National live traps, WI, USA; 15×15×48 cm and 13×13×40 cm) and weighed using cloth bags and Pesola® spring scales. Each individual received a permanent identification number stamped onto fingerling fish tags (National Band & Tag Co., Newport, KY, U.S.A.) attached to both ears, which they received at first capture (immigrants) or shortly after weaning (residents). Black hair dye (Clairol, Hydriance - black pearl No. 52) was painted on the back of each squirrel in a unique mark for visual identification from a distance. A tissue sample was taken from the tip of the ear for all

adult males for DNA analysis.

Each adult female had a 5-7 hour diurnal estrous period approximately 4 days after emergence from hibernation (Murie & Harris, 1982; Murie, 1995b). Pre-estrous females were trapped each day before they enterend estrus to examine the swelling and openness of their vulva, an indicator of reproductive status (Murie, 1995a). Males' reproductive maturity was checked at first and subsequent captures. Sexually mature males had descended testes and darkly-pigmented scrota and immature males had undescended testes and pink or light grey scrota.

Observations were made from 3m high observational towers using 7-10 power binoculars. From the day of the first female's emergence from hibernation until the end of the three week mating season (late-April to mid-May), I arrived at the field site 30 minutes before ground squirrels started emerging for the day and stayed until 30 minutes after the last ground squirrel had gone underground for the night. All sleeping burrow locations (estimated from locations of evening immergence and morning emergence) were recorded. Detailed notes were taken on all mating activity, including location and time of underground consortships. Columbian ground squirrel copulations occur underground (Murie, 1995b), therefore I assumed a copulation occurred when an estrous female and male remained in the same burrow system for more than five minutes (Raveh et al., 2010a). I used this information to quantify the number of copulations per male, his average rank, and the number of males each female mated with.

Female Columbian ground squirrels prepare and use natal burrows in which young are kept during lactation. Natal burrows were identified by observing pregnant females stocking burrows with dry straw and emerging from the same burrow in the

morning a few days before parturition (Murie et al., 1998). Pregnant females were then

trapped 2-3 days prior to parturition and moved to a field laboratory about 2km away

from the study colony. The females were housed in polycarbonate cages (48 x 27 x 20

cm) (for details see Murie et al., 1998; Skibiel et al., 2009), and checked four times a day

for the presence of neonates. Four to twelve hours after birth, the mother and neonates

were weighed. Neonates were sexed and a small tissue sample was taken from the right

or left hind toe or tip of the tail. The tissue was preserved in alcohol for later analyses of

microsatellite DNA. Mothers and their litters were released back into the field site, near

their natal burrow, about 24 hours after giving birth. After checking on their natal

burrow, most mothers returned to the release site and retrieved their pups. If the mother

didn't retrieve her pups, the pups were placed in the natal burrow with their mother.

Approximately 27 days after birth, the pups emerged from the burrows. They were

trapped on the day of emergence, weighed, identified from marks left by the tissue

sampling, their sex was confirmed, and they received ear tags for future identification.

Paternity analysis was described in detail by Raveh et al. (Raveh et al., 2010a)

and carried out in the Coltman Lab at the University of Alberta. In summary, DNA was

extracted from the previously gathered tissue, which was preserved in 99% EtOH, using

DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands). Thirteen variable

microsatellite loci were amplified using polymerase chain reaction (PCR). Paternity was

assigned using CERVUS 3.0 (Marshall et al., 1998; Kalinowski et al., 2007). All

offspring were born in the lab, therefore maternity was known. When there were 2 or

less mismatches with both parents the paternity was accepted. Paternity to all of the

offspring (226) with a 95 to 99% confidence (assuming mother-father-offspring

relationship) was assigned.

Data Analysis and Statistics

Data were analyzed using SAS 9.2 (SAS Institute Inc., 2007). All mothers used in analyses were greater than 1 years of age. Female mating sequences were observed on the day of estrus. The average rank for each male each year was calculated using the known female mating sequences from that year. If a male mated with a female at the first mating position, he would get a rank of one with each succeeding male receiving the same rank as the position he was able to obtain in the female's mating sequence (ie. at the second mating position, he would received a rank of two). All the ranks the male was able to obtain in one mating season were averaged together and he was given an "average rank." Analyses were conducted both on the number of offspring the male fathered at birth as well as the number surviving to weaning due to possible influence of maternal effects during lactation, which may affect the male's fitness if he mated with females who were unable to care for their offspring before they were weaned (Skibiel et al. 2009).

I first examined non-heritable characteristics that have been known influence the reproductive variance in a population. I analyzed whether a male's age and the number of competitor males that mated with the same female, since both have been linked to reproductive success (Broussard et al., 2003a; Raveh et al., 2010a). A Pearson correlation compared both these characteristics to the number of females the male mated with, his average rank and reproductive success.

I conducted a standardized partial regression analysis to examine the relative importance number of female mates and average rank to male reproductive success. The

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number of offspring the male produced was used as the dependant variable, and the male's average rank and the number of females the males mated with as the independent variables. I then re-ran the analysis using the offspring surviving to weaning as the dependent variable.

Multicollinearity problems may arise when using regression techniques by inflating the variance among the variables in the model. In order to assess the degree of collinearity between the average rank and the number of females the male mated with, I used diagnostic indices including the variance inflation factors (VIF) and the collinearity condition index (CI) (Petraitis et al., 1996).

Territorial males are dominant over non-territorial males and tend to be the first mate of the females in their territory (Manno & Dobson, 2008). The first male that mates with a female sires a greater percentage of the litter; therefore a male's territory holding status may affect his reproductive success. T-tests were conducted to determine if a there was a difference between territorial and non-territorial males on the number of offspring he sired, the number of females he was able to mate with, the average rank he was able to obtain, and his age.

RESULTS

The Coltman Lab successfully genotype all reproductive males and offspring to determine the paternal success of the males; 6 males in 2005, 8 males in 2006, 7 males in 2007 and 9 males in 2008. A GLM was conducted with male ID and year as random variables to test whether there was a difference in the number of offspring males produced each year. There was no significant year effect on male success ($f = 1.09$, $P =$

0.346, df = 31), therefore the males were treated as independent values each year. There

was a total of 300 matings over the four years with 76 complete mating sequences (one

mating sequence per female per year).

I found that there was a positive significant correlation between the number of

female mates and the number of offspring that the male sired at birth (r = 0.73952, n =

30, P < 0.0001). Older males tended to copulate with more females (r = 0.39461, n = 30,

P = 0.0341). Males that mated with females with more partners tended to have a higher

average rank for the season (r = 0.54268, n = 30, P = 0.0019). As the male increased in

rank (later in the female mating sequence), he both increased the number of offspring he

sired (r = -0.39354, n = 30, P = 0.0314) and increased the number mates he obtained (r =

-0.39737, n = 30, P = 0.0297).

I used a standardized partial regression analysis model to partition reproductive

success into variation in number of female mates and average rank in queue. I found that

the coefficients for the number of mates was more than 3 times greater than that for

average for offspring at birth and an even larger difference for offspring at weaning

(Figure 1).

Collinearity appeared inconsequential, as variation inflation factors (VIF) were

under 10 (Johnston, 1984) and condition indices (CI) less than 20 (Hair et al., 1998;

Belsley et al., 2004). I found no multicollinearity problems between the average number

of mates and average rank at birth (VIF = 1.19, CI = 1.0) and at weaning (VIF = 1.19, CI

= 1.0)

Territorial males sired more offspring than non-territorial males looking at both

the number of offspring born and the number of offspring who survived to weaning.

There were more matings obtained by territorial males mated than non-territorial males, and they had a lower average rank (mated sooner in the mating order) than the non-territorial males. Territorial males also tended to be older than non-territorial males. (Table 1).

DISCUSSION

This study addressed evaluated the importance of the number of mates (Bateman's 3rd principle) and average rank had on the male Columbian ground squirrels reproductive success. It also looked at how traits such as territoriality, age, and mating order affected variation in reproduction for Columbian ground squirrels males. I found that both the male's average rank and number of females he was able to copulate with influenced his reproductive success. Furthermore, the number of mates acquired had stronger influence than male's average rank in the mating queues on his reproductive success, both at birth and at weaning. Past research (Raveh et al., 2010a) had concluded that first mating was the most important factor for a male's reproductive success and only suggested that the males also gain fitness with additional matings. This study, however, found that that additional matings are more important on reproductive success than that of rank. While both factors appear important to male reproductive success, variation in number of mates appear to have a stronger influence on success than rank in female's queue. This may occur because most territorial males fill two roles; although territorial males mate earlier in the queue, they become non-territorial competitors on other male territories.

In Columbian ground squirrels, the relative benefit of first male advantage decreases as the female increases the number of males she mates with (Raveh et al., 2010a). Sperm competition, therefore, may play a large part in the reproductive success of the male. Males can benefit from preventing competitors access to females, which can be done with post-copulatory behaviors such as mate guarding (Manno & Dobson, 2008; Raveh et al., 2010a). If, however, the male spends too much time, or 'wastes time' (Parker, 1974), on post-copulatory behavior, he may loose out on future matings. As I have found, reproductive success depends more heavily on the number of females mated rather than the number of other males that the female copulates with, therefore, males might have to optimize the duration of post-copulatory guarding behaviors.

Age is another factor that has been found to influence the reproductive success of the Columbian ground squirrel (Raveh et al., 2010a). These squirrels can live up to 13 years of age, with a decline in breeding success after 5 years of age. The average age of the breeding males in my colony was only 4.27 years, with 52% of the males being 3-4 years of age. I found that age was correlated with the number of offspring he sired.

Columbian ground squirrels are known to have a first male advantage in siring offspring and territory-holding males are more likely to receive first matings with a female (Manno and Dobson 2008). I found that territorial males are also more likely to mate with females early in her mating order and mate with a greater number of females. Both can increase the number of offspring produced in a year, although number of matings was more strongly correlated with annual reproductive success. Males may trade-off between the number of potential female mates and their rank in a female's copulation queue. Sperm competition for fertilization of eggs can add a further layer of

complexity in determining the number of offspring produced. Since the number of mates has a stronger influence on the male's reproductive success than increasing his rank, it is important to look at different mating tactics, i.e. territorial vs. non-territorial, that allow the male the best opportunity for paternity.
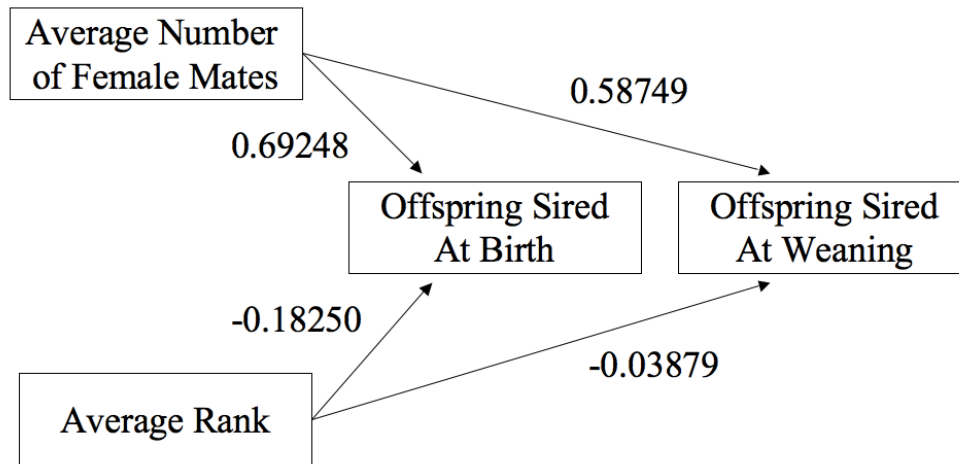
Figure 1. The influence of average rank and number of mates on reproductive success

in the Columbian ground squirrel (*Urocitellus columbianus*). Reproductive

success was defined as the sired number of offspring at birth (A) and the sired

number of offspring surviving to weaning (B).

	Non-Territorial (n = 7)	Territorial (n = 23)	T - value	df	P -value
Offspring Sired at Birth	0.86 ± 0.46	8.65 ± 0.97	-7.29	27.9	< 0.0001
Offspring Sired at Weaning	0.14 ± 0.14	5.65 ± 0.84	-6.46	23.2	< 0.0001
Female Mates	4.14 ± 0.99	10.91 ± 0.59	5.66	28	< 0.0001
Age	2.67 ± 1.20	4.70 ± 0.39	-2.48	27	= 0.0198
Rank	3.64 ± 0.39	2.64 ± 0.10	2.47	6.82	= 0.044

Table 1: The influence of territoriality on breeding characteristics of the Columbian ground squirrel (*Urocitellus columbianus*) that influence reproductive success. Numbers represent averages +- one standard deviation.

P = 0.05

Chapter Three

Odds of Mating and Efficiency of Producing Offspring for Territorial and Non-Territorial Male Columbian Ground Squirrels *(Urocitellus columbianus)*

Abstract. The widespread discovery of multiple mating tactics, particularly among males, highlights the diverse ways sexual selection may operate within a population. Alternative mating tactics can be preserved if they give males equal fitness, or if there are individuals who are incapable of employing the more successful strategy due to physiological or external constraints (described as "making the best of a bad job"). I studied alternative mating tactics in Columbian ground squirrels (*Urocitellus columbianus*). Reproductively mature male Columbian ground squirrels adopt one of two mating tactics; defending a territory or not defending a territory. I examined whether the proportion of matings, proportion of offspring sired, odds of obtaining a mating, and efficiency of siring offspring differed between males adopting the two mating tactics in a Columbian ground squirrel population. I found that territorial males received a higher proportion of matings and offspring and had better odds at receiving a mating at the first four mating positions. There was no significant difference between territorial males and non-territorial males at efficiency of impregnating a female if given equal mating opportunity. The non-territorial mating tactic does not appear to provide equal fitness to the territorial tactic, and non-territorial males appear to make the best of a bad job.

INTRODUCTION

Alternative mating tactics have been found in many animal species (Waltz &
Wolf, 1984) and may be associated with different morphologies (Emlen, 1997),
developmental stage (Neff et al., 2003), or behavioral dominance (Waltz, 1982).
Variation in mating tactics may be closely associated with particular mating systems. For
example, the variety and kind of tactics of monogamous species might differ from
polygynous species. While seminal studies of mating systems examined species-typical
mating patterns (e.g., Emlen & Oring, 1977a), the widespread discovery of multiple
mating tactics in one species, particularly among males, highlights the diversity of ways
that sexual selection may operate within a population (e.g., sneaker and satellite males,
Oliveira et al., 2001)

Animal mating systems are increasingly identified as polygynandrous, mating
patterns in which both males and females mate multiple times. Confirmation of
polygynandry has come from studies that use microsatellite DNA to identify paternity
(e.g., Hanken & Sherman, 1981; Pearse et al., 2002; Waser & De Woody, 2006;
DeYoung et al., 2009). For species in which females produce only one offspring per
breeding cycle, social polyandry or polygynandry may occur, in which females mate with
several males, but genetically the product is a single offspring with a single male parent
(social and genetic mating systems as described by Dobson et al., 2010). When females
produce multiple offspring, the outcome can be multiple paternity of a single clutch or
litter of offspring (e.g., Hanken & Sherman, 1981; Lane et al., 2008; Raveh et al., 2010a).
Whether a reproductive event results in single or multiple paternity, however, there is

competition among males for offspring, sometimes termed sperm competition (Parker, 1970).

Males in a strongly polygynous species tend to develop a more intense competition among each other than males in monogamous species (Clutton-Brock & Harvey, 1978). Males in strongly polygynous species can encounter different constraints on reproductive success. Constraints can be intrinsic (physiological) and include such characteristics as body size, age and growth rate (e.g., Leary et al., 2005). For example, large male broad-headed skinks are able to prevent smaller males from gaining access to females in order to mate (Cooper & Vitt, 1997). Constraints can also be external (environmental), including distances between mates and other males. In most systems where males have constraints, males may have evolved a range of reproductive tactics such as mate guarding (Sherman, 1989a), resource or guarding (Suzuki & Nagano, 2006), having the appearance or behavior of females (a 'sneaky male' strategy) (Rowland, 1979), or displays of male dominance to attract female choice (Bradbury & Gibson, 1983).

Alternative male tactics can be maintained in a population if the alternatives have equal success or if there are individuals who are incapable of employing the more successful strategy due to physiological or external constraints, therefore using a tactic that is considered the "making the best of a bad job" (Bateson, 1976). In order to maximize their reproductive opportunities, males should adopt a tactic based on their current competitive ability, determined by size, age, or energy reserves (Emlen & Oring, 1977a; Waltz & Wolf, 1984). If a male is unable to obtain a mate by using the conventional tactic of the species, he may opt for a subordinate alternative-mating tactic.

I studied mating and paternity success in the Columbian ground squirrel (*Urocitellus columbianus*). This species is particularly appropriate for a study of alternative mating tactics due to the reproductively active males falling into two categories of behavioral dominance. Dominant males defend territories at the beginning of a short (2-3 week) annual mating period, during which each female mates on a single day (Manno & Dobson, 2008). Subordinate males do not defend a territory, but live within or adjacent to the territories of dominant males. The subordinate males are also younger than territorial males, and thus represent an initial developmental stage in reproductive behavior. Territorial males are behaviorally dominant and win fights and chases within their territories particularly when females are in estrus (Murie & Harris, 1978; Manno & Dobson, 2008). Subordinate males generally lose fights and chases, but still manage to remain within the colony and have access to mating females.

Female ground squirrels frequently mate with multiple males, and about 68% of litters have multiple paternity (Raveh et al., 2010a). The first male to mate with a female has a considerable reproductive advantage; siring about 85% of singly sired litters and siring approximately 58% of offspring from multiply inseminated litters. Females generally mate with the territorial male whose core activity area overlaps the center of the female's home range, or with the nearest territorial male if no male's territory overlaps her home range (Manno and Dobson 2008). Thus, territorial males have a mating and inseminating advantage over non-territorial males, but there is still opportunity for up to 4 subsequent matings to produce offspring after the first male copulates. Individual females have a maximum of eight mates and a mean of around four mates (Raveh et al. 2010).

After the first mating with the territorial male, males from other territories and subordinate males both compete for any subsequent matings with the same female. Thus, there are two male tactics for obtaining matings in Columbian ground squirrels: 1) holding a territory to increase the likelihood of mating first, 2) pursuing mating opportunities with females by subordinate non-territorial males.

I examined whether being a subordinate male is an equivalent alternative mating tactic to being a territorial male, or whether subordinate males are simply making the best of a bad job. First, I analyzed secondary and subsequent matings of multiply-mating females for the relative prevalence of different males: territorial males and subordinate males. Territorial males are behaviorally dominant over subordinate males, even on territories other than their own (Manno and Dobson 2008). Thus, I tested whether subordinate males were as likely to obtain second and later matings as territorial males that are not on their own territory. I also determined whether subordinate males have an equal chance at paternity as dominant males, or whether they suffer a disadvantage in sperm competition. I compared the number of copulations to the number of offspring sired, controlling for the mating rank in a females queue, since the probability of reproductive success at each rank is dependent upon the success at earlier ranks. I also pooled reproductive attempts at various ranks to test whether subordinate males were as efficient as dominant males at siring offspring after copulating with a female.

METHODS

Study Population

I observed a colony of Columbian ground squirrels (*Urocitellus columbianus*) in

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Sheep River Provincial Park, Alberta, Canada (50°N, 110°W, elevation 1500m) from April to mid-July over a four-year period (2005-2008). Both age and matriarchal lineage were known for each individual with one exception, an immigrant male at least three years of age when first captured. Females emerged a few days to three weeks after the males, who emerged in mid-April each year. All individuals were trapped within two days of emergence using peanut butter in live traps (National live traps, WI, USA; 15×15×48 cm and 13×13×40 cm). Identification number, weight, reproductive status and general observations were recorded. The identification number was stamped onto fingerling fish tags (National Band & Tag Co., Newport, KY, U.S.A.) attached to both ears shortly after weaning (residents) or upon first capture (non-residents). Weight was taken using specialized cloth bags and Pesola® spring scales. The male's reproductive status was checked by examining whether the testes had descended into the scrotal sac, and examining the color of the scrotum. Sexually mature males had a black-pigmented scrotum and immature males were still light grey or pink in color. I also examining the swelling and dilation of the female's vulva to assess the reproductive status of pre-estrous females (Murie, 1995a). Approximately four days after emergence from hibernation, adult females went through a diurnal estrous period lasting 5-7 hours (Murie & Harris, 1982; Murie, 1995b).

Detailed behavior observations were taken from 3m high observational towers using 7-10 power binoculars, beginning the day of the first female's emergence in late-April until the end of the three-week mating season in mid-May. At least two seasoned researchers and one trained field assistant arrived at the field site 30 minutes before the first ground squirrel emerged for the day. Observers stayed at least 30 minutes after the

last ground squirrel immerged underground for the night to acquire accurate sleeping burrow information. I estimated sleeping burrows from location of morning emergence and evening immergence. The first mating was given to the male who emerged with the female on the day of oestrus (Raveh et al., 2010a). A mating sequence was formed by recording all copulations, assuming a copulation occurred when an oestrus female and male remained in the same burrow system for more than five minutes (Raveh et al., 2010a). I took detailed records of all mating and territorial interactions, including location and duration, through the mating season (see Manno & Dobson, 2008). Males were considered non-territorial, or subordinate, if they failed to defend a territory area and were inferior in the majority of interactions with a territorial male.

Females dug natal burrows during the lactation period, which I discovered by observing pregnant females frequently visiting and stocking a burrow with dry straw. Females also used their natal burrow as a sleeping burrow, and emerged from them in the morning a few days before parturition (Murie et al., 1998). Gestation is 23-25 days (Murie & Harris, 1982), so I moved pregnant females to the lab 21 days after oestrus. Females were housed in polycarbonate cages (48 x 27 x 20 cm) (for details see Murie et al., 1998; Skibiel et al., 2009) and were supplied food twice a day for the duration of their stay. I looked for the presence of neonatal young no less than four times a day. The weight of the mothers and neonates was taken four to twelve hours after birth and the sex of the neonates was determined at that time. The neonates had small tissue collected for DNA analysis.

Between 24 and 48 hours after birth, depending on time of day and weather, mothers and their litters were released back into the field site near their natal burrow.

Females first checked their natal burrow before returning to retrieve their pups. If the mother didn't return for her pups, the pups were placed in the natal burrow with their mother. Approximately 27 days after birth, the pups emerged from their natal burrows and were considered to be at the end of the weaning period. Data were gathered on the day of first emergence and included weight, identification (left by tissue sampling marks), and sex confirmation. They also received ear tags for future identification.

Paternity

I gathered DNA from the neonates by clipping a small tissue sample from the right or left hind toe or tip of the tail. Tissue samples were taken from the tip of the ear for all adult males and females who did not have tissue samples gathered at birth. All tissue was preserved in 99% EtOH for later analyses of microsatellite DNA.

The Coltman Lab at the University of Alberta successfully genotyped all reproductive males and I was able to assign paternity to all of the offspring (226) with a 95% to 99% confidence. Paternity analysis were described in detail by Raveh et al. (2010a). In summary, DNA was extracted from the previously gathered tissue using DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands), 13 variable microsatellite loci were amplified with the use of polymerase chain reaction (PCR), and paternity was assigned using the program CERVUS 3.0 (Marshall et al., 1998)

Data analysis

All data were analyzed using the statistical program R 2.9.2 (Urbanek & Iacus, 2009). I tested whether subordinate males were as likely as off-territory dominant males

to obtain the second mating position (rank 2) or later mating in a population consisting of n_f females, n_s subordinate males, and n_d dominant males. To quantify the difference in opportunity that dominant males have over the subordinate males, I assumed m_i were the number of matings for rank i, and m_i matings were distributed over the $n_s + n_d$ males according to the multinomial distribution. Each male then had a particular probability for a rank i mating with a specific female who had at least i number of matings. The average probability for a subordinate male was denoted by p_s and p_d was the average of the probability for a dominant male. Therefore $n_s p_s + n_d p_d = 1$. The odds (q) that rank i went to a subordinate male, given that the mating will go to either a dominant or subordinate male, was:

$$q = \frac{\dfrac{p_s}{p_s + p_d}}{1 - \dfrac{p_s}{p_s + p_d}}$$

I used X to denote the random variable that counted the number of matings for all the subordinate males. Then X was binomially distributed with parameters $n = m_i$ and $p = n_s p_s$. An exact confidence interval for p, denoted by (a,b), was then calculated by the Clopper-Pearson method (Clopper & Pearson, 1934). Subsequently, $a < p < b$ if and only if $\dfrac{n_d}{n_s} \times \dfrac{a}{1-a} < q < \dfrac{n_d}{n_s} \times \dfrac{b}{b-1}$, therefore if (a,b) with $0 \le a < b < 1$ is a $100(1 - \alpha)\%$

confidence interval for p then $(\dfrac{n_d}{n_s} \times \dfrac{a}{1-a}, \dfrac{n_d}{n_s} \times \dfrac{b}{b-1})$ was a $100(1 - \alpha)\%$ confidence interval for q. If $q < 1$, a dominant male had better odds at obtaining the mating at that rank.

The ability to sire offspring from a mating may vary between dominant and subordinate males. I looked at matings at each rank rather than looking at matings combined across all ranks. I observed m_i matings at rank i, and s_i of these m_i matings were by subordinate males. I then assembled the resulting offspring to the males of rank i matings in a list L_i of length m_i such that the first s_i elements in the list belonged to the subordinate males. Using this, I calculated the average number of offspring for both the subordinate ($\overline{O}(S)_i$) and dominant ($\overline{O}(D)_i$) males in the observed rank i matings

$$\overline{O}(S)_i = \frac{1}{s_i} \sum_{j=1}^{s_i} L_k[j] \text{ and } \overline{O}(T)_i = \frac{1}{m_i - s_i} \sum_{j=s_i+1}^{m_i} L_k[j]$$

If subordinate males had the same efficiency as territorial males at siring offspring from a copulation, then the observed values of $\overline{O}(S)_i - \overline{O}(D)_i$, which for the purpose of this study will be called the reproductive statistic, should not have been particularly large or small when compared to the distribution of such values calculated from all possible permutations of the entries in the list L_i. To quantify the efficiency of matings I conducted a randomization test using the reproductive statistic $\overline{O}(S)_i - \overline{O}(D)_i$. The randomization generated a distribution for $\overline{O}(S)_i - \overline{O}(D)_i$ which I was able to then quantify the extremity of my observed values.

RESULTS

I determined the mating sequences of 76 females and 30 males, of which seven (23.3%) were subdominant males over a four-year period, 2005-2008. Over this time, 300 matings were observed, with 226 of them being the second mating (rank 2) or later. Although subordinate males make up 23.3% of the male breeding population, they

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received only 11.94% (Figure 1) of the second or later matings and sired only 3.3% of the total offspring (Figure 2). Dominant males off their territory received more matings and offspring than subordinate males.

With a confidence interval set at 90%, I found that dominant males had higher odds of obtaining the rank 2 mating (q = 0.202; CI = (0.067, 0.481)). This was the same for the third (q = 0.587; CI = (0.304, 1.057)) and fourth (q = 0.280; CI = (0.092, 0.676)) mating positions. At the fifth mating position, however, the subordinate male had higher odds of mating with the female, though not significantly (q = 1.095; CI = 0.464, 2.367).

Although, dominant males received more offspring, they were not significantly more efficient at impregnating a female than subordinate males at the second rank (r = reproductive statistic; r = -0.297, P = 0.395), third rank (r = -0.436, P = 0.095), fourth rank (r = -0.234, P = 0.434), or the fifth rank (r = 0.307, P = 0.953) (Figure 3).

DISCUSSION

In order to understand the role that subordinate males play in the Columbian ground squirrel mating system, I examined whether non-territorial males were using an equal alternative mating tactic or were "making the best of a bad job." Subordinate males received proportionally fewer mates and offspring than dominant males regardless of whether the dominant male was on his own territory. There was also a strong first male advantage with the male's reproductive success decreasing as his position in the female's mating queue increased. I found that subordinates males have lower odds at obtaining any of the first four matings; however, subordinate males had better odds than a dominant male of mating with the female at mating at the fifth position. At the fifth position,

subordinate males were equally as efficient as dominant males at gaining matings with females.

In contrast to the findings of a prior study on Columbian ground squirrel mating behavior (Manno & Dobson, 2008), territory holding males were not guaranteed the first mating from the females on their territory. Furthermore, a few subordinate males were able to secure the key first mating position. All territories had at least one female residing on them, and unlike the study by Manno and Dobson (2008), all females resided on a male's territory. It is possible that more than one female on a single male's territory came into oestrus on the same day, forcing all but one female to mate first with a male other than the territory holder. Territorial males did, however, receive the majority of first matings from females on their territory. It may be a good strategy for male Columbian ground squirrels to focus on obtaining female-rich territories.

Dominant males both on and off their territory were more successful at obtaining matings than subordinate males except at the fifth rank. This raises the question of why subordinate males were able to acquire matings later in the mating sequence. Dominant males may have choosen not to waste time or energy mating with a female at a suboptimal rank. Chance of siring any offspring is low at the fifth position, and a dominant male's time might be better spent defending his territory and becoming familiar with other pre-oestrus females. A subordinate male, however, has less to lose and more to gain by mating with a female in the fifth position since he has little chance to mate with another female at a more optimal rank. Dominance might not accurately display a male's quality. A subordinate male may have better genes (eg., unrelated subordinate male vs. dominate relative Simmons, 1991) or possess other characteristics that may be

of benefit to females (Qvarnström & Forsgren, 1998). There may also be a benefit of having a variety of genotypes within a litter, or security of fertilization for the female (Charmantier et al., 2004). Furthermore, females tended to mate with males they had repeated contact with previous to entering oestrus. Subordinate males may be able to come into contact with more females since they do not defend territories. In my study colony, territorial males claimed only about half of the livable area, and all claimed territories had females residing on them.

Even though subordinate males were not as successful at acquiring a mating as dominant males, dominance was not a factor for the proportion of offspring sired at any rank. Females did not appear to bias sperm utilization in favor of dominant males.

In order to fully understand the selective pressures on a particular mating tactic, it is important to look at the costs of employing each tactic (Caro & Bateson, 1986). Costs of defending a territory may include the inability to become familiar with females in other parts of the colony, the missed opportunities to mate with females during times of physical male interaction, and the physical injuries and energetic cost of excluding other males from the territory. Subordinate males benefit by gaining reproductive experience that may allow them to be more successful than males of the same age that do not attempt to breed, which has been suggested as a female Columbian ground squirrel strategy (Broussard et al., 2003b). Those males that delay breeding, however, may have a better chance at avoiding aggression from territorial males and surviving to the following breeding season. It may be necessary to evaluate the costs and benefits of the alternative mating tactics over an entire lifetime.

I found that the reproductive success of male Columbian Ground Squirrels is

largely dependent on the territoriality and mating tactics of breeding adults. Female

choice may also be an influence on male reproductive tactics. Females tend to mate first

with dominant males, therefore, defending a territory has become the conventional tactic

for male Columbian ground squirrels. The subordinate males, however, adopt a best of a

bad job tactic and are able to obtain some reproductive success and experience. If a

subordinate male is able to obtain a copulation, he is just as likely as a dominant male to

sire offspring at that mating position, suggesting that there is no cryptic female choice for

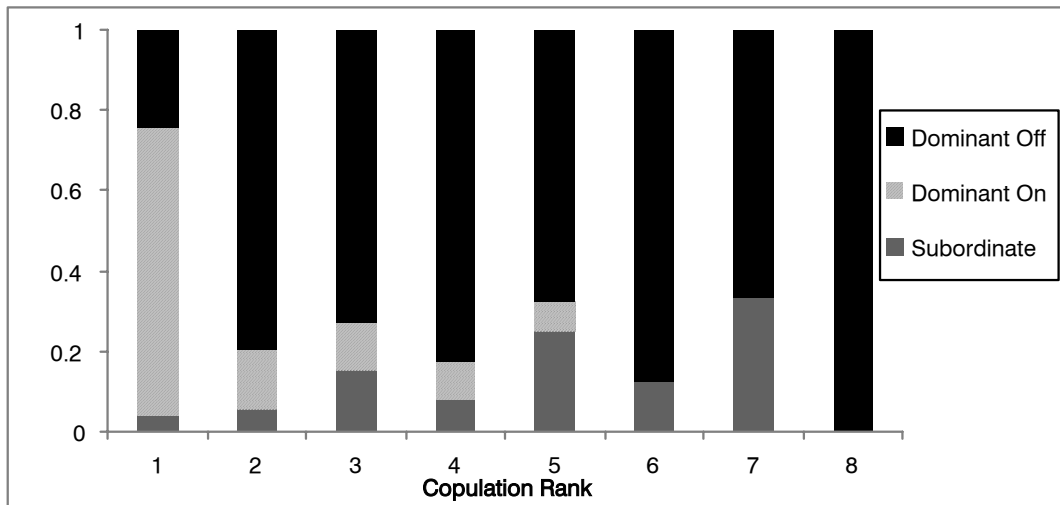dominant males to fertilize more of her offspring.

A.
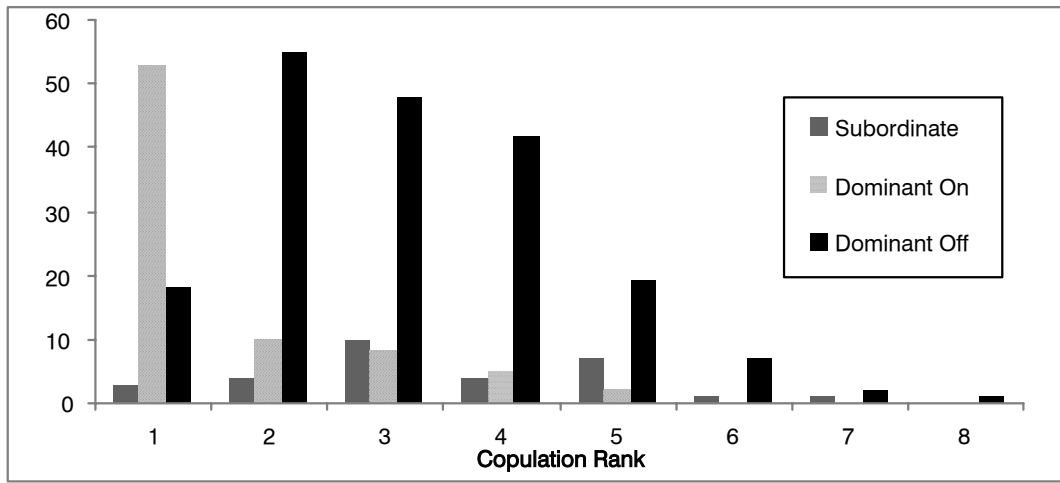
B.

Figure 1: Number (A) and proportion (B) of copulations by subordinate (non-territorial) males, dominant males off their territory, and dominant males on their territory per mating position (rank in queue).
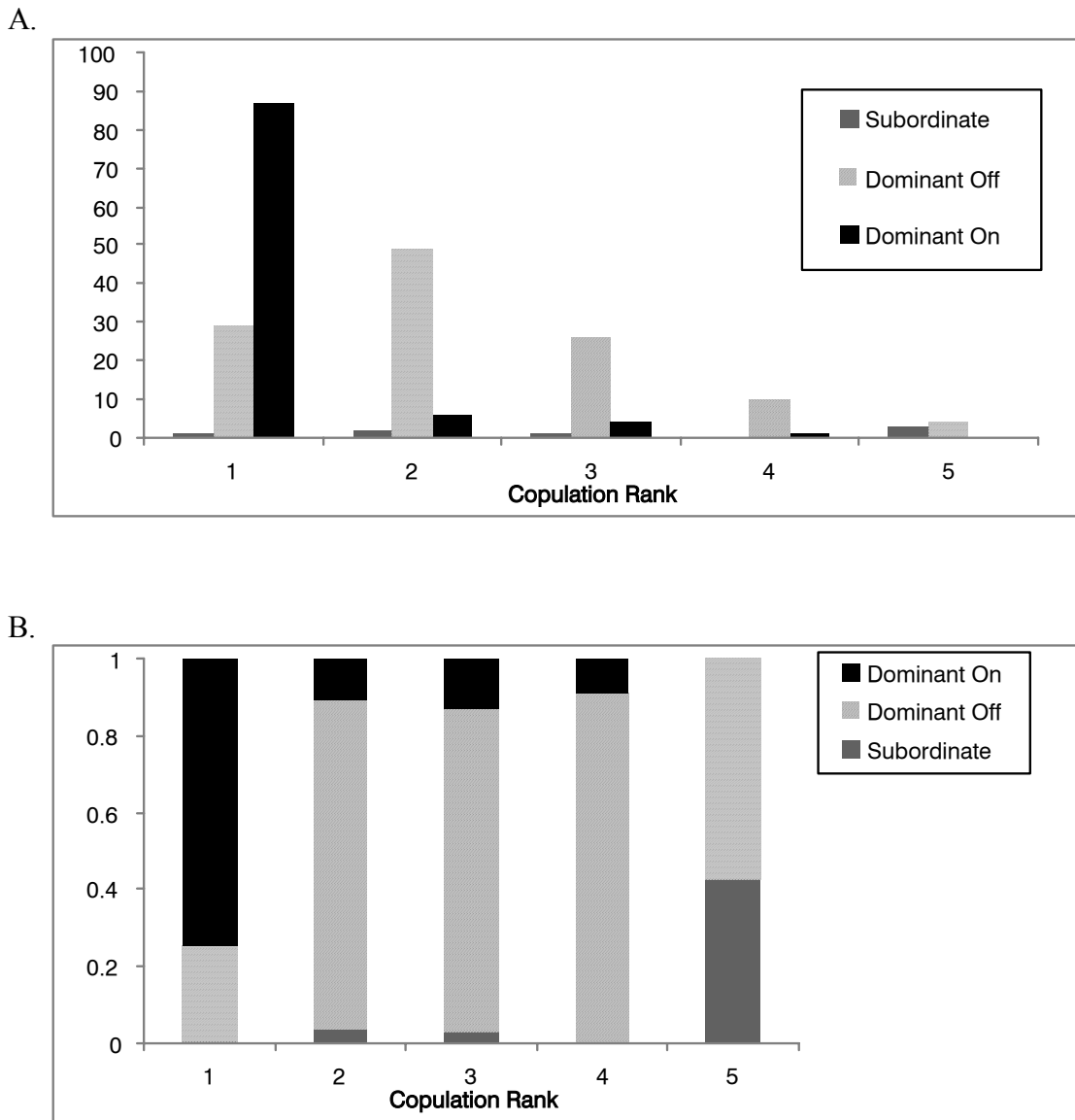
A.

B.

Figure 2: Number (A) and proportion (B) of offspring by subordinate (non-territorial) males, dominant males off their territory (non-resident) and dominant males on their territory (resident) per mating position (rank in queue). No offspring were born after the fifth mating.

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